Heart Rate Variability and Baroreflex Sensitivity: Factors and Reference Ranges

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ABSTRACT

Objective: Heart rate variability (HRV) and baroreflex sensitivity (BRS) are influenced by various internal and external factors, making it necessary to establish universal reference values. We aimed to identify independent factors affecting short-term HRV parameters and BRS, as well as determine appropriate reference ranges, which are lacking in Asian populations.

Materials and Methods: A total of 117 healthy Thai participants (aged 20-72 years; final n=117) were recruited based on strict criteria, including normal medical history, physical examination (ascertained by a physician), and normal blood profile. Spontaneous 5-minute HRV parameters (time and frequency domains) and spontaneous cross-correlation BRS were measured.

Results: Age and resting heart rate (HR) are independently and inversely correlated with BRS and all HRV parameters, except LFnu (normalized low-frequency component) and LF/HF ratio (ratio of low- to high-frequency component). Sex differences were observed only in LF, which was higher in men. However, multiple regression analysis showed that sex did not significantly contribute as an independent variable to either HRV or BRS. Remarkably, BRS exhibited moderate to strong correlations with all HRV values, indicating its prominent role in influencing HRV and surpassing the impact of age.

Conclusion: Age and resting HR were identified as independent factors influencing 5-minute HRV and BRS. Our findings suggest that decreased baroreflex function associated with aging may contribute to reduced HRV among the elderly. Furthermore, we established reference ranges for each 5-minute HRV parameter and BRS, categorized by age group (20-39, 40-59, and ≥60 years old). These reference values offer valuable clinical insights, particularly for Asian populations where such normative ranges were previously unavailable.

Keywords: Heart rate variability; baroreflex sensitivity; reference values; healthy subjects; cardiovascular autonomic assessment (Siriraj Med J 2023; 75: 713-724)

INTRODUCTION

Heart rate variability (HRV) and baroreflex sensitivity (BRS) are quantitative markers that represent cardiovascular autonomic functions.[1-3] HRV measures the oscillation of interbeat (RR) intervals, which reflects the central sympathetic and parasympathetic modulation of the heart.[4] BRS refers to the relationship between changes in systolic blood pressure (SBP) and corresponding RR intervals. It is widely accepted that hemodynamic parameters and derivatives, including HRV and BRS, are influenced by various internal and external factors, such as age, sex, ethnicity, recording procedures, surrounding

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temperature and environment.[1,2,5-11] Consequently, it is necessary to establish reference ranges for HRV and BRS that are specific to each laboratory to ensure meaningful interpretation, and facilitate inter-laboratory comparisons.[12] However, there are considerable variations in HRV and BRS reference values, even among Asian individuals[10,13-15], likely due to disparities in recording duration, analysis methods, and other factors.[5,12,16]

HRV and BRS have been increasingly employed in the clinical setting, mostly in clinical trials, e.g., as risk assessment tools in coronary artery diseases.[17,18] HRV is sometimes used as an additional investigation in patients with atypical postural orthostatic tachycardia syndrome or POTS.[19] Moreover, their recording practicality have made both HRV and BRS widely used for other purposes besides clinical applications. For example, HRV is used as an objective stress measurement in e-sport players[20] and training monitoring parameters in sports science.[21] This popularity, however, resulted in a huge variation in procedure and outcome, leading to difficulties in interpretation, particularly when comparing among studies without reference values.

Resting, short-term assessments of HRV (5 minutes) and BRS (10 minutes) are noninvasive and practical for clinical investigations.[1,2,12,17-19,22] However, standardized 5-minute HRV reference values for Asian adults are currently lacking age stratification[13] or fail to demonstrate age dependence.[20] While a few studies in Thailand have compared HRV and BRS between patients and controls or different groups[23-25], normal values for the Thai population have not yet been established, limiting the clinical use in the region. Our aim was to determine factors that influence short-term HRV parameters and BRS, and to establish reference values for healthy Thai adults.

MATERIALS AND METHODS

This study was approved by the Siriraj Institutional Review Board (COA no. Si 679/2012).

Participants

We enrolled healthy Thais, aged 20-80 years, from Bangkok. All participants provided written informed consent and met the following criteria: normal BMI (18.5-22.9 kg/m^2)[26], and blood profile within the last six months, including complete blood count (CBC), fasting blood glucose (FBG), blood urea nitrogen (BUN), creatinine, liver function tests, and lipid profile: triglyceride (TG) <200 mg/dL and total cholesterol <200 mg/dL, or ≥200mg/dL with calculated LDL (cal-LDL) <160 mg/dL, and HDL >50 mg/dL (women) or >40 mg/dL (men). The exclusion criteria was as follows: diabetes mellitus; cardiovascular diseases (e.g., hypertension); abnormal electrocardiogram (ECG); pulmonary diseases, including obstructive sleep apnea; psychiatric diseases; use of drugs that affect the autonomic nervous system (ANS), with the exception of statins and baby aspirin; current smokers or individuals who had quit smoking <1 year; heavy alcohol drinkers (>2 standard drinks per day); individuals with occupations that affect the ANS, such as security guards and athletes; acute illness; and pregnancy. All female participants were assessed outside their menstruation period.

Data collection

Participants avoided alcoholic beverages for ≥48 hours, and caffeinated beverages, vitamins, herbs, and intense exercise for ≥12 hours. Upon arrival at the Cardiovascular Autonomic Function Laboratory, Department of Physiology, participants underwent an interview and a comprehensive physical examination conducted by a physician. Recorded data comprised participant demographics, including sex, age, BMI, exercise habits, alcohol consumption, menstruation, resting blood pressure (BP), and heart rate (HR) using an automatic sphygmomanometer.

The autonomic assessment took place in a quiet room, with a temperature of 23-24°C, humidity between 50-60%, and commenced at least ≥2 hours after the last meal. Noninvasive continuous hemodynamic parameters were measured using the Finometer® Pro device with an ECG module (lead II) and analyzed with the BeatScope® Easy program (Finapres Medical System BV, Amsterdam, The Netherlands), at a sampling rate of 200 Hz.

The protocol began with a 10-minute period of rest in a supine position, with system calibrations performed between the 6th and 10th minute. Subsequently, continuous measurements of blood pressure (BP) and ECG were recorded for 12 minutes, during which participants remained awake, quiet, and relaxed.

Data and statistical analysis

HRV analysis was performed using LabChart®7Pro software (ADInstrument, Castle Hill, Australia). A 5-minute segment of stable ECG data without, ectopic beats was manually selected to derive 11 time- and frequency-domain HRV parameters and BRS, as shown in Table 2.

Statistical analyses were performed using SPSS software (IBM Corporation, New York, USA). Data normality was assessed using the Kolmogorov-Smirnov test. Results were reported as mean±SD, with additional median and interquartile range (P_{25}-P_{75}) provided for non-normally distributed data. For comparisons between

groups, the unpaired t-test or one-way ANOVA with *post hoc* Tukey's test was used for normally distributed data, while the Mann-Whitney U test or Kruskal-Wallis test with *post hoc* Dunn's test was applied for non-normally distributed data, as appropriate. A P value <0.05 was considered statistically significant.

Pearson correlation analysis was utilized to determine the linear correlation between continuous data and HRV parameters or BRS. Correlation coefficients (r) falling within the range $0.36 \leq |r| < 0.68$ or $|r| \geq 0.68$ were regarded as indicating moderate or strong correlations, respectively.[27] Factors that exhibited significant differences in parameters between groups or significant correlations with HRV or BRS values were further included in a stepwise multiple regression analysis to identify independent determinants. Lastly, reference values for each parameter were determined based on percentile values and categorized as normal, borderline, or abnormal as described in Table 5.

RESULTS

Participant general characteristics

Of the 144 initially interviewed healthy participants, 27 individuals were excluded due to abnormal blood profiles or physical examination findings. The general characteristics of the remaining 117 participants (44 men and 73 women) are summarized in Table 1. The mean age was similar between men and women. Men exhibited higher BMI, BP, and respiratory rate (RespR), but had significantly lower resting HR. All blood profile data were within normal limits, consistent with the inclusion criteria (data not shown). The participants were divided into three age groups: 20-39, 40-59, and 60-79 years (n=56, 40, and 21, respectively). In the two younger age groups, the average ages did not differ significantly between sexes, while in the oldest group, women were older than men (average age, women vs. men, 64.41 vs. 60.50 years, n=17 vs. 4, respectively) (Supplemental Materials, Table S.1).

Comparisons of HRV and BRS among independent groups

The comparison of HRV parameters and BRS among different age groups is presented in Table 2. Most parameters showed a declining trend with age, except for LFnu, HFnu, and LF/HF, which were not dependent on age. The 40-59 age group generally did not differ significantly from the 60-79 age group, except for SDNN, Total power, and LF, which were higher in the 40-59 age group. Interestingly, we observed no significant differences between sexes in all parameters except for LF, which was higher in men (Table S.2).

We also examined HRV and BRS differences in relation to other characteristics. However, no significant differences were found when caffeine drinkers vs. non-caffeine drinkers, non-heavy alcohol drinkers vs. non-alcohol drinkers, regular exercise vs. sedentary individuals,

TABLE 1. Participants' general characteristics.

Characteristics	Men (n = 44)	Women (n = 73)	P value
Age (year)	39.00 ± 12.82	44.11 ± 14.94	ns[a]
BMI (kg/m^2)	21.29 ± 1.21, 21.65 (20.22 - 22.32)	20.75 ± 1.09	0.0127[b]
SBP (mmHg)	118.16 ± 10.50	109.89 ± 11.77, 108.00 (101.00 - 115.50)	0.0002[b]
DBP (mmHg)	73.39 ± 8.10	69.01 ± 8.69	0.0079[a]
MAP (mmHg)	88.31 ± 8.19	82.64 ± 8.97	0.0009[a]
HR (/min)	67.25 ± 8.20	72.10 ± 9.75, 72.00 (66.00 - 76.50)	0.0053[b]
RespR (/min)	15.98 ± 9.69, 13.00 (12.00 - 16.00)	15.38 ± 2.14, 16.00 (14.00 - 16.00)	0.0040[b]

Data are mean±SD. Medians (P_{25} - P_{75}) are additionally shown for non-normally distributed data. [a]Unpaired t-test (normally distributed data); [b]Mann-Whitney U test (non-normally distributed data); ns, not statistically significant or $P \geq 0.05$.

TABLE 2. Comparison of HRV parameters and BRS among age groups.

Parameters	20-39 years (n=56)	40-59 years (n=40)	60-79 years (n=21)	*P* value
HRV				
Time domain parameters				
SDNN (ms)	56.98 ± 28.43, 46.51 (38.04 – 70.12)	46.42 ± 18.22, 42.31 (34.29 – 56.85)	32.34 ± 13.69*,#	<0.0001[b]
SDSD (ms)	50.88 ± 30.92, 41.07 (33.40 – 64.85)	31.76 ± 16.69*	22.78 ± 11.59*, 19.15 (15.98 – 26.16)	<0.0001[b]
RMSSD (ms)	50.80 ± 30.86, 41.01 (33.36 – 64.74)	31.71 ± 16.66* 19.12 (15.95 – 26.12)	22.74 ± 11.56*,	<0.0001[b]
pNN50 (%)	26.43 ± 20.55, 21.54(10.60 – 41.89)	10.76 ± 13.96*, 4.77(1.58 – 17.98)	5.82 ± 9.20*, 2.11(0.80 – 6.02)	<0.0001[b]
Frequency domain parameters				
Total power (ms^2)	4083.40 ± 4348.02, 2414.42 (1235.23 - 4844.84)	2366.20 ± 2036.57, 1671.50 (1302.74 - 2742.20)	1215.93 ± 1336.86*,#, 900.07(487.06 - 1325.66)	<0.0001[b]
LF (ms^2)	1173.82 ± 1509.87, 681.23 (307.11 - 1398.26)	495.05 ± 385.00*, 373.62(254.98 - 729.60)	270.82 ± 309.08*,#, 184.38(104.17 - 304.04)	<0.0001[b]
LFnu	42.93 ± 15.51	56.73 ± 15.84*	50.90 ± 19.91	0.0436[a]
HF (ms^2)	1491.10 ± 2040.50, 763.31 (418.14 - 1551.51)	444.77 ± 571.81*, 286.37 (167.51 - 535.86)	226.89 ± 225.34*, 151.41 (97.97 - 212.00)	<0.0001[b]
HFnu	51.12 ± 16.14	40.00 ± 15.71*	44.95 ± 18.67	0.0058[a]
LF/HF	1.09 ± 0.78, 0.86 (0.45 - 1.52)	1.89 ± 1.77*, 1.22 (0.79 - 2.21)	1.62 ± 1.45, 1.42 (0.67 - 2.00)	<0.0289[b]
BRS (ms/mmHg)	19.15 ± 9.87, 15.72 (12.35 - 22.89)	11.20 ± 5.15*, 10.37 (8.68 - 13.08)	8.21 ± 2.58*	<0.0001[b]

Data are mean±SD. Medians (P_{25} - P_{75}) are additionally shown for non-normally distributed data. [a]One-way ANOVA followed by *post hoc*; [b]Kruskal-Wallis test followed by *post hoc* Dunn's test (non-normally distributed data); *,#Significant difference compared to age groups *20-39 years or #40-59 years, respectively; ns, not statistically significant or $P \geq 0.05$.

Abbreviations: SDNN, standard deviation of the NN interval (NN stands for normal-to-normal RR interval); RMSSD, the root mean square of differences between adjacent NN intervals; SDSD, standard deviation of differences between adjacent NN intervals; pNN50, proportion of NN50 to total NN intervals (NN50 stands for number of adjacent NN interval pairs with a difference greater than 50 ms); VLF, very low frequency (≤ 0.4 Hz); LF, low frequency (0.04-0.15 Hz); HF, high frequency (0.15-0.4 Hz); LFnu, normalized unit of LF; HFnu, normalized unit of HF.

women in the follicular vs. the luteal phase of the menstrual cycle, and morning vs. afternoon assessment time (Table S.3-S.7).

Correlation analysis

Correlation analysis was conducted between each HRV parameter or BRS and all factors, including hemodynamic and blood profile measures. Age showed a moderate negative correlation with all absolute HRV parameters (excluding LFnu, HFnu, and LF/HF) and BRS. Additionally, a weak negative correlation was observed between age and HFnu, while a weak positive correlation was found between age and LFnu or LF/HF. Resting HR, but not BP, exhibited a moderate inverse correlation with three time-domain HRV indices, namely SDSD, RMSSD, and pNN50. Furthermore, BRS displayed a stronger negative correlation with SBP than resting HR (Table 3). Other factors not shown in the table, including blood profile data, either showed no significant correlation with HRV parameters or BRS ($P \geq 0.05$), or displayed significant but weak correlations ($P < 0.05$, $|r| < 0.36$).

Stepwise multiple regression

To determine independent influences on each HRV or BRS parameter, stepwise multiple regression analysis was conducted. All factors with any significant correlation or differences between groups were included in the analysis, resulting in a total of 22 parameters: sex (men=0, women=1), age, BMI, weight, height, SBP, DBP, MAP, HR, RespR, ambient temperature, humidity, FBG,

TABLE 3. Correlations between HRV parameters or BRS vs age, BMI, resting BP, and HR.

Parameters	Age	BMI	SBP	DBP	MAP	HR
HRV						
Time domain parameters						
SDNN	***r* = -0.45** ****	ns	*r* = -0.19 *	ns	ns	*r* = -0.34 **
SDSD	***r* = -0.50** ****	ns	*r* = -0.29 **	ns	*r* = -0.20 *	***r* = -0.37** ****
RMSSD	***r* = -0.50** ****	ns	*r* = -0.29 **	ns	*r* = -0.20 *	***r* = -0.37** ****
pNN50	***r* = -0.53** ****	ns	*r* = -0.30 **	ns	*r* = -0.22 *	***r* = -0.40** ****
Frequency domain parameters						
Total power	***r* = -0.38** ****	ns	ns	ns	ns	*r* = -0.32 **
LF	***r* = -0.37** ****	ns	ns	ns	ns	*r* = -0.23 *
LFnu	*r* = 0.21 *	*r* = 0.34 ***	*r* = 0.22 *	ns	*r* = 0.18 *	ns
HF	***r* = -0.40** ****		*r* = -0.24 **	ns	ns	*r* = -0.26 **
HFnu	*r* = -0.26 **	*r* = -0.26 **	*r* = -0.26 **	*r* = -0.19 *	*r* = -0.24 *	*r* = -0.19 *
LF/HF	*r* = 0.23 *	*r* = 0.22 *	*r* = 0.24 **	*r* = 0.21 *	*r* = 0.23 *	ns
BRS	***r* = -0.59** ****	ns	***r* = -0.42** ****	ns	*r* = -0.33 ****	*r* = -0.27 ****

r, Pearson's correlation coefficient; ns, not statistically significant or $P \geq 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. Significant correlations with $|r| > 0.36$ are shown in bold type.

TG, cal-LDL, ALT, Hb, Hct, RBC, WBC, neutrophil (N), and eosinophil (%E). Table 4 displays the multiple linear equations and their adjusted coefficient of determination (Adj. R2) for each HRV and BRS value. The independent variables in each equation are presented in order of their contribution size to the regression model. Age was the main contributing variable for all time-domain HRV parameters, Total power, LF, HF, and BRS, followed by resting HR. Interestingly, sex did not emerge as a significant variable in any of the equations, suggesting that any differences in HRV parameters between sexes may be an epiphenomenon. It is worth noting that none of the adjusted R^2 exceeded 0.50, indicating that the factors included in the study accounted for less than 50% of the variance in each parameter. It is possible that other factors not considered in our study may play a role.

Relationship between HRV parameters and BRS

BRS exhibited a strong positive correlation with all time-domain HRV parameters. It also displayed positive correlations with most frequency-domain parameters, except for LFnu and LF/HF ratio, which showed an inverse correlation (Table S.8). Among the HRV parameters, RMSSD (r=0.86), SDSD (r=0.86), pNN50 (r=0.85), and HF (r=0.80), which are associated with parasympathetic activity, showed the strong positive correlations with BRS. LF exhibited a moderate correlation (r=0.59). Given

TABLE 4. Stepwise multiple regression results for HRV parameters and BRS.

	Regression equation				Adj. R^2
HRV					
Time domain parameters					
SDNN	= 159.929 *(90.992)*	- 0.836 Age *(40.820)*	- 1.170 HR *(24.619)*		0.335
SDSD	= 170.202 *(100.290)*	- 0.985 Age *(55.108)*	- 1.381 HR *(33.375)*		0.407
RMSSD	= 169.864 *(100.261)*	- 0.984 Age *(55.129)*	- 1.377 HR *(33.344)*		0.407
pNN50	= 117.848 *(104.949)*	- 0.748 Age *(69.339)*	- 1.065 HR *(43.356)*		0.468
Frequency domain parameters					
Total power	= 17092.348 (47.557)	- 99.590 Age *(26.480)*	- 153.131 HR (19.307)		0.258
LF	= 4487.946 *(27.694)*	- 31.256 Age *(22.031)*	- 36.928 HR *(9.484)*		0.190
LFnu	= -97.336 *(9.417)*	+ 5.091 BMI *(14.765)*	+ 0.337 SBP *(5.916)*		0.143
HF	= 6645.337 *(34.556)*	- 46.026 Age *(27.188)*	- 58.678 HR *(13.627)*		0.236
HFnu	= 190.121 *(35.468)*	- 4.392 BMI *(11.759)*	- 0.604 HR *(10.511)*	- 0.303 Age *(8.997)*	0.177
LF/HF	= 2.063 *(9.345)*	+ 0.013 TG *(12.495)*	- 0.248 WBC *(7.015)*		0.139
BRS	= 53.412 *(94.140)*	- 0.377 Age *(76.833)*	- 0.356 HR *(21.172)*		0.436

Adj. R^2, adjusted coefficient of determination; italicized numbers in parentheses are F-to-remove of the parameter immediately above.

these robust associations, we conducted a reanalysis by incorporating HRV and BRS into the stepwise multiple regression calculation for each other (Table S.9A and S.9B). The inclusion of both variables resulted in improved predictive power (Adj. R^2) in every model. Interestingly, BRS emerged as the most influential variable for predicting all HRV parameters, replacing the variance explained by age (Table S.9A). Furthermore, RMSSD, a vagal HRV parameter, contributed the most to the BRS value (Table S.9B).

Age-specific reference values of HRV parameters and BRS (Table 5)

The reference values for HRV parameters and BRS were categorized by age group, except for LFnu and LF/HF, which were age-independent (Table 4). These values were organized into five intervals based on percentile, as described in the Materials and Methods section. As expected, the age-dependent parameters were highest in the youngest age group.

DISCUSSION

In this study, we examined short-term HRV (5 minutes) and spontaneous cross-correlation BRS in 117 healthy Thai volunteers (44 men and 73 women, 20-72 years). Our findings revealed that BRS and most HRV parameters, namely, SDNN, SDSD, RMSSD, pNN50, Total power, LF, HF, and HFnu, were negatively and independently correlated with age and resting HR. However, factors such as sex, most blood profile components, caffeine intake, alcohol drinking, exercise, menstrual phase, and assessment time did not show any significant associations with HRV parameters and BRS.

Major determinants of HRV and BRS are age and resting heart rate.

Age and resting heart rate are major determinants of HRV parameters and BRS in healthy subjects. The negative correlation between age and all parameters, except for LFnu and LF/HF, was observed in both the correlation study and stepwise multiple analysis (Table 3 and 4). These results are consistent with several studies reporting a decline in HRV and BRS with age in healthy participants[5,14,28-31] with our comparison of HRV parameters among age groups (Table 2). Our rigorous inclusion and exclusion criteria ensured the selection of a homogeneous pool of healthy subjects, indicating that the negative correlation with age represents a normal aging process rather than a pathology.[32] The absence of an age association with LFnu and LF/HF was supported by the stepwise multiple regression results (Table 4), aligning with the findings of Remaekers *et al.*[33] Furthermore, the differences were primarily observed between the youngest group (20-39 years) and the two older groups. The average or median values of HRV and BRS in the age group 40-59 and ≥60 were generally similar, especially parasympathetic-associated HRV parameters: RMSSD, pNN50, and HF. The two older groups only differed significantly in SDNN, Total power, and LF (Table 2). Although this discrepancy could be attributed to the small sample size in the oldest age group, a study has shown that older subjects (60-77) did not differ significantly from the oldest middle-age group (40-59), which supports our results.[28] Possible explanations include a faster decline in HRV values with age in younger individuals[32] and a more linear correlation between sympathetic modulation, global autonomic regulation HRV (LF and SDNN, respectively) and age compared to parasympathetic modulation.[14,34]

Another significant independent factor affecting HRV and BRS, as observed in our data, is resting HR. While this factor is less frequently reported[6,33,35], we found a negative correlation between resting HR and most HRV parameters and BRS, similar to the age effect but of smaller magnitude (Table S.9A and S.9B). Our results are generally in agreement with Ramaekers *et al.*[33], who reported negative correlations between HR and SDNN, SDSD, pNN50, RMSSD, Total power, LF, and HF, except for LFnu and LF/HF ratio. Since resting HR is associated with cardiac autonomic tone, while HRV parameters are more related to cardiac autonomic regulation[4], the negative correlation suggests reduced cardiac autonomic modulation at higher autonomic tone. Alternatively, it may reflect limited variability at narrower RR intervals.[36]

BMI did not show a significant association with most HRV parameters and BRS in our study, and it only weakly influenced LFnu and HFnu. In contrast, a study by Vallejo *et al.*[37] in 30 females (21-35 years; BMI ranging from <19 to >30kg/m^2) found that both age and BMI significantly affected HRV. The discrepancy may be attributed to the narrower range of BMI in our healthy participants (18.5-22.9kg/m^2), or the fact that the other study did not include resting HR in their analysis (which could be a determinant variable of BMI).

HRV parameters and BRS are probably not sex dependent.

Most HRV parameters and BRS were not significantly different between men and women, except for significantly higher LF in men (Table S.2). Additionally, sex did not appear in any of the stepwise multiple regression equations (Table 4), indicating that sex was not an independent factor determining HRV parameters and BRS in healthy

TABLE 5. Normal, borderline, and abnormal values for HRV parameters and BRS.

Parameters		Age 20-39 years (n = 56)	40-59 years (n = 40)	> 60 years (n = 21)
SDNN (ms)				
	A_H	≥ 122.62	≥ 84.38	≥ 65.70
	B_H	91.87 - 122.61	68.28 - 84.37	43.17 - 65.69
	Normal	**27.54 - 91.86**	**26.89 - 68.27**	**20.75 - 43.16**
	B_L	22.01 - 27.53	20.48 - 26.88	17.49 - 20.74
	A_L	≤ 22.00	≤ 20.47	≤ 17.48
SDSD (ms)				
	A_H	≥ 144.20	≥ 66.57	≥ 50.14
	B_H	86.01 - 144.19	47.27 - 66.56	41.48 - 50.13
	Normal	**21.56 - 86.00**	**15.04 - 47.26**	**14.11 - 41.47**
	B_L	15.87 - 21.55	10.94 - 15.03	10.33 - 14.10
	A_L	≤ 15.86	≤ 10.93	≤ 10.32
RMSSD (ms)				
	A_H	≥ 143.91	≥ 66.44	≥ 50.04
	B_H	85.86 - 143.90	47.20 - 66.43	41.42 - 50.03
	Normal	**21.53 - 85.85**	**15.02 - 47.19**	**14.08 - 41.41**
	B_L	15.85 - 21.52	10.92 - 15.01	10.32 - 14.07
	A_L	≤ 15.84	≤ 10.91	≤ 10.31
pNN50 (%)				
	A_H	≥ 69.21	≥ 44.07	≥ 28.81
	B_H	56.11 - 69.20	27.41 - 44.06	22.74 - 28.80
	Normal	**1.86 - 56.10**	**0.49 - 27.40**	**0 - 22.73**
	B_L	0.67 - 1.85	0 - 0.48	0
	A_L	≤ 0.66	*	*
Total power (ms^2)				
	A_H	≥ 16429.82	≥ 7089.93	≥ 4396.11
	B_H	8407.85 - 16429.81	5220.90 - 7089.92	2308.46 - 4396.10
	Normal	**681.03 - 8407.84**	**760.36 - 5220.89**	**403.65 -2308.45**
	B_L	474.08 - 681.02	398.73 - 760.35	333.98 - 403.64
	A_L	≤ 474.07	≤ 398.72	≤ 333.97
LF (ms^2)				
	A_H	≥ 4492.49	≥ 1574.98	≥ 1122.30
	B_H	3006.39 - 4492.48	996.83 - 1574.97	416.35 - 1122.29
	Normal	142.66 - 3006.38	149.08 - 996.82	73.44 - 416.34
	B_L	74.83 - 142.65	78.57 - 149.07	59.40 - 73.43
	A_L	≤ 74.82	≤ 78.56	≤ 59.39
LFnu				
	A_H		≥ 82.24	
	B_H		71.53 - 82.23	
	Normal		**23.29 - 71.52**	
	B_L		17.34 - 23.28	
	A_L		≤ 17.33	

TABLE 5. Normal, borderline, and abnormal values for HRV parameters and BRS. (Continue)

Parameters		Age 20-39 years (n = 56)	40-59 years (n = 40)	> 60 years (n = 21)
HF (ms²)				
	A_H	≥ 6835.77	≥1734.42	≥ 816.01
	B_H	3284.54 - 6835.76	868.83 - 1734.41	461.89 - 816.00
	Normal	**218.61 - 3284.53**	**92.57 - 868.82**	**78.78 - 461.88**
	B_L	57.65 - 218.60	36.49 - 92.56	55.68 - 78.77
	A_L	≤ 57.64	≤ 36.48	≤ 55.67
HFnu				
	A_H	≥ 77.99	≥ 67.40	≥ 80.39
	B_H	72.25 - 77.98	55.73 - 67.39	72.84 - 80.38
	Normal	**31.41 - 72.24**	**18.41 - 55.72**	**25.10 - 72.83**
	B_L	24.50 - 31.40	12.22 - 18.40	16.72 - 25.09
	A_L	≤ 24.49	≤ 12.21	≤ 16.71
LF/HF				
	A_H		≥ 6.47	
	B_H		2.88 - 6.46	
	Normal		**0.34 - 2.87**	
	B_L		0.22 - 0.33	
	A_L		≤ 0.21	
BRS (ms/mmHg)				
	A_H	≥ 45.19	≥ 19.96	≥ 12.44
	B_H	34.08 - 45.18	14.90 - 19.95	10.95 - 12.43
	Normal	**10.52 - 34.07**	**5.34 - 14.89**	**4.87 - 10.94**
	B_L	7.08 - 10.51	4.70 - 5.33	4.37 - 4.86
	A_L	≤ 7.07	≤ 4.69	≤ 4.36

Normal, P_{10}-P_{90}; borderline low (B_L) ≥ $P_{2.5}$ to < P_{10}; borderline high (B_H) > P_{90} to ≤ $P_{97.5}$; abnormally low (A_L) < $P_{2.5}$; abnormally high (AH) > $P_{97.5}$; *, not defined.

subjects. The difference in LF between men and women could be attributed to the negative correlation between LF and resting HR (Table 3), as men tend to have lower resting HR than women (Table 1). Although many reports have described sex differences in HRV, especially in young adults[12,13], most of these studies did not utilize multiple regression analysis in their investigations.

BRS is highly correlated with HRV.

BRS has been found to have a positive correlation with HRV in both normotensive and hypertensive subjects. It is also the most important determinant in predicting HRV in multiple regression analysis[38,39], which is consistent with our findings. Although BRS and HRV both reflect cardiovascular autonomic functions, they represent different aspects and are not redundant. Therefore, it is reasonable to consider their correlations.[17]

The parasympathetic components of HRV, such as RMSSD, SDSD, pNN50, and HF, exhibited higher correlations with BRS compared to sympathetic or global autonomic components like LF, SDNN, and Total power (Table S.8). These distinct correlations support the notion that activation and deactivation of sympathetic or parasympathetic control during blood pressure fluctuation are not mirror images. While the SA node is controlled by both parasympathetic and sympathetic cardiac fibers, the vagal response is much faster, leading to immediate changes in RR intervals in response to blood pressure fluctuations.[2] Furthermore, it is important to note that our study, despite the 'baroreflex

sensitivity' name, specifically focused on the cardiovagal branch of the baroreflex arc, as we derived responses from RR intervals, while excluding changes in sympathetic vasomotor tone. Thus, it is not surprising that our results align with other studies showing a high correlation between BRS and cardiac vagal control.[40]

The high correlations observed between HRV parameters and BRS can provide insights into the mechanisms underlying reduced HRV with aging (Table 4). Studies associating HR and BP oscillations have suggested a close relationship between HRV and baroreceptor function.[41,42] In our study, we attempted to integrate these two observations by including BRS in the multiple regression analysis to predict each HRV parameter and vice versa. The inclusion of BRS replaced all HRV variance previously accounted for by age and emerged as the most influential factor, exhibiting the highest proportion of squared semi-partial correlation for each HRV parameter (Table S.9A). In other words, the age-related decline in HRV can also be explained by the reduced d BRS. Therefore, a decline in baroreceptor or baroreflex function may contribute to the diminished HRV observed in older individuals.

Age-specific reference values of HRV parameters and BRS

The present study presents reference values for 5-minute HRV and cross-correlation BRS in a sample of healthy Thai individuals (Table 5). We identified age and resting HR as independent factors influencing HRV and BRS, while sex did not show a significant association. Therefore, we categorized the reference values into three age groups without considering sex stratification. The reference values were presented based on percentiles since most of the HRV and BRS data exhibited nonparametric distribution.

While many studies in Asian populations defined normal ranges for short-term HRV in terms of mean ± SD, with or without age specification[13,14,43], the only other study reporting age-adjusted reference values in percentile in Asian individuals (in Brazil) was conducted by Dantas *et al.*[44] Comparing our reference values in in the frequency domain with the Asian Brazilian data, we observed higher values. These differences could be attributed to variations in analytical methods or environmental factors among the study subjects.

We provided age-adjusted reference values for BRS using the spontaneous cross-correlation sequence method, which has been suggested to be more reliable than the sequential method.[22] To the best of our knowledge, our reference values for cross-correlation BRS may be the only ones reported for an Asian population. However, Tang *et al.*[15] recently published spectral BRS reference values for a Chinese population. When compared to the ATRAMI study[17], which reported BRS values in post-myocardial infarction patients, our lower limit for abnormal BRS (AL) was higher than the cut-point associated with increased cardiac mortality (3ms/mmHg) in those patients. This disparity can be explained by the ATRAMI study inclusion of non-healthy subjects, with a higher average age (57 years), and the use of phenylephrine injection to determine BRS.

Limitations

Our sampling rate for BP and ECG signal provided by the Finometer® Pro device was limited to 200 Hz maximum[45], which was lower than the Task Force's recommendation at 250-500 Hz.1 However, there is evidence that 200 Hz is acceptable for HRV and BRS analysis, especially for healthy subjects.[46]

We were unable to provide reference values based on age and resting HR, even though they are both independent variables in HRV and BRS regression equations, due to limited sample size, especially in the oldest age group. Another limitation of our study is that the reference values are specific to the spontaneous 5-minute HRV recording and cross-correlation BRS method. While these methods are valuable and practical in clinical settings, further research is needed to establish reference values for 24-hour HRV recording and spectral analysis BRS, specifically for the Thai population.

CONCLUSION

Our study provides age-dependent reference values for spontaneous 5-minute HRV and cross-correlation BRS in healthy Thai adults, aged 20-72, along with an understanding of the factors influencing them. We found that HRV parameters, except LFnu and LF/HF ratio, as well as BRS, were independently and negatively correlated with age and resting HR, while sex did not show a significant association. Moreover, our results suggest that the decline in HRV with age may be partially attributed to age-related reductions in baroreflex function. The reference values and insights gained from our data analysis will serve as a foundation for the interpretation of HRV and BRS values in autonomic research and clinical practice.

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Conflict of interest statement

None to declare.

Abbreviations: %E, percent eosinophil; Adj. R2, adjusted coefficient of determination; AH, abnormally high; AL, abnormally low; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; BH, borderline high; BL, borderline low; BMI, body mass index; BP, blood pressure; bpm, beat per minute; BRS, baroreflex sensitivity; BUN, blood urea nitrogen; cal-LDL, calculated low-density lipoprotein cholesterol; CBC, complete blood count; DBP, diastolic blood pressure; dl, deciliter; ECG, electrocardiogram; FBG, fasting blood glucose; h, hour; Hb, hemoglobin; Hct, hematocrit; HDL, high-density lipoprotein cholesterol; HF, high frequency (0.15-0.4 Hz); HFnu, high frequency power in normalized unit; HR, heart rate; HRV, heart rate variability; Hz, Hertz; kg, kilogram; LF, low frequency (0.04-0.15 Hz); LFnu, low frequency power in normalized units; m2, square meter; MAP, mean arterial pressure; mg, milligram; mmHg, millimeter of mercury; ms, millisecond; N, neutrophil count; NN interval, the interval between two successive normal R waves; NN50, number of adjacent NN interval pairs with a difference greater than 50 ms; pNN50, proportion of NN50 to total NN intervals; r, Pearson's correlation coefficient; R2, coefficient of determination; RBC, red blood cell count; RespR, respiratory rate; RMSSD, root mean square of differences between adjacent NN intervals; RR interval, the interval between two successive R waves; SA node, sinoatrial node; SBP, systolic blood pressure; SD, standard deviation; SDNN, standard deviation of the NN interval; SDSD, standard deviation of differences between adjacent NN intervals; TG, triglyceride; VLF, very low frequency (≤0.4 Hz); WBC, white blood cell count

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