Reproducibility and Gender-Related Differences of Heart Rate Variability during All-Day Activity in Young Men and Women

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Background: Only few data are available on reproducibility over time in healthy young men and women and the corresponding gender-related changes of heart rate variability (HRV) measurements.

Methods: We studied temporal and spectral HRV indices obtained from 24-hour Holter recordings in 32 healthy volunteers (14 men and 18 women, mean age 29 ± 3 years) during 2 days of their usual all-day activity.

Results: Time-domain measures and the spectral low-frequency (LF) and high-frequency (HF) components as well as the LF/HF ratio were comparable on both test days. Significantly higher values on test day 2 were observed only for the spectral very-low-frequency (VLF) component and for the resulting total power. Compared to men, women had higher day- and nighttime vagus-associated HRV indices, including root mean square of successive differences (RMSSD), pNN50 (NN50 count divided by the total number of all NN intervals), and HF power, and lower day- and nighttime VLF and LF power with lower LF/HF ratio and total power.

Conclusions: Temporal indices and the LF and HF spectral HRV measures are reproducible over usual all-day activity in young healthy subjects. Young women have higher day- and nighttime vagal tone than men with similar age range.

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standard heart rate variability parameters; reproducibility; gender-related changes; young men and women

Heart rate variability (HRV) measurements are used as a simple, noninvasive electrocardiographic method to assess the autonomic status allowing us to evaluate the sympathovagal balance at the sinoatrial level. They provide information about sympathetic and parasympathetic autonomic function in normal and pathological hearts.^{1–3}

Reproducibility over time and age- and gender-related changes of HRV measurements is essential for clinical utility and applications of this method. In recent years, several studies have evaluated these different aspects in patients with heart disease,^{4–6} and in younger subjects with normal hearts.^{7–10} However, conflicting results have been provided on HRV time- and frequency-domain parameters when comparisons were performed between men and women.^{11–22} The purpose of this

study was to evaluate reproducibility and to determine potential gender-related differences of standard HRV parameters during all-day activity in a group of young men and women with no overt heart disease.

MATERIAL AND METHODS

Study Subjects

We analyzed HRV parameters from 24-hour Holter recordings obtained from 32 subjects, 14 men and 18 women, mean age 29 ± 3 years. All the subjects were volunteers and were composed of 16 medical students of our Medical Faculty, 10 residents and 6 technicians. All subjects had a careful history taken and were examined by a cardiologist

in order to rule out any potential cardiovascular problem.

Study Protocol

The study protocol consisted of two test days, on which each participant underwent a 24-hour Holter recording. Test day 1 was separated from test day 2 by an interval of 48 hours. During the test days the subjects were asked to continue their normal work and leisure activities. The subjects were instructed to have on both test days approximately 7 to 8 hours of sleep, to wake up around 7:00 AM, and to have food and beverages in the morning, at noon (around 1:00 PM), and in the evening (around 7:00 PM).

Analysis of HRV

All HRV parameters were taken from 24-hour Holter recordings and analyzed according to the recommendations of the Task Force of the European Society of Cardiology (ESC) and the North American Society of Pacing (NASPE).²³ The three-channel 24-hour Holter recordings were all manually analyzed from a Del Mar Avionics software (HRV Analyzer, Del Mar Avionics, Irvine, CA, USA). Normal sinus beats, supraventricular or ventricular ectopic beats, artifacts, or unclassified were detected. A strict selection of normal RR (NN) intervals in the 24-hour recordings was made. RR intervals before and after ectopic beats and intervals that varied by more than 20% were excluded from the analysis. The software calculated the mean NN interval, the time-domain and spectral HRV indices. The performed measurements of HRV included time-domain and frequency-domain indices.

Temporal Measures

The 24-hour time-domain indices comprised statistical and geometric measures. The standard deviation of all NN intervals (SDNN), the standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording (SDANN), the root mean square of successive differences (RMSSD), and pNN50 (NN50 count divided by the total number of all normal RR intervals) were used as statistical measures and the HRV triangular index (HRVi) as geometric measure. Statistical measures were expressed in milliseconds (ms). SDNN and the HRVi are both estimates of overall HRV, and RMSSD and pNN50 reflect alterations in autonomic tone that are predominantly vagally mediated.^{23,24}

Frequency Domain Analysis

Frequency domain analysis was performed by a nonparametric method, the fast Fourier transformation. Spectral components were evaluated in terms of frequency given in Hertz (Hz) and amplitude assessed by the area or power spectral density of each component (given in msec²). The following spectral bands were determined: the very-low-frequency band (VLF), the low-frequency band (LF), the high-frequency band (HF), the LF/HF ratio, and the total power. Spectral components were expressed in absolute values (ms²) and in natural logarithms (ln) of the power because of the skewness of the distributions. Furthermore, LF and HF powers were given in normalized units (nu). Normalization was performed by subtracting from the total power the VLF component, reducing thereby the effects of noise due to artifacts and minimizing the effects of the changes in total power on the LF and HF components.

Statistical Analysis

Comparisons between HRV parameters obtained on test days 1 and 2 were made first for all subjects and then between male and female participants for the whole and for the daytime and nighttime recordings. Repeated measures analysis of variance (ANOVA), as well as the Student's *t*-test when appropriate, were used to detect significant differences between data on both test days and between men and women. Differences were considered significant at a *P* value < 0.05. Results are expressed as mean ± SEM.

RESULTS

All volunteers fully completed both test days. During their testing, the subjects had their normal usual lives, including working time, lunch time, time spent at home, and number of hours of sleep (mean 7.8 ± 0.4 hours). No subject was taking at that time any particular medication.

Reproducibility of Time-Domain and Geometric Measures

Mean values of NN intervals, time-domain statistical and geometric measures for all study subjects are depicted in Table 1. Mean NN interval values and time-domain indices were similar on both test days for all subjects together. On test day 2,

Table 1. NN Interval Values and Time-Domain Measures in All Study Volunteers

	Day 1 (n = 32)	Day 2 (n = 32)	P Values
NN interval (ms)	745 ± 26	725 ± 24	NS
SDNN (ms)	135 ± 10	116 ± 9	NS
SDANN(ms)	117 ± 10	95 ± 10	NS
RMSSD (ms)	35 ± 3	34 ± 3	NS
pNN50 (%)	12.8 ± 2.4	12.7 ± 9.2	NS
HRV index	36 ± 13	38 ± 11	NS

All values are expressed as mean ± SEM.

NN interval = normal RR interval; SDNN = standard deviation of all NN intervals; SDANN = standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording; RMSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; pNN50 = NN50 count divided by the total number of all NN intervals; TV = total variability over 24 hours; HRV = heart rate variability.

there was, however, a tendency to somewhat lower values for SDNN and SDANN, but the differences were not significant. Parameters for vagal tone, including RMSSD and pNN50, were very stable. The HRV index also showed comparable values during test days 1 and 2.

Reproducibility of Spectral Measurements

Table 2 shows spectral measurements performed on test days 1 and 2. Values for LF and HF, in abso-

Table 2. Frequency Domain Indices in All Study Volunteers

	Day 1 (n = 32)	Day 2 (n = 32)	P values
VLF (ms ²)	574 ± 131	1250 ± 235	<0.05
ln VLF (ln ms ²)	6.1 ± 0.02	6.9 ± 0.02	<0.01
LF (ms ²)	693 ± 96	673 ± 101	NS
ln LF (ln ms ²)	6.4 ± 0.02	6.3 ± 0.02	NS
LF nu	69 ± 30	69 ± 20	NS
HF (ms ²)	349 ± 72	288 ± 48	NS
ln HF (ln ms ²)	5.5 ± 0.02	5.4 ± 0.02	NS
HF nu	30 ± 30	31 ± 20	NS
Ratio LF/HF	2.8 ± 0.4	2.7 ± 0.3	NS
ln ratio LF/HF	1.17 ± 0.03	1.17 ± 0.02	NS
TP (ms ²)	1616 ± 226	2211 ± 327	<0.01
ln TP (ln ms ²)	7.2 ± 0.2	7.5 ± 0.1	<0.01

All values are expressed as mean ± SEM.

VLF = very low frequency; LF = low frequency; HF = high frequency; LF/HF ratio = low-to-high frequency ratio; TP = total power; ln = natural logarithm ; nu = normalized units.

lute, logarithmic, and normalized units were similar on both test days. Consequently, the ratio LF/HF showed no significant changes on both test days. Analysis of the VLF component resulted in significant differences between test days 1 and 2. Values, both in absolute ($P < 0.05$) and in logarithmic units ($P < 0.01$), were significantly higher on test day 2. These changes induced significantly higher values of the total power, also both in absolute ($P < 0.001$), and in logarithmic units ($P < 0.001$).

Effects of Gender on Time-Domain and Geometric Measures

Tables 3 and 4 illustrate the comparisons of mean NN interval values, time-domain, and spectral components between the male and female study subjects on test days 1 and 2. When considering the 24-hour recordings on test days 1 and 2, no significant gender-related differences were observed for mean NN interval values and time-domain indices reflecting global HRV. Thus, SDNN, SDANN values, and the HRV index were similar in men and women. However, time-domain indices for vagal tone showed significantly higher values for RMSSD ($P < 0.05$) and pNN50 ($P < 0.05$) in the female subjects on test days 1 and 2.

Effects of Gender on Spectral Measurements

As seen in Table 4, spectral components had a different behavior in the female group. On both test days, the VLF and LF components were lower and the HF component was higher in women than in men, expressed in absolute ($P < 0.05$), in ln values ($P < 0.05$), and in normalized units ($P < 0.05$). Thus, the resulting LF/HF ratio, expressed in absolute ($P < 0.01$) and in logarithmic units ($P < 0.01$), and the total power, expressed in absolute ($P < 0.05$) and in logarithmic units ($P < 0.05$), were lower in women on test days 1 and 2 when compared to the corresponding values in the male volunteers. Compared to test day 1, both men and women had significantly higher values of the VLF component, expressed in absolute ($P < 0.05$) and in logarithmic units, on test day 2 ($P < 0.05$), with resulting higher values of the total power, also both in absolute ($P < 0.05$) and in logarithmic units ($P < 0.05$).

Table 3. Comparisons of NN Interval Values and Temporal Measures between Men and Women

	Men (n = 14)		Women (n = 18)	
	Day 1	Day 2	Day 1	Day 2
NN interval (ms)	734 ± 25	722 ± 40	766 ± 44	752 ± 40
SDNN (ms)	130 ± 18	113 ± 10	138 ± 12	118 ± 14
SDANN (ms)	110 ± 17	85 ± 10	122 ± 14	103 ± 16
RMSSD (ms)	32 ± 5	33 ± 5	38 ± 5*	36 ± 3*
pNN50 (ms)	11.1 ± 3.8	11.1 ± 3.9	14.1 ± 3.3*	13.8 ± 2.9*
HRV index	36 ± 14	38 ± 14	37 ± 11	36 ± 12

All values are expressed as mean ± SEM.

*P < 0.05 when comparing day 1 and 2 values of the female subjects to the corresponding values of the male subjects.

Abbreviations as in Table 1.

Circadian Gender-Related Profiles of Measured HRV Parameters

Table 5 shows day- and nighttime circadian variations for NN interval values, temporal-, and frequency-domain indices. All nighttime values significantly increased on test days 1 and 2 as compared with daytime values in both men and women ($P < 0.05$). Women tended to have on both test days during daytime slightly lower and during nighttime slightly higher NN interval values than men. However, the differences did not reach significance ($P = 0.06$). Women had on both test days when comparing with men lower VLF and LF ($P < 0.05$ for day- and nighttime) and higher RMSSD ($P < 0.05$ for daytime and $P > 0.01$ for nighttime), pNN50

($P < 0.01$ for day- and nighttime), RMSSD ($P < 0.05$ for daytime and $P < 0.01$ for nighttime), and HF ($P < 0.05$ for day- and nighttime) values, with a resulting lower LF/HF ratio ($P < 0.05$ for day- and nighttime), and total power ($P < 0.05$ for day- and nighttime).

The hourly circadian variations of NN intervals, SDNN, RMSSD, LF, HF, and the LF/HF ratio in a male and a female study volunteer are displayed in Figure 1. Peak values for most parameters were reached during sleep between 2:00 and 4:00 AM. All values markedly decreased in the morning between 7:00 and 8:00 A.M. The most characteristic and striking differences were observed for the lower LF and higher HF values in the female study volunteer when compared to the male subject.

Table 4. Comparisons of Spectral Components between Men and Women

	Men (n = 14)		Women (n = 18)	
	Day 1	Day 2	Day 1	Day 2
VLF (ms ²)	699 ± 140	1612 ± 451*	476 ± 105†	960 ± 206*†
ln VLF (lnms ²)	6.5 ± 0.01	7.4 ± 0.02**	6.2 ± 0.02†	6.9 ± 0.02*†
LF (ms ²)	829 ± 172	814 ± 178	587 ± 99†	563 ± 111†
ln LF (lnms ²)	6.7 ± 0.02	6.7 ± 0.01	6.4 ± 0.01†	6.3 ± 0.02†
LF nu	76 ± 3	78 ± 4	63 ± 4†	61 ± 4†
HF (ms ²)	272 ± 94	224 ± 51	408 ± 106†	337 ± 74†
ln HF (lnms ²)	5.6 ± 0.02	5.4 ± 0.01	6.0 ± 0.02†	5.8 ± 0.01†
HF nu	24 ± 3	22 ± 2	37 ± 4†	35 ± 4†
Ratio LF/HF	3.8 ± 0.7	3.6 ± 0.3	1.9 ± 0.3††	1.9 ± 0.3††
ln ratio LF/HF	1.3 ± 0.02	1.3 ± 0.02	0.6 ± 0.01††	0.6 ± 0.01††
TP (ms ²)	1801 ± 443	2651 ± 647**	1472 ± 222†	1910 ± 271*†
ln TP (lnms ²)	7.5 ± 0.02	7.9 ± 0.02*	7.3 ± 0.01†	7.6 ± 0.02*†

All values are expressed as mean ± SEM.

*P < 0.05 and **P < 0.01 when comparing day 2 values of the male and female subjects to their corresponding day 1 values;

†P < 0.05 and ††P < 0.01 when comparing day 1 and 2 values of the female subjects to the corresponding day 1 and 2 values of the male subjects.

Abbreviations as in Table 2.

Table 5. Daytime and Nighttime NN Interval and HRV Values in Men and Women

	Daytime (8:00 AM–9:00 PM)				Nighttime (10:00 PM–7:00 AM)			
	Men		Women		Men		Women	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
NN (ms)	655 ± 10	647 ± 11	616 ± 9†	613 ± 13†	883 ± 29*	863 ± 28*	971 ± 39*	953 ± 30*
SDNN (ms)	121 ± 8	108 ± 9	125 ± 11	110 ± 10	143 ± 6*	121 ± 9*	148 ± 8*	128 ± 8*
SDANN (ms)	100 ± 5	81 ± 4	115 ± 8	95 ± 8	124 ± 8*	97 ± 8*	129 ± 6*	113 ± 6*
RMSSD (ms)	26 ± 2	27 ± 3	31 ± 2†	29 ± 2†	42 ± 6*	43 ± 6*	48 ± 6*††	46 ± 5*††
pNN50 (ms)	8.8 ± 1.7	8.9 ± 1.6	11.9 ± 1.4††	11.9 ± 1.4††	14.3 ± 1.5*	14.3 ± 1.6*	17.0 ± 1.7*††	16.5 ± 1.3*††
HRV index	29 ± 5	30 ± 4	31 ± 4	31 ± 5	46 ± 5*	49 ± 4*	46 ± 4*	46 ± 3*
In VLF (lnms ²)	6.4 ± 0.03	7.2 ± 0.03	6.0 ± 0.04†	6.7 ± 0.01†	6.7 ± 0.02*	7.6 ± 0.01*	6.3 ± 0.03*†	7.0 ± 0.02*†
In LF (lnms ²)	6.6 ± 0.03	6.6 ± 0.03	6.3 ± 0.08†	6.2 ± 0.03†	6.9 ± 0.02*	6.8 ± 0.06*	6.5 ± 0.05*†	6.5 ± 0.03*†
In HF (lnms ²)	5.3 ± 0.05	5.3 ± 0.03	5.8 ± 0.03†	5.6 ± 0.02†	5.8 ± 0.04*	5.5 ± 0.03*	6.2 ± 0.01*†	6.0 ± 0.01*†
In ratio LF/HF	1.2 ± 0.03	1.3 ± 0.01	0.5 ± 0.02†	0.6 ± 0.01†	1.6 ± 0.3*	1.9 ± 0.3*	0.6 ± 0.2*†	0.8 ± 0.2*†
In TP (lnms ²)	7.3 ± 0.04	7.4 ± 0.04	7.2 ± 0.02†	7.5 ± 0.02†	7.7 ± 0.02*	8.0 ± 0.04*	7.4 ± 0.01*†	7.7 ± 0.02*†

All values are expressed as mean ± SEM. *P < 0.05 when comparing day 1 and 2 nighttime values of the male and female subjects to their corresponding day 1 and 2 daytime values; †P < 0.05 and ††P < 0.01 when comparing day 1 and day 2 daytime and nighttime values of the female subjects to the corresponding day 1 and 2 values of the male subjects. Abbreviations as in Tables 1 and 2.

DISCUSSION

The results of this study on reproducibility and gender-related differences of HRV parameters in young healthy men and women showed first, that time-domain indices, geometric measures, and spectral components, except the VLF component, were highly reproducible considering that all recordings were performed in all-day life conditions. Second, when compared to men women had higher values of RMSSD and pNN50, a lower VLF and LF, and a higher HF component, present throughout the day and night cycle, suggesting thereby a higher parasympathetic autonomic modulation.

Comparisons with Prior Studies

The only reference values for normal standard HRV measures were given by the Task Force of the ESC and the NASPE²³ based on Bigger's et al.²⁵ previously published data. When comparing our results to these reference standard measurements performed in healthy middle-aged subjects, we found in our younger subjects generally somewhat lower values for time and spectral indices, except for the vagus-associated components (RMSSD, pNN50, and HF), which were higher, probably due to the female participants, and the HRV index, which was comparable. In an earlier study⁷ on reproducibility of standard HRV parameters, all values were correctly reproducible within subjects. However, there were marked interindividual variations suggesting differences in the magnitude of fluctuations in cardiac autonomic tone in normal subjects. Heart rate values were less subject to inter- and intraindividual variations. These results showed that HRV was influenced by vagal tone and HR by sympathetic activity. In another study⁸ in 14 normal subjects, all time- and frequency-domain parameters were highly reproducible. Furthermore, most of the measured variables had an excellent intraclass correlation coefficient (>0.8), suggesting marked individual reproducibility and there was also a very strong positive correlation between vagally time-domain-mediated measures (RMSSD and pNN50) and the HF component of the spectral domain. The reproducibility of HRV parameters has also been determined in short-term recordings and found to be highly reproducible.^{9,10}

Figure 1. Circadian profiles of hourly measured values of NN intervals, SDNN, RMSSD, LF and HF power, and the LF/HF ratio in a 29-year-old male and in a 27-year-old female study volunteer. SDNN = standard deviation of all NN intervals; RMSSD = root mean square of successive differences; ln = natural logarithm; LF = low-frequency power; HF = high-frequency power.

Reproducibility of the VLF Component

The VLF component was higher on test day 2 in both the male and female subjects. The exact significance of the VLF component is still not clear. Although the VLF component has been described as a marker of sympathetic activity and dependent on parasympathetic tone and thus associated with increased risk for cardiac and arrhythmic death,^{17,26} some researchers suggested that physical activity was a major determinant of the VLF component, demonstrating the dependence of the VLF power on the amount and natural variations of physical activity.²⁷ Because our study protocol was based on everyday activity, the possibility exists that slight variations in this activity on test day 2 could be responsible for the observed fluctuations of the VLF component. In this sense, this component appears to be much less reproducible than the LF and HF spectral indices.

Cardiac Autonomic Differences between Men and Women

In our study, women had higher values of short-term time-domain measures reflecting increased vagal tone. This was, in addition, corroborated by a lower LF, a higher HF, and a resulting lower LF/HF ratio. These differences were observed during the day- and during the nighttime recordings. Thus, our data confirmed a primarily higher parasympathetic state in women than in men, at least in this age group. Earlier and more recent studies^{11–15} have generally shown higher values for SDNN and SDANN, both reflecting global HRV, in men with no gender differences for vagus-associated parameters, such as RMSSD and pNN50, except in the recent study by Bonnemeier et al.¹⁵ These authors found higher RMSSD values in younger men, which is a rather confounding observation considering that the risk of coronary artery disease

development is higher in young men than in young women. Studies performed on spectral HRV parameters showed that men had higher values than women of the VLF and LF components, both associated with sympathetic activity.^{13,16–19,25} Women tended to have higher HF, a marker of vagal modulation, and lower VLF and LF values with lower LF/HF ratios than men.^{19–22} Taking into account the clearly established correlations between time- and frequency-domain parameters,^{1,23,28} it is somewhat astonishing to state these discrepant findings between time-domain indices in men and spectral components in women. Compared to these data our results were much more uniform. Indeed, in our study global HRV was similar in both genders and most parameters from time and spectral domain reflecting vagal activity were higher in women. The differences in HRV values in women appear to be due to a lower sympathetic activity. This higher parasympathetic tone may reflect the relative protection against arrhythmias and development of coronary artery disease in women. Possible explanations for these differences include differences in HR dynamics in women and the protective role of estrogens.^{19,22,29} In this sense, in a previous study Huikuri et al.²² found that hormone replacement therapy may have favorable effects on the autonomic regulation in postmenopausal women by increasing baroreflex sensitivity and HRV.

Heart Rate, HRV Measures, and Gender-Related Differences

In our study NN interval values showed a clear circadian variation. Women tended to have during daytime slightly lower (higher HR) and during nighttime slightly higher NN (lower HR) interval values than men; however, the differences did not reach significance. Thus, the increased vagal tone in our female study participants was not significantly reflected in the observed HR levels, indicating thereby some discrepancy between mean HR, respectively NN interval values, and HRV measures. This disparity in the behavior of mean HR and HRV measures has been previously reported in various normal and pathologic situations.^{30–32} Thus, in a previous study no significant changes were found in HR values despite significant power spectral modifications in response to postural changes before and after volume depletion³⁰ or after baroreceptor loading with a negative pressure neck chamber.³¹ In patients with conges-

tive heart failure, HR is often increased due to elevated plasma catecholamines. However, HRV is reduced and particularly the LF power, a mixture of both autonomic inputs, which has been found to be paradoxically reduced or abolished with severe heart failure.³² These and our findings suggest that mean HR and HRV measures do not have direct correlations. In this sense, as observed in our study participants significant and important changes in HRV measures can occur in the absence of overt changes in HR. This disparity is due to different control mechanisms responsible for the regulation of HR and HRV measures. For instance, HR may be modulated by changes in cardiac stroke volume or in blood pressure. Thus, the day and night variations in HR, as seen in our study, gave little information on the precise cardiac autonomic state of our young study participants when compared with the subtle changes we observed through the analysis of HRV parameters.

Study Limitations

First, our study was based only on a small sample of participants. Second, all results reflect a young age group and direct comparisons with other age groups were not performed. In this sense, an extrapolation of the effects of a higher vagal tone in women and its relative cardioprotection to other age groups should be done carefully. Third, this study was performed during all-day activity and thus reflects multiple physical and mental changes, all factors that may have had some influence on our results. However, all participants strictly confined to the established study protocol and avoided particularly to perform intense physical activity that would have produced important HR increases and changes in HRV.

CONCLUSION

Temporal indices and the LF and HF spectral HRV measures are reproducible over usual all-day activity in young subjects without overt heart disease. Young women have higher day- and nighttime vagal tone than men with similar age range, resulting in higher cardiovascular protection.

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