Reference Values for Time- and Frequency-Domain Heart Rate Variability Measures

Stefan Sammito, Irina Böckelmann

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Reference values for time- and frequency-domain heart rate variability measures

Short title: Reference values for heart rate variability measures

Sammito, Stefan, Dr. med.[1,2]; Böckelmann, Irina, Prof. Dr. med.[2]

[1]Bundeswehr Joint Medical Service Headquarters – Section Health Promotion, Sports and Nutrition Medicine

[2]Occupational Medicine, Faculty of Medicine, Otto von Guericke University, Magdeburg

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Corresponding authors:

Bundeswehr Medical Service Headquarters, Department VI, Section Health Promotion, Sports and Nutrition Medicine, Cdr (MC) Dr. med. Sammito, Stefan

Von-Kuhl-Str. 56, 56070 Koblenz, Germany

drsammito@web.de, Phone: +0049 6131 8844857, Fax: +0049 6131 8844870

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Abstract:

Background: The analysis of heart rate variability (HRV) has become an established procedure in recent decades. Due to the fact that there are no appropriate reference values available, HRV findings can still only be compared within a group or in individuals in longitudinal studies.

Objective: The objective of the present study was to examine a group of healthy subjects of different ages and genders and identify reference values for common HRV parameters.

Methods: A long-term 24h-ECG of 695 voluntary subjects was recorded by using a 2-channel Holter system during daily routine activities.

Results: Reference values for men and women in ten-year age groups were calculated for SDNN, RMSSD, SDANN, pNN50, LFnu, HFnu, LF/HF ratio, SD1 and SD2. The 5th and the 95th percentiles were given for each gender and for the age groups 20-30, 30-40, 40-50 and 50-60 years. We observed a consistent decrease in HRV with increasing age as well as a gender dependency of HRV findings.

Conclusion: We studied a large group of healthy subjects and identified reference values for commonly used HRV measures for 24-hour measurements. The reference values differed considerably from the values published in 1996 in the Guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. In future, steps should be taken to expand the data base and define reference values for the age groups under 20 and over 60 years. It would be desirable to obtain reference values for short-term recordings (e.g. 5-minute recordings) too.

Keywords:

autonomic nervous system; heart rate; analysis; sympathetic; parasympathicus

Abbreviations:

BMI	body mass index
ECG	electrocardiogram
HF	high frequency
HFnu	high frequency power normalized unit
HRV	heart rate variability
LF	low frequency
LFnu	low frequency power normalized unit
pNN50	Percentage of consecutive NN intervals that deviate from one another by more than 50 ms
RMSSD	Root Mean Square of successive differences of NN intervals
SDANN	Standard deviation of the average of all consecutive 5-minute NN intervals
SDNN	Standard deviation of NN intervals

Introduction:

The measurement and analysis of heart rate variability (HRV), which is based on the variation between consecutive NN intervals, has become an established procedure over the past two decades[1-4] since the publication of the first guidelines[5]. There have been not only advances in recording technology (smaller, more mobile, more accurate devices)[6] but NN intervals can now be measured by small chest strap and pulse watch systems[7-8]. Technological developments have decreased the costs of recording and analysis and have facilitated applications in an outpatient setting.

In 1996, Malik et al. introduced possible "normal values" in the Guidelines of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[5]. However they pointed out that these values had been measured only in a small number of subjects and recommended further studies. In a 2010 review that included 44 previously published studies, Nunan et al.[9] reported mean values unrelated to age for the five-minute short-term recording of commonly used HRV measures. They identified mean lower values than those published in the above guidelines of 1996[5]. Due to the fact that HRV is not only gender-related[10-12] but also age-related[10, 13-14], the values of Nunan et al.[9] being not correlated with age are applicable only to a limited extent.

Since there are no appropriate reference values available, HRV findings can only be compared within a group or in individuals in longitudinal studies. The objective of the present study is to examine a group of healthy subjects of different ages and genders and identify reference values for common time- and frequency-domain HRV measures as well as for both Lorenz plot variables.

Methods:

A long-term ECG of voluntary subjects was recorded by using a 2-channel Holter system (Schiller MT-101, Firma Schiller AG, Switzerland) over a period of 24 hours during daily routine activities, and the intervals between the individual R-waves were calculated with a sampling frequency of 1,000 Hz. During recording time, subjects were asked to follow their normal daily routines except performing physical exercise or taking a shower/bath. This corresponds to the recommendations in the Guidelines of the Task Force[5] as well as to current recommendations on measuring NN intervals for HRV analysis[7].

Exclusion criteria were diagnosis of coronary heart disease with or without stent implantation, heart attacks, strokes, night shifts during recording time, diagnosed diabetes mellitus (irrespective of type), use of psychiatric medication, and pregnancy. Subjects younger than 20 years and older than 60 years were also excluded.

All subjects were informed about the study in writing prior to participation and signed informed consent forms. The study protocol conforms to the ethical guidelines of the Declaration of Helsinski and was presented to and approved by the ethics committee of Otto-von-Guericke-University Magdeburg, Germany.

A total of 695 subjects were included in the study. Most of the subjects were volunteer participants from the normal population of Magdeburg, Germany (n = 267). The other subjects came from preventive studies in several different workplaces: bank employee (n = 89), military service (n = 57), doctor´s assistant (n = 46), students (n = 45), government employees (n = 44), public transportation service (n = 38), employees at the university (n = 38), police service (n = 33), rescue service (n = 30), nurses

(n = 5) and doctors (n = 3). On the day of 24 h-ECG-measurement the subject spend the day as normal as usual, and they didn´t have night shift.

After the data was recorded, it was analysed by the Schiller MT-200 Holter_ECG program (Version 2.54, Firma Schiller AG, Switzerland) on a personal computer. The long-term ECG was analysed both by computer and manually respectively visually by a physician.

After the NN intervals were exported, an HRV analysis was performed in Kubios HRV Version 2.0 (University of Kuopio, Finland)[15] with artifact correction (settings: "custom" and "0.3") and a trend component (method: "smooth priors" and "Lamda = 500")[16]. The frequency analysis was conducted by using a Fast Fourier Transformation (window width 256 s, overlap ratio 50%). The following frequency bands were used: low frequency (LF) from 0.04 to 0.15 Hz and high frequency (HF) from 0.15 to 0.40 Hz[5]. Recordings with a total length of less than 23 hours were excluded.

In addition to time-domain measures (SDNN, RMSSD, SDANN, pNN50), the LFnu, the HFnu and the LF/HF ratio as frequency-domain measures and SD1 and SD2 as HRV parameters of the Poincaré plot were calculated[7]. It must kept in mind that LFnu and HFnu are linearly dependent and that both parameters always add together to 100 (LFnu + HFnu = 100). Also SD1 and SD2 are related to linear indexes and can be transferred to SDNN respectively to RMSSD.

In order to compute age- and gender-related reference values, the subjects were allocated to the following 10-year age groups according to their gender (male/female):

- age ≥ 20 and < 30 years,
- age ≥ 30 and < 40 years,
- age ≥ 40 and < 50 years and
- age ≥ 50 and < 60 years.

A total of 695 subjects were included in the study. 319 subjects were men with an average age of 40.2 ± 10.4 years, a body height of 179.8 ± 7.2 cm and a body weight of 85.9 ± 13.6 kg (body mass index [BMI]: 26.6 ± 3.8 kg/m²). 376 of the subject were women with an average age of 40.4 ± 11.3 years, a body height of 167.3 ± 6.9 cm and a body weight of 69.0 ± 12.6 kg (BMI: 24.7 ± 4.4 kg/m²). Only body height, body weight and BMI show significant differences between men and women ($p < 0.001$). The blood pressure was 132.4 ± 15.3 mm Hg systolic and 83.3 ± 10.4 mm Hg diastolic for men and 124.5 ± 15.3 mm Hg systolic and 81.5 ± 10.6 mm Hg diastolic for women (significant difference between gender, $p < 0.001$ [systolic blood pressure] and $p = 0.027$ [diastolic blood pressure]). More men than women were smoker and ex-smoker (28.8 % vs. 17.8% respectively 17.6 % vs. 13.3%, $p < 0.001$). 44.4% of the men and 45.5% of the women performing athletic training with an average of 3.0 ± 1.4 (men) and 2.3 ± 1.3 times per week (women, $p < 0.001$ between both gender). The number of subjects in the different age groups is shown in Table 1.

Table 1

The 5th, 25th, 50th, 75th and 95th percentiles were calculated for all age groups separately for men and women. A regression analysis of each HRV measure was performed for the total group, separately for men and women and additional for each gender and for each age group with age as an independent variable to proof the effect of age. The normal distribution was assessed by a Kolmogorov-Smirnov test,

and because no normal distribution was found, differences between men and women in the various HRV measures were assessed by a Mann-U-Whitney-Test. Additional Kruskal-Wallis-Tests were calculated for each age group separately for men and women to identify significant differences between the reference values. A p-value of <0.05 was considered significant. Calculations were performed with IBM SPSS 23 (IBM, Amonk, NY, USA).

Results:

A significant difference between men and women was observed for all measured HRV parameters (SDNN, RMSSD, pNN50, SDANN, LFnu, HFnu, LF/HF, SD1 and SD2). The differences were still significant for HRV parameters SDNN, LFnu, HFnu and LF/HF ($p < 0.05$, data not showed) in each age-group. Other HRV parameter differences between men and women were not consistently.

In most age groups, male subjects showed higher values (with regard to the 95th percentile) for the HRV measures (see Table 2 – Table 4). For the entire group (data not showed) and when male and female subjects are calculated separately, however, age has a significant influence on HRV. Except for the LF/HF ratio, findings from all HRV measures declined with increasing age (also with regard to the 95th percentile). Only in the LF/HF ratio did the HRV findings of both men and women first increase (up to the group of 40–50 years) and then fall again in the group of 50–60 years. If age is used as an independent variable in a regression analysis for each age group and for each gender separated there isn´t an significant effect of age on the HRV parameters in the most age groups (data not showed). Only in the women age group 40-50 years and in the women age group 20-30 years in 8 out of 9 and respectively in 5 out of 9 of the HRV parameters age showed also a significant effect inside the age group.

Table 2 – Table 4 give an overview of the percentile values of all HRV measures with the relevant age-related regression values (separately for men and women) and the significance level (p) for the gender-related difference. Men and women showed significant differences for all HRV parameters in all age groups ($p < 0.001$). Figure 1 and 2 showed separate scatter-plots for all HRV parameters for men and women.

Table 2-4

Figure 1-2

When comparing the HRV findings for SDNN, SDANN and RMSSD, we observed differences in comparison to the 1996 normal values unrelated to age or gender (Table 5). It must be taken into account, however, that the normal values published in 1996 were mean values and standard deviations. In our study, the 50th percentile for SDNN is considerably lower than the previously published reference values. The 50th percentile for RMSSD is considerably higher[5]. With SDNN, the 95th percentile of all age and gender groups was also considerably lower than 141 ± 39 ms[5], which was described in the Guidelines of the Task Force. On the other hand, the 95th percentile for RMSSD and SDANN, however, was clearly above the 1996 normal values in all age and gender groups.

Table 5

Discussion:

For the first time, reference values for selected HRV measures have been presented on the basis of a healthy and relatively large sample by using percentile values for 24-hour long-term recordings. HRV findings for a subject can now be related to a healthy reference group.

The reference values we identified differ considerably from the normal values published in 1996 in the Guidelines of the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology[5] (Table 5). It must be kept in mind, however, that the normal values published at that time were based on a relatively small sample, that neither gender nor age were taken into consideration, and that recording technology has improved decisively over the past two decades. For example, Körber et al. demonstrated in the year 2000 that there were significant differences in NN intervals recorded on analogue and digital Holter ECG devices[6]. In addition, the accuracy of R-wave peak detection has continuously improved over the past years. Today, recordings with a sampling frequency of 1,000 Hz can be performed easily, whereas ECG recordings in the 1990s mostly had frequencies of 64 or 128 Hz. Higher sampling frequencies increase the accuracy of HRV analysis. This explains deviations from the normal values published in 1996 and the necessity of obtaining up to date reference values. As already mentioned above, the normal values of the Task Force represent only means and standard deviations[5].

Excluded from our study were persons who had suffered or were suffering from diseases with a known impact on HRV (coronary heart diseases with or without stent implantation, heart attack[1], stroke[2] and diagnosed diabetes mellitus irrespective of the type[3-4]) as well as persons currently on psychiatric medication, pregnant women[17], and persons working a night shift during the recording period. Changes in HRV have been reported for a series of mental conditions and/or the medication (for example, drugs for anxiety and panic disorders, post-traumatic stress disorder, epilepsy and depression[18-22]). There have been no conclusive studies on other mental conditions[23]. By excluding all subjects taking psychiatric medication, we attempted to obtain a sample that included only subjects who were healthy in terms of mental con-

ditions. Since our inquiry was based solely on patient history provided by the subjects, we cannot guarantee that a condition not yet diagnosed was mentioned or that an already existing illness was not concealed. Pregnant women were also excluded from the study since there is evidence that pregnancy influences HRV[17]. By excluding these factors, we ensured to the highest possible extent a healthy sample without chronic diseases that would influence HRV.

In concordance with other studies[10-14], we were able to demonstrate that both age and gender have an influence on HRV. For this reason, age- and gender-related reference values are necessary. In this respect, our data differ significantly from the "normal values" of 1996[5]. Our data also show that HRV decreases with age and that there are also gender-related differences for all HRV measures.

This study does have some limitations, however. With a total of 695 subjects, we ensured a large sample for our analysis. We were, however, unable to provide any relevant reference values for subjects aged 20 years or younger and subjects aged 60 years or older since there were not enough subjects in these age categories to include them in the study. Larger samples for each ten-year age category would also be desirable in order to increase the significance of our reference values. The objective of future research efforts in this context should focus on closing existing gaps in the age structure and increasing the sample size.

An additional limitation can be seen in the fact, that the study population wasn´t a representative cohort of the normal population. Because several diseases were declared as excluded criteria, it will be very difficult to get a representative cohort of the normal population especially in the older age groups. The comparison of anthropometric parameters (body height, body weight, BMI, blood pressure) and lifestyle factors

(physical activity, smoking) showed correspondence with data from a representative survey in Germany[24]. The study population differs in blood pressure (men: 132.4 / 83.3 mm Hg vs. 124.8 – 129.8 / 70.0 – 79.7 mm Hg; women: 124.5 / 81.5 mm Hg vs. 113.3 – 123.7 / 66.6 – 74.5 mm Hg),[25] regularly sport activity (men: 44.0 % vs. 61.4 – 82.4 %; women: 45.0 % vs. 60.3 – 74.3 %)[26] and smoking status (men: 28.8 % vs. 30.2 – 47.0 %; women: 17.8 % vs. 27.9 – 40.0%)[27]. All other parameters were similar to the representative cohort[24]. Therefore it can be supposed that although the study population is not a representative cohort, it matches with a normal population with respect to HRV.

The reference values presented in this study are only to be used for an analysis of HRV based on measurements of the NN intervals over a period of 24 h under every-day conditions. They cannot be applied to an HRV analysis involving heavy physical strain (e.g. sports activities) as HRV decreases on account of increased heart rates during stress tests[28]. Nor can these results be applied to short-term recordings (e.g. 5-min recordings) since the findings of numerous HRV measures depend on re-cording time[20, 29].

Conclusions:

For the first time, age- and gender-related reference values are now available for 24-hour HRV analyses using common HRV measures. In future, the data base must be expanded and reference values must be defined for the age groups under 20 and over 60 years. It would also be desirable to obtain reference values for short-term recordings (e.g. 5-minute recordings).

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Clinical perspectives:

For the first time, age- and gender-related reference values are now available for 24-hour HRV analyses using common HRV measures in time-domain (SDNN, RMSSD, SDANN, pNN50), in frequency-domain (LFnu, HFnu, LF/HF ratio) and HRV parameters of the Poincaré plot (SD1 and SD2). These references are now available for men and women in age groups from 20 to 60 years.

It is now possible to interpret HRV of a healthy patient means of references values. So this new medical knowledge improves the usable of HRV in the clinical practise as well as in outpatient settings instantly.

In future, the data base must be expanded and reference values must be defined for the age groups under 20 and over 60 years. It would also be desirable to obtain reference values for short-term recordings (e.g. 5-minute recordings).

Figure legends:

Figure 1: Scatter plot of SDNN (A/B), RMSSD (C/D), pNN50 (E/F) and HFnu (G/H) for men (left) and women (right) with Regression analysis with age as co-variable.

Figure 2: Scatter plot of LFnu (A/B), LF/HF (C/D), SD1 (E/F) and SD2 (G/H) for men (left) and women (right) with Regression analysis with age as co-variable.

Table 1: Number of subjects, divided into male (M) and female (F), presented according to their age, total number n = 695.

Gender	Age group	Number
M	20-30	71
	30-40	73
	40-50	106
	50-60	69
F	20-30	97
	30-40	75
	40-50	110
	50-60	94

Table 2: Percentile values of time-domain HRV measures, divided into male (M) and female (F), categorised according to age group, with regression analysis (with age as co-variable) and p-value for gender-related differences. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n = 695.

			Percentile						
			5th	25th	50th	75th	**95th**	Regression analysis with age as co-variable	Gender-related difference
SDNN [ms]	M	20-30	**32.36**	43.78	56.54	68.27	**92.55**	-0.758*** ($r^2 = 0.258$)	**p < 0.001**
		30-40	**29.92**	39.66	48.98	56.35	**70.21**		
		40-50	**24.91**	33.02	41.52	49.61	**68.80**		
		50-60	**18.83**	26.64	33.64	41.72	**55.76**		
	F	20-30	**27.89**	40.83	46.34	56.56	**77.24**	-0.658*** ($r^2 = 0.288$)	
		30-40	**26.07**	34.53	42.39	53.52	**66.42**		
		40-50	**20.17**	28.78	33.71	40.57	**52.92**		
		50-60	**18.43**	24.98	29.47	36.05	**48.92**		
RMSSD [ms]	M	20-30	**24.84**	34.43	48.13	69.63	**100.02**	-0.798*** ($r^2 = 0.181$)	**p = 0.014**
		30-40	**21.97**	29.87	40.71	49.56	**74.33**		
		40-50	**18.25**	24.46	33.39	44.12	**66.75**		
		50-60	**14.66**	22.77	28.77	35.12	**56.18**		
	F	20-30	**22.37**	35.12	42.66	58.58	**89.81**	-0.717*** ($r^2 = 0.216$)	
		30-40	**20.16**	28.03	36.50	50.06	**71.14**		
		40-50	**15.48**	22.90	30.00	36.30	**53.27**		
		50-60	**15.94**	20.81	25.80	33.07	**54.46**		
pNN50 [%]	M	20-30	**4.76**	10.95	19.09	37.58	**41.58**	-0.485*** ($r^2 = 0.231$)	**p = 0.018**
		30-40	**3.19**	7.19	13.23	20.59	**30.81**		
		40-50	**1.31**	3.51	10.27	17.05	**30.10**		
		50-60	**0.62**	2.80	4.86	9.49	**24.13**		

	F	20-30	**3.37**	10.89	15.06	25.67	**37.56**	
		30-40	**1.75**	6.41	11.43	19.78	**31.50**	0.442***
		40-50	**0.79**	3.18	6.69	12.29	**21.94**	(r^2 = 0.268)
		50-60	**0.63**	2.24	4.19	8.03	**17.72**	
SDANN [ms]	M	20-30	**95.06**	125.61	149.06	181.15	**232.19**	
		30-40	**94.91**	116.45	136.84	162.51	**221.53**	-1.336***
		40-50	**77.64**	102.13	121.79	146.22	**184.38**	(r^2 = 0.122)
		50-60	**66.24**	91.52	118.64	142.74	**172.74**	**p < 0.001**
	F	20-30	**84.03**	111.63	132.85	150.66	**213.85**	
		30-40	**69.93**	95.64	116.19	142.39	**182.48**	-0.894***
		40-50	**72.92**	92.00	109.97	135.48	**168.91**	(r^2 = 0.094)
		50-60	**67.74**	84.93	105.16	131.65	**163.92**	

Table 3: Percentile values of frequency-domain HRV measures, separately for male (M) and female (F), categorised according to age group, with regression analysis (with age as co-variable) and p-value for gender-related differences. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n = 695.

			Percentile						
			5th	25th	50th	75th	**95th**	Regression analysis with age as co-variable	Gender-related difference
LFnu [---]	M	20-30	**38.51**	60.91	68.06	77.08	**83.59**	0.233*** (r^2 = 0.053)	**p < 0.001**
		30-40	**58.40**	66.00	75.46	79.85	**87.58**		
		40-50	**57.49**	68.87	77.07	82.15	**89.27**		
		50-60	**56.25**	68.35	75.49	80.95	**86.54**		
	F	20-30	**42.57**	53.89	61.48	69.18	**78.38**	0.303*** (r^2 = 0.089)	
		30-40	**45.42**	60.35	67.81	74.97	**81.38**		
		40-50	**45.99**	66.69	72.26	78.78	**84.48**		
		50-60	**49.74**	62.05	72.06	78.14	**82.87**		
HFnu [---]	M	20-30	**16.41**	22.92	31.94	39.09	**61.49**	-0.233*** (r^2 = 0.053)	**p < 0.001**
		30-40	**12.42**	20.15	24.54	34.00	**41.60**		
		40-50	**10.73**	17.85	22.85	31.13	**42.51**		
		50-60	**13.46**	19.05	24.51	31.65	**43.75**		
	F	20-30	**21.62**	30.82	38.52	46.11	**57.43**	r =- 0.303*** (r^2 = 0.089)	
		30-40	**18.62**	25.03	32.19	39.65	**54.58**		
		40-50	**15.52**	21.22	27.74	33.31	**54.01**		
		50-60	**17.13**	21.86	27.94	37.95	**50.26**		
LF/HF [---]	M	20-30	**0.63**	1.56	2.13	3.36	**5.10**	0.033*** (r^2 = 0.038)	**p < 0.001**
		30-40	**1.40**	1.94	3.08	3.96	**7.06**		
		40-50	**1.35**	2.21	3.36	4.60	**8.32**		
		50-60	**1.29**	2.16	3.08	4.25	**6.44**		

	F	20-30	**0.74**	1.17	1.60	2.25	**3.63**	0.032*** (r^2 = 0.079)
		30-40	**0.83**	1.52	2.11	3.00	**4.37**	
		40-50	**0.85**	2.00	2.60	3.71	**5.44**	
		50-60	**0.99**	1.64	2.58	3.57	**4.84**	

Table 4: Percentile values of HRV parameters of the Poincaré plot, separately for male (M) and female (F), categorised according to age group. With regression analysis (with age as co-variable) and p-value for gender-related differences. * p < 0.05, ** p < 0.01, *** p < 0.001, n = 695.

			Percentile						
			5th	25th	50th	75th	**95th**	Regression analysis with age as co-variable	Gender-related difference
SD1 [ms]	M	20-30	**17.80**	24.74	34.40	49.61	**71.27**	-0.567*** (r^2 = 0.181)	**p = 0.011**
		30-40	**15.80**	21.45	29.04	35.51	**53.11**		
		40-50	**13.15**	17.66	24.01	31.69	**47.76**		
		50-60	**10.62**	16.48	20.56	25.20	**40.13**		
	F	20-30	**16.06**	25.16	30.42	41.68	**63.74**	-0.509*** (r^2 = 0.216)	
		30-40	**14.41**	20.05	26.03	35.71	**50.88**		
		40-50	**11.14**	16.49	21.55	25.99	**38.00**		
		50-60	**11.45**	14.94	18.44	23.65	**38.77**		
SD2 [ms]	M	20-30	**153.90**	210.44	246.83	294.22	**362.62**	-2.339*** (r^2 = 0.158)	**p < 0.001**
		30-40	**159.37**	192.30	225.48	259.99	**355.56**		
		40-50	**129.04**	162.75	198.61	235.24	**291.21**		
		50-60	**108.63**	142.51	185.20	223.29	**269.46**		
	F	20-30	**145.49**	185.34	212.28	244.67	**326.42**	-1.570***	

	30-40	**126.56**	160.86	191.03	224.71	**289.21**	(r^2 = 0.129)
	40-50	**125.69**	152.92	176.15	211.31	**258.22**	
	50-60	**116.38**	135.88	165.41	205.36	**251.54**	

Table 5: Normal values for several indices of heart rate variability from the Guidelines of the European Society of Cardiology and North American Society of Pacing and Electrophysiology [5]

Variable	**Units**	**Normal values (mean ± SD)**
SDNN	ms	**141 ± 39**
SDANN	ms	**127 ± 35**
RMSSD	ms	**27 ± 12**