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Aging and nonlinear heart rate control in a healthy population

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Beckers, Frank, Bart Verheyden, and André E. Aubert. Aging and nonlinear heart rate control in a healthy population. *Am J Physiol Heart Circ Physiol* 290: H2560–H2570, 2006. First published December 22, 2005; doi:10.1152/ajpheart.00903.2005.—In recent years more studies are using nonlinear dynamics to describe cardiovascular control. Because of the large dispersion of physiological data, it is important to have large studies with both male and female participants to establish a range of physiological healthy values. This study investigated the effect of gender and age on nonlinear indexes. Nonlinear scaling properties were studied by using 1/*f* slope (where *f* is frequency), fractal dimension, and detrended fluctuation analysis short- and long-term correlations ($DFA\alpha_1$ and $DFA\alpha_2$, respectively). Nonlinear complexity was described with correlation dimension (CD), Lyapunov exponent (LE), and approximate entropy (ApEn). The population consisted of 135 women and 141 men (age, 18–71 yr). Twenty-four hour ECG recordings were obtained by using Holter monitoring. The recordings were split into daytime (8 AM–9 PM) and nighttime (11 PM–6 AM). A day-night variation was present in all nonlinear heart rate variability (HRV) indexes, except for the CD in the female population. During the night the percentage of CD values of surrogate data files differing from the CD value of the original data increased. All nonlinear indexes were significantly correlated with age. Deeper analysis per age category of 10 yr showed a stabilization in the age decline of the fractal dimension and ApEn at the age of ≥40 yr. The vagal pathways seemed to be more involved in the generation of nonlinear fluctuations. Higher nonlinear behavior was evident during the night. No clear difference between men and women was found in the nonlinear indexes. Nonlinear indexes decline with age. This can be related to the concept of decreasing autonomic modulation with advancing age.

heart rate variability; nonlinear dynamics; gender; circadian rhythm

THE PHYSIOLOGICAL BACKGROUND of heart rate fluctuations (heart rate variability; HRV) has been described extensively with the use of statistical methods and linear spectral analysis methods. The power spectral density description of HRV shows two distinct peaks: one in the so-called low-frequency (LF) band (0.04–0.15 Hz in humans) and another in the high-frequency (HF) band (0.16–0.4 Hz in humans). HF fluctuations have been attributed to vagal modulation, and LF fluctuations appear to be jointly mediated by sympathetic and vagal influences, together with the baroreflex mechanism (51a). It is well-known that time and frequency domain indexes quantifying HRV are reduced in many pathological conditions. Myocardial infarction (29), diabetes (17), coronary artery disease (21), and end-stage heart failure (18) are some of the most clearcut examples in which HRV was found to be associated with survival. The decreased autonomic modulation with advancing age is also reflected in a decrease in linear HRV indexes (10, 44, 45).

In healthy subjects, a significant difference between day and night HRV was reported (45), reflecting the higher vagal modulation during the night. In normal, healthy conditions, gender differences have been observed in HRV in various age classes (10, 23, 44, 45). Compared with men, women are at lower risk of coronary heart disease and of serious arrhythmias (58). Clinical manifestations of coronary artery disease in women lag by ~10 yr, and coronary events such as sudden cardiac death may lag as much as 20 yr (13). It has been suggested that a beneficial autonomic control of heart rate at ages <45 yr might be partly responsible for this advantage in the female population (45).

In recent years, new dynamic methods of HRV quantification have been used to uncover nonlinear fluctuations in heart rate that are otherwise not apparent. These nonlinear variations would enable the cardiovascular system to respond more quickly to changing conditions. Several methods have been proposed to quantify these fluctuations on the basis of the scaling properties of the heart rate variations [fractal dimension (FD) (28), 1/*f* slope (60) (where *f* is frequency), detrended fluctuation analysis (40)], the properties of the complex system in phase space [Lyapunov exponents (LE) (47), correlation dimension (CD) (8), pointwise CD (51), approximate entropy (ApEn) (5, 43)], or other nonlinear properties [recurrence plots (11, 35), Poincaré plots (53), and symbolic dynamics (56)].

Previous studies have tried to assess gender- and age-related differences in some nonlinear components of HRV (27, 42, 63). A decrease in nonlinear behavior was observed with increasing age (27, 42, 63), as were differences between day and night values (42, 63). However, most of these studies have major limitations, especially concerning the short-duration ECG recordings used (39, 48), but also the comparison of small groups (27) and the mix of male and female subjects (9) limit the use of these studies for comparison.

Analysis methods derived from nonlinear system dynamics have opened up a new approach for studying and understanding the characteristics of cardiovascular dynamics. Nonlinear analysis methods differ from the conventional HRV methods because they are not designed to assess the magnitude of variability but rather the quality, scaling, and correlation properties of the signals. A defining feature of healthy function is adaptability, the capacity to respond to unpredictable stresses and stimuli. A nonlinear responsiveness would offer greater flexibility than a linear responsiveness. The meaning of the indexes used for nonlinear dynamics is not as clearcut as those derived from spectral analysis. Future research will need to focus on determining clear physiological meanings for all indexes. Most studies conducted thus far have concluded that decreased nonlinear behavior of heart rate is associated with pathological states.

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On the basis of smaller studies in different pathologies, one can indicate possible physiological links to the nonlinear indexes: FD is a measure of the regularity of a signal. It corresponds to the self-similarity of the signal. It has been linked to vagal modulation of heart rate (63). A $1/f$ slope of -1 is an indication that the tachograms represent scaling behavior. This slope has been associated with sympathetic modulation (20), although this was not found by others (59, 60). ApEn is an index of overall complexity and predictability of a time series. The more regular and predictable the time series, the lower will be the value of ApEn. On the other hand, a more random RR time series will lead to higher ApEn values. ApEn is mostly linked with vagal modulation (4). Detrended fluctuation analysis quantifies the fractal scaling properties of the time series. The scaling exponents obtained with detrended fluctuation analysis can be seen as self-similarity parameters, which are characteristic for fractal behavior. Values of these exponents of ~ 1 are an indication of scaling behavior. The long-range correlations should correspond to sympathetic modulation, whereas short-term correlations should correspond to both sympathetic and vagal modulation (57). The largest LE quantifies the sensitivity of the system to initial conditions. This is characterized by the average rate of divergence of two neighboring trajectories in phase space and gives a measure of predictability. A negative LE implies that the orbits approach a common fixed point. A LE of zero means that the trajectories maintain their relative position, and a positive LE implies that the orbits diverge, representing a chaotic attractor. In previous studies this index was believed to be uncorrelated to autonomic modulation (20). The CD is a quantitative measure of the phase space and describes the complexity of the system and has been moderately linked to both sympathetic and vagal modulation (26) or only vagal modulation (20).

The concept of "normal" or "control" values is difficult in HRV analysis. To label a patient with a reduced or increased HRV, baseline values before the onset of the pathology should be available, which is often not possible. Because of the large dispersion of physiological data (especially HRV data), it is important to have large studies with both male and female participants to establish a range of physiological healthy values.

The purpose of the present study was, first, to investigate the effect of gender and age on nonlinear indexes; second, to study day-night variations in nonlinear indexes; third, to correlate traditional time and frequency domain measures of HRV with methods derived from nonlinear dynamics to make physiological correlates; and fourth, to define a physiological range of these nonlinear indexes in a healthy population.

METHODS

Population

A detailed medical history was obtained from all participants. Patients underwent a standard physiological examination. All patients presented with normal ECGs that were verified during analysis of the Holter recording. Patients were not on drug treatment. Only subjects with no history of diabetes, hypertension, cardiovascular, neurological, or psychiatric diseases were allowed to participate in this study. A total of 276 healthy subjects entered the study. They were recruited at two centers of occupational medicine (Medi Leuven and IDEWE) and in a group of volunteers of the Christelijke Mutualiteiten (health insurance institution). The population consisted of 135 women and 141 men between the age of 18 and 71 yr. All subjects gave informed consent to the protocol approved by the local Ethical Committee.

Data Acquisition

Twenty-four hour ECG recordings were obtained using Holter monitoring (ELA Medical, sample rate 200 Hz). Only recorders with time tracking were used. After RR peak detection and visual inspection by the operator for verifying the peak detection, a file containing the consecutive RR intervals was exported for later processing with HRV analysis software (3). The 24-h recordings were split into daytime (8 AM–9 PM) and nighttime (11 PM–6 AM).

Linear Analysis

Linear HRV parameters were calculated in agreement with the standards of measurement proposed by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (51a). In case of ectopic beats, two linear filters were applied to correct for data points outside a limit interval (3).

Mean and SD of the RR tachogram (SD), the square root of the mean of the sum of the squares of differences between consecutive RR intervals (rMSSD), and the percentage of intervals that vary more than 50 ms from the previous interval (pNN50) were calculated in the time domain.

After resampling of the tachogram at 2 Hz with the use of a cubic spline approximation [for details, see Aubert et al. (3)] power spectra were obtained by using fast Fourier transformation. The direct current component was removed by subtracting the mean value of the data set. A Hanning window of 256 points (corresponding to 128 s, gliding window with 50% overlap) was used. Two frequency bands were defined: a LF band from 0.04 to 0.15 Hz and a HF band from 0.16 to 0.4 Hz. Within each frequency band the spectral power was expressed in absolute values of low, high, and total power (in ms^2), a low-to-high ratio, and in normalized units $\{\%LF = [LF/(\text{total power})] \times 100$ and $\%HF = [HF/(\text{total power})] \times 100\}$.

To check the stationarity of the time series, the mean value and the SD of the total recording, as well as of each data segment of 256 points, were calculated. The variation of the mean value from one segment to another was kept <1 SD (3). Also, a nonparametric "run" test was used. In this case a criterion has to be fulfilled. Both procedures have been explained in detail previously (3). Only stationary parts of the signal were used for further spectral analysis.

Nonlinear Analysis

The nonlinear components of HRV were computed by using several methods.

Fractal dimension. The first method, fractal dimension or FD, is based on the algorithm of Katz (28). This algorithm describes the planar extend of the time series. The higher the FD, the more irrregular the signal.

1/f slope. The $1/f$ slope of the log(power)-log(frequency) plot was obtained from the linear regression from 10^{-4} to 10^{-2} Hz. The plots had an uneven density that might give greater weight for data in the higher-frequency range. Therefore, we used a logarithmic interpolation of the log-log plot, giving a balanced number of points for linear interpolation. A slope of -1 is an indication of scaling behavior.

ApEn. ApEn quantifies the entropy of the system (4). Entropy refers to system randomness, regularity, and predictability and allows systems to be quantified by rate of information loss or generation. ApEn more specifically measures the likelihood that runs of patterns that are close will remain close for subsequent incremental comparisons. It was calculated according to the formula of Pincus (43) with fixed input variables $m = 2$ and $r = 15\%$ as suggested by Goldberger et al. (15) (m being the length of compared runs, and r is a filter). Higher values of ApEn indicate a more complex structure in the time series.

Detrended fluctuation analysis. Detrended fluctuation analysis quantifies fractallike correlation properties of the time series and uncovers short-range and long-range correlations. The root-mean-square fluctuation of the integrated and detrended data are measured within observation windows of various sizes and then plotted against window size on a log-log scale (40). The scaling exponent DFAα indicates the slope of this line, which relates log(fluctuation) to log(window size). Both the short-term (4–11 beats) DFAα_1 and the long-term (>11 beats) DFAα_2 scaling exponents were calculated. The scaling exponent can be seen as a self-similarity parameter, which is characteristic of a fractal. Values of α around 1 are an indication of scaling behavior.

Correlation dimension. In the presence of chaos, an attractor in phase space characterizes the dynamics of the system, and its complexity can be quantified in terms of the properties of the attractor. In our study, the time delay for the reconstruction of the attractor was calculated for each recording separately with the method of the autocorrelation function (2). The correlation dimension or CD can be considered as a measure for the number of independent variables needed to define the total system in phase space (8). The embedding dimension was varied between 2 and 30. When a finite value is found for the CD of a time series, correlations are present in the signal. To conclude whether these correlations are linear or nonlinear, a surrogate time series needs to be calculated from the signal. The surrogate time series has a random phase but the same power spectrum as the original signal. A significant difference between the CD of the surrogate and the original time series indicates that there are nonlinear correlations present in the HRV signal. The difference between the CD of the original data and the CD of the surrogate data is defined by an S value. S values >2 indicate significant differences; S values <2 indicate no significant differences.

Largest Lyapunov exponent. Finally, the largest Lyapunov exponent LE was calculated on the basis of the algorithm of Rosenstein et al. (47), which allows the calculation of this parameter on short data sets. The trajectories of chaotic signals in phase space follow typical patterns. Closely spaced trajectories converge and diverge exponentially relative to each other. For dynamic systems, sensitivity to initial conditions is quantified by the LEs. LEs characterize the average rate of divergence of these neighboring trajectories. A positive LE can be taken as a definition of chaos provided the system is known to be deterministic (47). Larger values of the LE indicate more complex behavior.

Statistical Analysis

Statistical analysis was performed with SPSS Windows version 8.0 (Scientific Packages for Social Sciences, Chicago, IL). All data were tested for normality by using the Kolmogorov-Smirnov test. To correct for skewed distributions, a logarithmic transform was used on the spectral power indexes and rMSSD. pNN50 was transformed by using the square root.

Differences between groups were analyzed by Student's t-test or by ANOVA calculated for day and night in both men and women.

To test the association between the spectral and nonlinear indexes, we calculated a two-tailed Pearson correlation coefficient (r). Because HRV is known to decrease with age (45), partial correlation coefficients were calculated. Correlations between −0.25 and 0.25 were disregarded because they indicate only little or no relationship (12).

RESULTS

Mean values for linear and nonlinear HRV indexes are listed in Tables 1 (male population) and 2 (female population). The influence of gender is depicted in Table 3 for all indexes. The male and female populations were similar in age distribution; however, men presented with an overall higher body mass index (BMI) compared with the women (24.9 ± 3.1 vs. 23.4 ± 3.8 kg/m^2, respectively; $P < 0.005$).

Day-Night Variation

A day-night variation was present in all nonlinear HRV indexes except for the correlation dimension in the female population. The value of the largest LE was positive both in males and females, and both during daytime and nighttime. The value of the CD increased slightly during the night (3.97 ± 0.72 to 4.37 ± 1.30 in the male population; $P < 0.05$; 4.15 ± 0.75 to 4.41 ± 1.29 in the female population; P = NS). During the night the percentage of CD values of surrogate data files differing from the CD value of the original data increased in both populations ($P = 0.001$). The values of FD and DFAα_1 increased significantly at daytime. DFAα_2 (Fig. 1), ApEn, and the LE value increased during night hours, and the 1/f slope became less steep. The day-night variations were visible in both the male and the female population.

All linear indexes showed a day-night variation, except SD and LF power did not show this in both our male and female population. Heart rate decreased during the night ($P < 0.001$). In general, daytime values of the linear indexes were lower than nighttime values. In both the male and female population, total power and HF power increased significantly during the night (both $P < 0.001$, Fig. 1). LF power showed a lower relative contribution compared with daytime values.

Gender Influence

Gender-related differences only existed in ApEn, DFAα_1, and the LE. These differences showed as well during the day as during the night. Values of ApEn were higher in the female population, and the LE and DFAα_1 were lower compared with the male population.

Heart rate in the female population was higher compared with the males both during the day and night. All absolute HRV indexes were higher in the male population. Frequency domain indexes showed a significantly lower HRV in female subjects. The gender difference was mainly caused by a difference in LF power. The relative contribution of LF power during the day was significantly higher in the male population compared with the female population ($P < 0.01$). During the night the gender difference in %LF power disappeared although LF power in absolute values in the male population was still significantly higher compared with the female population ($P < 0.001$). No significant gender differences were found in HRV indexes denoting vagal modulation (rMSSD, pNN50, and HF power) during the night. During daytime a small difference existed in rMSSD and pNN50 between the male and female subjects (both $P < 0.05$).

Age Dependence

All nonlinear indexes were significantly correlated with age (all $P < 0.001$) during daytime hours. This correlation was more pronounced in the female population. During the night the relation with age disappeared in some indexes, especially in the male population. (Tables 1 and 2).

Spectral powers, rMSSD, pNN50, FD, ApEn, DFAα_1, CD, and LE decreased with increasing age. The 1/f slope became steeper. Only DFAα_2 increased with increasing age ($r = 0.45$; $P < 0.001$, all data). Increasing age was associated with a

Table 1. *Values of linear and nonlinear HRV indexes during the day and the night in males*

	Male Day			Male Night			Day-Night Difference	
	Mean	SD	Correlation with age	Mean	SD	Correlation with age	Without mean RR as covariate	With mean RR as covariate
Time domain								
Mean RR, ms	743	93	NS	952	135	NS	‡	
SD, ms	115.9	39.7	−0.526	124.2	44.3	−0.562	NS	‡
rMSSD, ms	29.2	12.2	−0.586	43.0	21.4	−0.575	‡	†
	27.7 (10.9–75.5)			38.9 (11.4–110.3)				
pNN50 (%)	8.2	8.0	−0.628	18.3	16.2	−0.604	‡	*
	6.4 (0.2–42.1)			13.1 (0.1–65.6)				
Frequency domain								
Total power, ms^2	2,109.7	1,462.4	−0.692	3,296.8	2,511.5	−0.532	‡	†
	1,868.0 (190.6–8,453.0)			2414.3 (273.4–14,464.1)				
LF power, ms^2	857.1	538.7	−0.717	1,083.4	835.9	−0.528	NS	‡
	773.6 (59.8–2,707.8)			909.8 (65.6–5,588.6)				
%LF power	41.3	8.4	NS	33.2	7.4	NS	‡	‡
HF power, ms^2	209.7	216.5	−0.624	498.4	561.1	−0.594	‡	NS
	143.4 (10.0–1,146.9)			281.9 (15.6–3,363.9)				
%HF power	8.9	4.1	NS	13.1	6.8	−0.424	‡	NS
LF/HF	5.8	3.2	NS	3.4	2.2	−0.352	‡	NS
	5.3 (0.9–21.5)			2.9 (0.6–13.7)				
Nonlinear HRV								
1/*f* slope	−1.18	0.18	−0.268	−1.11	0.21	NS	†	NS
FD	1.27	0.09	−0.683	1.20	0.08	−0.544	‡	*
Ap En	0.74	0.16	−0.265	0.80	0.16	NS	†	NS
DFAα_1	1.53	0.14	NS	1.48	1.53	NS	‡	NS
DFAα_2	1.03	0.11	0.458	1.14	0.13	0.516	‡	‡
CD	3.97	0.72	−0.314	4.37	1.30	NS	*	NS
%CD difference	60			77			‡	
S value	1.9	0.7		2.5	0.9			
Lyapunov exponent	0.27	0.07	−0.291	0.30	0.10	NS	‡	†

Median (minimum, maximum) is added to indexes with no normal distribution. rMSSD, square root of the mean of the sum of the squares of differences between consecutive RR intervals; pNN50, percentage of intervals that vary more than 50 ms from the previous interval; LF, low frequency; HF, high frequency; FD, fractal dimension; DFAα_1 and DFAα_2, detrended fluctuation analysis short- and long-term scaling exponents, respectively; CD, correlation dimension; 1/*f*, 1/frequency; ApEn, approximate entropy; HRV, heart rate variability; *S* value, difference between the CD of the original data and the CD of the surrogate data; NS, not significant. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

higher heart rate only in the female population (day: $r = 0.35$; night: $r = 0.27$; both $P < 0.001$). For the nonlinear indexes, the age dependency was especially prominent during the daytime hours and more pronounced in the female population. FD was strongest related with age ($r = -0.56$; $P < 0.001$, all data, Fig. 2). Linear indexes in the male population were more strongly related with age than in the female population.

Deeper analysis per age category of 10 yr showed a stabilization in the age decline of the FD and ApEn at the age of ≥40 yr. DFAα_2 continued to increase up to 60 yr. The number of surrogate data with the original data remained stable over all ages (Fig. 3). The value of the 1/*f* slope became steeper, and the LE decreased at the age of >60 yr.

The day-night variation in most nonlinear indexes was also age dependent. Day-night differences in ApEn and DFAα_1 were most prominent in the age classes of <50 yr, whereas in the LE it was more prominent in the >50 yr age categories.

Also the decline in linear indexes stabilized around the age category of 40 yr.

Values for the male and female population converged at higher ages, and the gender differences for ApEn, FD, the LE and the linear parameters (LF, HF and total power) disappeared at ages >40 yr (Fig. 4).

Influence of Resting Heart Rate

All linear HRV indexes were strongly related to HR (or mean RR). FD increased and DFAα_2 decreased with increasing heart rate. The other nonlinear indexes are not influenced by heart rate. An inverse relation between heart rate and most linear HRV indexes existed. Only the percentages of LF and HF had a positive association with heart rate. Especially the indexes attributed to vagal modulation were strongly associated with heart rate (pNN50 and rMSSD: $r = -0.72$; HF: $r = -0.64$; all $P < 0.001$). This was also the case after examining the partial correlations for the male and female population separately.

After introducing mean RR as a covariate, gender differences during the night disappeared for the linear parameters. Only HF% and the LF/HF ratio showed a significant gender difference during the night. During the day the gender differences remained.

For the nonlinear indexes, correction for mean RR did not alter the gender differences. Introducing mean RR as a covariate in the day-night variation changes the significance levels both in the female and the male population (Tables 1 and 2).

Correlations Between Different HRV Indexes

Table 4 shows the partial Pearson's correlation coefficients, controlling for age, between nonlinear and linear HRV indexes.

ApEn was related with linear indexes describing vagal modulation after controlling for age [rMSSD, pNN50, and HF power in absolute and proportional (%) units; all $r > 0.38$; $P < 0.001$]. The FD was not related with linear HRV indexes. Without controlling for age, however, as is done in most

Table 2. *Values of linear and nonlinear HRV indexes during the day and the night in females*

	Female Day			Female Night			Day-Night Difference	
	Mean	Standard deviation	Correlation with age	Mean	Standard deviation	Correlation with age	Without mean RR as covariate	With mean RR as covariate
Time domain								
Mean RR, ms	703	83	0.350	892	109	0.274	‡	
SD, ms	99.1	22.5	NS	102.3	34.2	NS	NS	‡
rMSSD, ms	25.6	9.2	−0.409	38.2	16.3	−0.297	‡	NS
	24.6 (10.0–60.2)			35.8 (11.3–112.2)				
pNN50, %	5.9	6.1	−0.441	15.0	12.8	−0.317	‡	NS
	4.3 (0.1–33.4)			12.0 (0.1–62.8)				
Frequency domain								
Total power, ms^2	1,448.0	947.4	−0.585	2066.4	1,516.6	−0.305	‡	*
	1,201.9 (238.9–6,307.7)			1,723.6 (268.4–12,096.7)				
LF power, ms^2	572.1	389.7	−0.657	667.9	496.8	−0.322	NS	*
	494.2 (60.1–2,624.5)			558.1 (65.9–3,342.7)				
%LF power	38.7	7.4	−0.499	31.8	6.9	NS	‡	†
HF power, ms^2	164.1	157.4	−0.503	404.9	455.5	−0.343	‡	NS
	123.8 (19.7–1,118.4)			265.0 (16.6–3,957.9)				
%HF power	10.5	4.4	NS	17.8	7.9	NS	‡	‡
LF/HF	4.4	2.0	NS	2.3	1.5	NS	‡	‡
	3.8 (0.7–10.9)			1.8 (0.41–7.41)				
Nonlinear HRV								
1/*f* slope	−1.21	0.17	−0.367	−1.14	0.21	−0.271	†	NS
FD	1.28	0.08	−0.700	1.22	0.08	−0.532	‡	NS
Ap En	0.80	0.17	−0.455	0.92	0.20	−0.346	‡	‡
DFAα_1	1.45	0.13	−0.317	1.37	0.13	NS	‡	‡
DFAα_2	1.05	0.10	0.604	1.12	0.11	0.419	‡	NS
CD	4.15	0.75	−0.535	4.41	1.29	NS	NS	†
%CD difference	62			80			‡	
S value	2.1	0.8		2.2	1.2			
Lyapunov exponent	0.25	0.06	−0.319	0.28	0.09	NS	†	NS

Median (minimum, maximum) is added to indexes with no normal distribution. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

Table 3. *Gender differences of the linear and nonlinear HRV indexes*

	Gender Difference			
	Without adjustment for mean RR		With adjustment for mean RR	
	Day	Night	Day	Night
Time domain				
Mean RR, ms	‡	‡		
SD, ms	‡	‡	†	NS
rMSSD, ms	*	NS	NS	NS
pNN50, %	*	NS	NS	NS
Frequency domain				
Total power, ms^2	‡	‡	†	NS
LF power, ms^2	‡	‡	‡	NS
%LF power	†	NS	‡	NS
HF power, ms^2	NS	NS	NS	NS
%HF power	†	‡	‡	‡
LF/HF	‡	‡	‡	‡
Nonlinear HRV				
1/*f* slope	NS	NS	NS	NS
FD	NS	NS	NS	NS
ApEn	†	‡	*	‡
DFAα_1	‡	‡	‡	‡
DFAα_2	NS	NS	*	NS
CD	NS	NS	NS	NS
%CD difference	NS		NS	
Lyapunov exponent	*	*	*	*

*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

studies, strong relations existed with LF and HF power ($r = 0.48$ and $r = 0.43$, respectively; $P < 0.001$). The 1/*f* slope was related to LF power ($r = 0.29$; $P < 0.001$). The short-term correlation parameter DFAα_1 was related to almost every linear HRV parameter. The strongest association was found in the proportional contributions of LF and HF ($r = 0.67$ and $r = -0.67$, respectively; both $P < 0.001$) and LF/HF ratio ($r = 0.72$; $P < 0.001$). The long-term correlation index DFAα_2 was only related to LF power in absolute and relative units ($r = -0.27$ and $r = -0.64$, respectively; both $P < 0.001$).

The CD showed only a link to %HF power ($r = 0.28$; $P < 0.001$), and the LE did not show any relation with the linear HRV indexes, even without controlling for age.

The FD is correlated with every other nonlinear index except for the CD. ApEn is related to the 1/*f* slope and the FD. DFAα_1 was also related to the FD, ApEn, CD, and LE.

DISCUSSION

In this study the most commonly used nonlinear indexes (FD, ApEn, 1/*f* slope, CD, detrended fluctuation analysis, and the largest LE) were examined in a population of 276 healthy subjects between the age of 18 and 71 yr. We found a strong decrease in nonlinear behavior with advancing age. Gender differences in nonlinear dynamics were less pronounced.

Influence of Gender and Age

We did not find clear evidence of a higher nonlinear behavior of heart rate fluctuations in the female population. DFAα_1

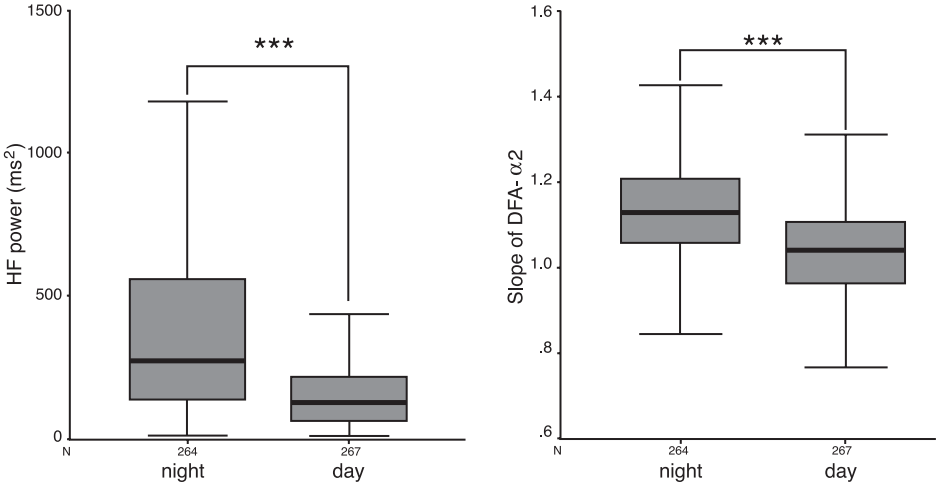

Fig. 1. Night-day difference of high-frequency (HF) power and detrended fluctuation analysis long-term scaling exponent (DFAα_2): all data. (***$P < 0.001$). Boxes represent the 75th percentile, median, and 25th percentile. Whiskers show the largest and smallest observed values.

and the LE were slightly lower in the female population. ApEn, on the contrary, was higher in the female population. These findings agree with Pikkujämsä et al. (41).

The major difference between the male and female population is in LF modulation of heart rate. This was also found previously (7, 10, 23). LF modulation has been linked to sympathetic modulation of heart rate. This means that the male population has an overall higher sympathetic drive than women. Especially during the day, the larger proportion of LF power in men is striking. Higher sympathetic activity has been related to a higher susceptibility to fatal arrhythmia and to the development of coronary artery disease (50). Therefore, hypothetically, the reduced LF power in women could protect against the development and incidence of coronary artery disease and arrhythmia. The exact contribution of this difference in autonomic cardiac control remains to be elucidated.

The higher heart rate found in women and the lower sympathetic drive in women are seemingly contrasting. However, one should keep in mind that heart rate is not only determined by sympathetic modulation but is a result of a complex interplay between sympathetic influences, vagal influences, baroreflex, and even physical fitness. The higher heart rate in women can also be related to a lower stroke volume. In a previous study, we found no significant correlations between BMI and heart rate or HRV indexes for the female population, whereas higher BMI was related to lower HRV indexes in men (45). Also, in the female population, the menstrual cycle could have an influence on the HRV indexes. Guasti et al. (16) described in 1999 that sympathetic modulation can be influenced different in different phases of the ovulatory cycle. Also, others later

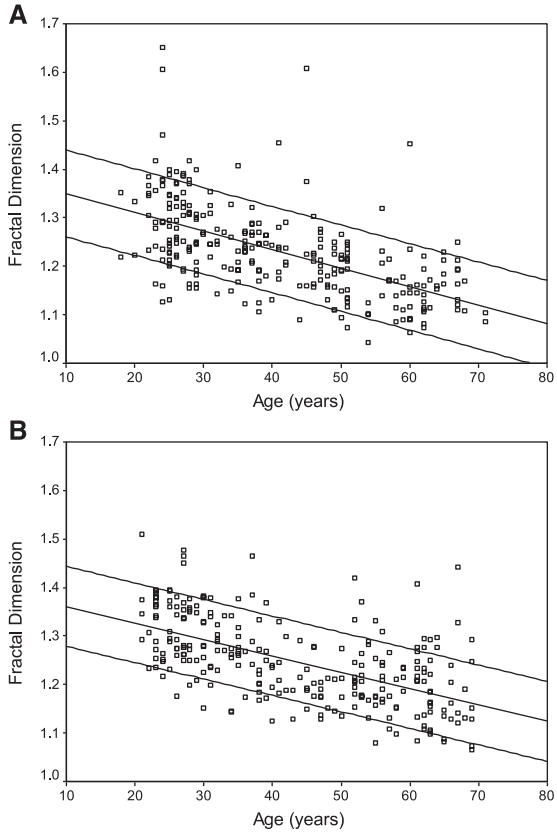

Fig. 2. Decrease of the fractal dimension (FD) with age. In the male population (*A*), $R^2 = 0.34$ ($P < 0.001$); in the female population (*B*), $R^2 = 0.34$ ($P < 0.001$). Top and bottom lines represent 90% confidence intervals (CI).

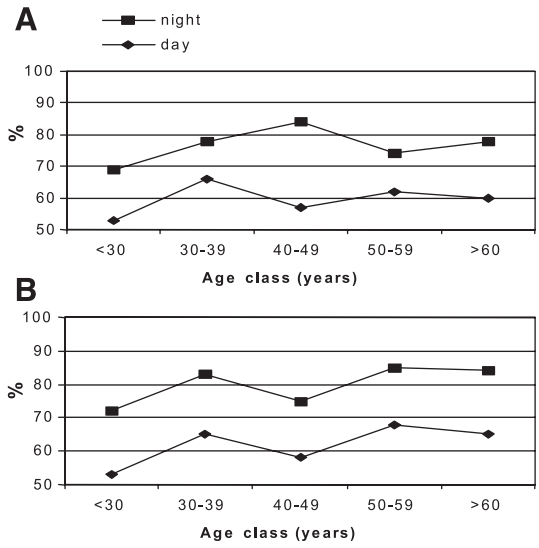

Fig. 3. Percentage of recordings showing a difference between the correlation dimension value of the surrogate data sets and the original data in the different age classes in male population (*A*) and female population (*B*).

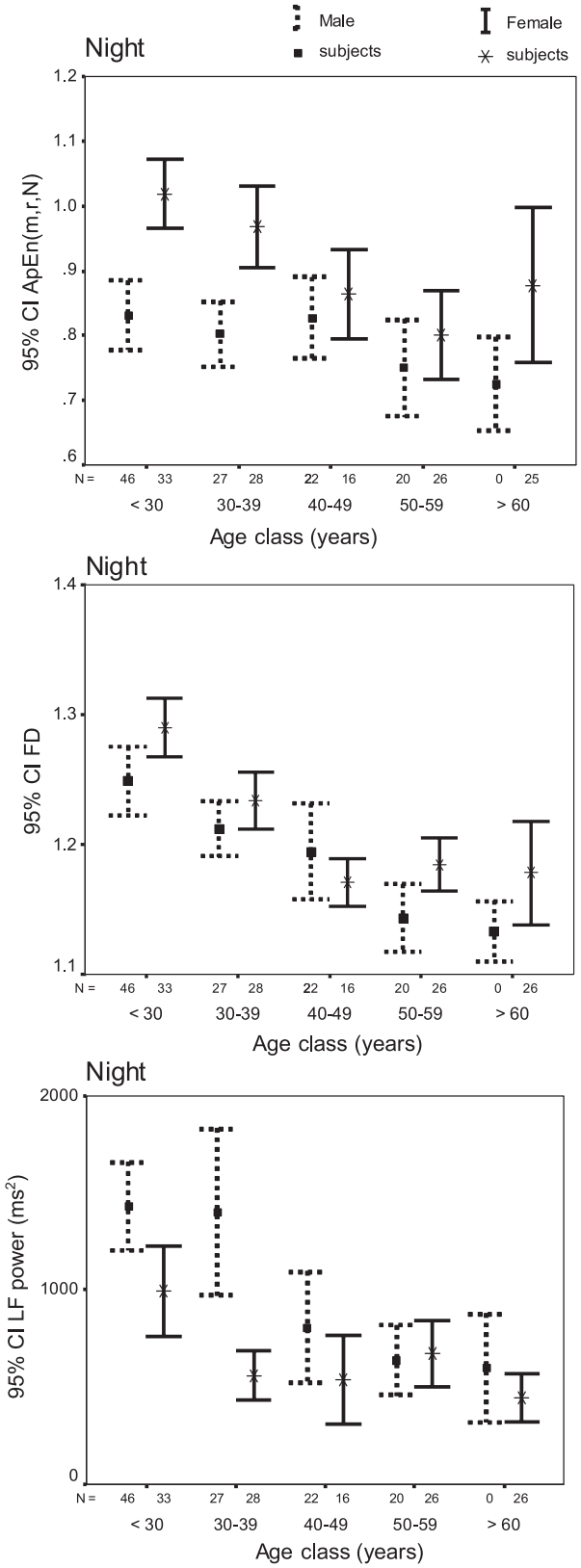

Fig. 4. Disappearing gender differences from the age of 40 yr onward for approximate entropy (ApEn), FD, and low-frequency (LF) power during the night.

described small changes in sympathetic activity with menstrual cycle (37, 49). Leicht et al. (32). on the contrary, could not find changes in HRV in the different menstrual cycles. Recently, Vellejo et al. (55) described that in HRV analysis in women, menstrual cycle was the least important factor influencing HRV. Age and BMI were more important factors (55). It should be noted that all these studies were performed in young female subjects (age <35 yr).

There was a strong correlation with age in most nonlinear indexes. Increasing age was associated with decreasing nonlinear behavior. This was shown in a decrease in FD, ApEn, CD, LE, and a steeper 1/*f* slope. The decrease with age was more prominent in the female population. Our results also show a convergence of the male and female data between the age of 40 and 50 yr, both for the linear and the nonlinear indexes. This has been attributed to the female hormone estrogen (45). Most women enter menopause during this age range. The age dependence of the linear HRV indexes was also observed by others (1, 10, 14, 31, 54, 63). Pikkujämsä et al. (41) did not find a relation with age for ApEn and DFAα_1 in both men and women; however it should be noted that the range of ages in their study was very limited (40–59 yr). In a previous study (42) with a wider age distribution, they did find a strong decline in ApEn with age and the 1/*f* slope. DFAα_2 also increased with advancing age, which is in accordance with our results. Iyengar et al. (24) found the opposite of our results for DFAα_1 and DFAα_2. Their mixed gender population (10 subjects) was measured while lying down, awake, and watching a movie. This and the small number of subjects can explain these different findings. Otsuka et al. (39) have reported a decrease in CD value with age in their male population, especially during the night hours. We also found a decrease in CD but only during the day. They used a constant time delay of 1.0 s for each recording, while we opted to calculate the optimal time delay for each recording separately. This could give different results. Yeragani et al. (63) also found a decreased FD in an elderly population, both in wake state and in sleep state. A decrease in cardiac nonlinear dynamics with age has also been observed by Kaplan et al. (27) in a limited population of 16 young and 16 old healthy subjects. The decrease in nonlinear behavior was also described by Acharya et al. (1) in a mixed population of male and female subjects.

The decrease of nonlinear behavior with increasing age can be related to the general concept of decreasing autonomic modulation with advancing age. Increasing age might thus result in an increased inability of the cardiac system to respond adequately to changing conditions.

Day-Night Variations

During the night there was a tendency for higher nonlinearity: the LE increased, the CD increased, the percentage in differences with surrogate data sets increased, DFAα_2 increased, ApEn increased, and the 1/*f* slope came closer to real 1/*f* behavior. Only the FD and DFAα_1 decreased. This was the case in both the male and the female population. Even after correction for mean RR there is a tendency toward increased nonlinearity during the night.

Time and frequency domain indexes support the previously observed increase in vagal modulation during the night: pNN50, rMSSD and the proportion of HF power increase

Table 4. *Partial Pearson correlation coefficients between linear and nonlinear HRV indexes, controlling for age*

	Mean RR, ms	1/*f* Slope	FD	ApEn	DFAα_1	DFAα_2	CD	Lyapunov Exponent
All Data								
Nonlinear HRV								
1/*f* slope								
FD	−0.52							
ApEn		0.39	0.36					
DFAα_1			−0.30	−0.49				
DFAα_2	0.40		−0.48					
CD				0.44	−0.27			
Lyapunov exponent			−0.27		0.34			
Time domain								
SD, ms	0.43			−0.40				
rMSSD, ms	0.72			0.38	−0.35			
pNN50, %	0.72			0.38	−0.35			
Frequency domain								
Total power, ms^2	0.62	0.30						
LF power, ms^2	0.44	0.29			0.32	−0.27		
%LF power	−0.45				0.67	−0.64		
HF power, ms^2	0.64			0.39	−0.32			
% HF power	0.34			0.54	−0.67		0.28	
LF/HF	−0.40			−0.37	0.72			

All correlations listed are $P < 0.001$.

during the night, while heart rate slows down. Although LF power increases in absolute value, its relative contribution decreases, again stressing the vagal dominance during the night. In this study, the transition phases of going to sleep and waking up were excluded from the analysis to provide data on relatively stable time periods. The morning and evening periods can also contain potentially important information. Especially the transition between sleep and awakening has been described previously as a time of high sympathetic activity and has been related to the occurrence of cardiac arrhythmia in this time frame (30). Future research will focus on the hourly day-night variations.

Relation with Linear Indexes

The influence of resting heart rate was also noted in our previous study (45). Also others (36, 42) later described the importance of considering the influence of resting heart rate and age in measuring HRV. The latter parameter should be taken into account when examining both linear and nonlinear HRV indexes.

ApEn was strongly related with indexes describing vagal modulation of heart rate. This was in accordance with observations in a previous study in heart failure patients (4). However, some studies did not find a relation with vagal indexes (52). Unlike previous reports (63), we did not find a relation of FD with HF power or other vagally related indexes. However, without controlling for age there was a strong relation with both HF and LF power. Previous studies have not taken age into account as a covariate. The 1/*f* slope was related to LF power, which seems logical because the 1/*f* power spectrum is largely determined by the LF power. Yamamoto and Hughson (59), however, suggested only a moderate influence of the sympathetic nervous system on the 1/*f* slope after experiments of β-blockade. Yamamoto et al. (60) rather suggested an influence of the vagal system due to the influence of atropine on the 1/*f* slope. Hagerman et al. (20), on the contrary, found no influence of β-blockade and cholinergic blockade on the 1/*f* slope. The CD was only moderately related to the proportion of HF power, while the LE seemed unrelated to any linear HRV index. The CD has also been studied by Kanters et al. (26) by using forced respiration. They concluded on a mixed influence of both sympathetic and vagal stimulation because no differences were found by using the forced respiration protocol. However, no comparison to surrogate data sets was made. The long-term correlation index was related to LF power, while the short-term correlation index was strongest related to the proportion of LF and HF power. This last relation was also found by Tulppo et al. (52) in baseline conditions and during head-up tilt and exercise. Our values of DFAα_1 and DFAα_2 are also in agreement with values found by Peng et al. (40) in a healthy population. These findings are also in full support of the article by Willson et al. (57), in which the DFAα_1 and DFAα_2 indexes where described as ratios of spectral powers. DFAα_1 was related to 2/[1 + (HF/LF)], and DFAα_2 was related to 2/[1 + (LF/VLF)], with VLF representing the power in the very low frequency region below 0.04 Hz. These formulas also describe the link with the LF/HF ratio for DFAα_1 and the link with LF power for DFAα_2 that we found.

Our results indicate that the nonlinear fluctuations in humans are only moderately influenced by the sympathetic nervous system but more by the vagal nerves. The majority of the nonlinear indexes show a clear relation with indexes representing vagal modulation (ApEn, DFAα_1, and FD without correction for age). The 1/*f* slope and DFAα_2 were more related to sympathetic modulation.

Practical Applications

In this study the nonlinear indexes have been linked to vagal modulation of heart rate control. The rationale behind the idea of nonlinear fluctuations is that the cardiovascular system would adapt easier and faster to necessary changes. For this the choice of the vagal (fast acting) pathways seems logical. Because the nonlinear control seems to be decreased during the day, it stresses the importance of the vagal pathways in en-

abling the nonlinear fluctuations to be expressed or even for the sympathetic system to inhibit the generation of the nonlinear fluctuations. The triggers of this increased need for nonlinear control during the night might come from the increased respiratory sinus arrhythmia, or even from changes linked to different sleep stages.

The use of nonlinear indexes as risk stratifiers might be an important future application. Already several studies have been published that demonstrate the huge potential of these methods. Mäkikallio et al. (34) found an increased risk in patients with decreased $DFA\alpha_1$ index and a steeper $1/f$ slope ($-1.5 < \beta < -1$). This was later confirmed by Huikuri et al. (22). Promising results have been obtained in patients with panic disorders (46, 62). Yeragani et al. (61) recently proved that in panic disorder decreased baroreflex sensitivity and an increased blood pressure variability is accompanied by a higher degree of nonlinear complexity. Heart failure patients on the contrary have depressed nonlinear behavior compared with healthy subjects. Guzetti et al. (18) even demonstrated that the $1/f$ slope had prognostic value in this patient group. Earlier, Peng et al. (40) had already demonstrated a lower $DFA\alpha_1$ and a higher $DFA\alpha_2$ value in heart failure patients. Lin et al. (33) found beneficial effects of β-blocker therapy in patients with advanced congestive heart failure, represented by a $1/f$ slope closer to -1 (-1.70 before therapy vs. -1.22 after therapy), representing the typical $1/f$ behavior and an increase in the $DFA\alpha_1$ parameter from 0.78 to 1.13 after β-blocker therapy. Heart transplant patients presented a decreased system complexity (8, 19). Hypertension also leads to decreased nonlinear behavior of HRV (25), while blood pressure fluctuations in this group presented a lower degree of nonlinear control (38). It is becoming clear that nonlinear control is decreased in patients at higher risk of lethal arrhythmia. Risk assessment for patients after myocardial infarction or in heart failure or the prediction of lethal arrhythmia (22, 34, 51, 56) is important for clinical assessment of disease severity.

Study Limitations

1) No activity log was recorded during the 24-h Holter recording. This means that we have no information about possible differences in the young and the elderly population (6). The subjects were asked to refrain from intense physical activity to avoid interference from exercise episodes; also, no specific interventions to stimulate or block autonomic pathways were performed. *2*) Respiration was not recorded during the 24-h Holter recording. We used visual inspection of the power spectra to verify the location of the respiratory component in the HF region. *3*) No information about sleep (quality, hours of sleep) was recorded. Night hours were chosen the same for all subjects, ensuring the same data lengths for analysis. Hours were chosen from 11 PM to 6 AM, ensuring the inclusion of the periods with enhanced vagal modulation, and avoiding the waking up period.

Summary of Conclusions

We found evidence of the involvement of the autonomic nervous system in the generation of nonlinear fluctuations in healthy human subjects. The vagal pathways seemed to play a dominant role in the generation of these complex dynamics. This was expressed in higher nonlinear behavior during the night, when vagal influence is largest (higher HF power). No clear difference between men and women was found in the nonlinear indexes.

Nonlinear heart rate fluctuations also decline with age. This can be related to the general concept of decreasing autonomic modulation with advancing age. This again provides evidence for the involvement of the autonomic nervous system in the generation of these complex fluctuations.

We do not see the nonlinear analysis techniques as a replacement of the linear methods but rather as a completion of the model. The linear methods have an advantage over the nonlinear methods in that they are more suitable when shorter data sets are used. Spectral analysis is also superior in visually representing autonomic modulation. The interpretation of the spectral components is more intuitive and easier to understand. However, they cannot quantify the presence or absence of nonlinear behavior.

In our analysis of the nonlinear dynamic systems in 24-h Holter recordings, we have opted to calculate values for day and night separately and not to use the 24-h period as a whole because day-night fluctuations are present in autonomic control of heart rate. Analyzing the whole day as one period should therefore mask specific alterations caused by either sympathetic predominance (during the day) or vagal predominance (during the night).

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