Heart Rate Variability and Oxidative Stress Markers in Healthy Thai Subjects Aged 20-50 Years

ความแปรปรวนของอัตราการเต้นของหัวใจ และตัวชี้วัดภาวะเครียดออกซิเดชัน ในอาสาสมัครไทยที่มีสุขภาพดี อายุระหว่าง 20-50 ปี

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ABSTRACT

Heart rate variability (HRV) is an important parameter to determine autonomic nervous function which was associated with cardiovascular disease (CVD). Oxidative stress also contributes to the development of CVD. This study aimed to compare HRV and oxidative stress markers between the genders of healthy Thai subjects. Twenty healthy subjects (male = 9, female = 11) were measured HRV (time and frequency domains) in supine and tilt position. Blood samples were collected to measure blood biochemistry and oxidative stress markers. Results show that HRV frequency domain parameters in supine positions, LF and the LF/HF ratio in female were lower than those of in male (P<00.5) while there was no significant difference between male and female in tilting position. Oxidative stress markers were not different between genders. Conclusion, autonomic nervous systems modulate cardiac function including, heart rate which is reflected by HRV. In this study LF/HF ratio of male was significantly higher than those of female. The oxidative stress markers were not difference between genders. However, the numbers of subjects are very small and need to be increased.

บทคัดย่อ

การวัดความแปรปรวนของอัตราการเต้นของหัวใจ (HRV) เป็นตัวชี้วัดการทำงานของระบบประสาทอัตโนวัติ ซึ่งมีความสัมพันธ์กับโรคหัวใจและหลอดเลือด ภาวะเครียดออกซิเดชันก่อให้เกิดการพัฒนาของโรคหัวใจและหลอดเลือด ด้วย วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อเปรียบเทียบ HRV และตัวชี้วัดภาวะเครียดออกซิเดชัน ระหว่างเพศหญิงกับเพศชาย ในคนไทยที่มีสุขภาพดี จำนวน 20 คน (ชาย 9 คน และ หญิง 11 คน) วัด HRV ทั้ง time domain และ frequency domain ในท่านอนและท่ายืน เก็บตัวอย่างเลือดเพื่อวัดชีวเคมีในเลือด และตัวชี้วัดภาวะเครียดออกซิเดชัน ผลการศึกษาพบว่า HRV ในท่านอนหงาย frequency domain คือ LF และ HF/LF ในเพศหญิงต่ำว่าเพศชาย (p<0.05) ในขณะที่ท่ายืนไม่มีความแตกต่างทางสถิติ และพบว่าตัวชี้วัดภาวะเครียดออกซิเดชันไม่มีความแตกต่างทางสถิติระหว่างเพศ ระบบประสาทอัตโนวัติปรับการทำงานของหัวใจซึ่งสะท้อนให้เห็นโดย HRV ในการศึกษานี้พบว่าค่า LF และ LF/HF สูงกว่าในเพศหญิง ตัวชี้วัดภาวะเครียดออกซิเดชันไม่มีความแตกต่างระหว่างเพศ แต่ในการศึกษานี้มีจำนวนอาสาสมัครน้อยและต้องมีการเพิ่มจำนวน

Key Words: Heart rate variability, Oxidative stress markers

คำสำคัญ: ความแปรปรวนของอัตราการเต้นของหัวใจ ตัวชี้วัดภาวะเครียดออกซิเดชัน

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Introduction

The autonomic nervous system (ANS) plays an important role to control cardiovascular function. Autonomic imbalance regarding to increased sympathetic and decreased parasympathetic activities has been strongly implicated in the pathophysiology of CVD such as arrhythmogenesis and sudden cardiac death (Sztajzel, 2004). It is well established that CVD is the leading cause of morbidity and mortality in both male and female worldwide.

Heart rate variability (HRV) is a noninvasive method and an important parameter of autonomic nervous function in human. There is evidence to show that reduced HRV as the decreased vagal tone activity associated with patients with heart failure, myocardial infarction. Thus, modifiable lifestyle-related risk factor of CVD has been reported to increased HRV that can prevent CVD (Thayer *et al.*, 2010).

It is well established that reactive oxygen species (ROS) strongly associate with the pathogenesis of CVD (Dhalla *et al.*, 2000). Several studies have reported that oxidative stress involved in the development of human diseases such as cancer (Valko *et al.*, 2006), cardiovascular disorders (Dhalla *et al.*, 2000), neurological disorders (Floyd, 1999), immunologic diseases (Bashir *et al.*, 1993), atherosclerosis, hypertension (Kasparova *et al.*, 2005), diabetes (Haidara *et al.*, 2006), acute respiratory distress syndrome, idiopathic pulmonary fibrosis (Lamb *et al.*, 1999), chronic obstructive pulmonary disease and asthma (Sugiura and Ichinose, 2008). This study, HRV and oxidative stress markers in healthy Thai subjects need to be revealed and compare the value between genders. These results will provide the baseline data for next study that the effect of the aerobic exercise on HRV and oxidative stress markers in healthy Thai subjects.

Objectives of the study

The aim of this study was to assess the heart rate variability and oxidative stress markers in Thais healthy aged 20-50 years.

Materials and Methods

Study designs and population

The study was analytical and descriptive approved by the Human Research Ethics Committee, Khon Kaen University (HE561451) and informed consent was obtained from each participant. Twenty healthy subjects of both genders (male = 9 and female = 11) aged between 20-50 years were recruited. The number of subjects was calculated according to a previous study (Amano *et al.*, 2001). All subjects were completed a confidential health-screening questionnaire. They were healthy with BMI of 18.5–24.9 kg/m^2 (WHO, 2000) with no history of regular alcohol drinking or smoking. Those having history of cardiovascular (i.e. coronary heart disease, arrhythmia and chronic heart failure), neuromuscular, arthritic, pulmonary, severe microvascular, diabetes mellitus, hypertension or other debilitating diseases were not included in this study.

Experimental Protocols

Participants were asked to have 2 visits to our Laboratory Unit. On the first visit, physical examinations and measurements of anthropometry and HRV were obtained. In the second visit (2 day later), blood collections for fasting glucose, lipid profiles and oxidative stress markers were performed.

Body mass index (BMI)

Height and weight were measured for each participant, according to the WHO guidelines (WHO, 2000). Participants wore light clothing without shoes. Weight was determined using a digital scale, to the nearest tenth. Height was measured standing with feet together and arms relaxed at the sides. The BMI was calculated as weight (kg) divided by height (m^2).

Heart rate variability

Participants were prepared for electrode placement to measure RR interval via a 3-lead EKG. After 10 minutes of rest in the supine position on tilt table (V.S. engineering, Thailand), the EKG was recorded for at least 5 minutes in the same position and then the tilt table was adjusted to 70 degree. The EKG (lead II) was digitally recorded continuously using a desktop computer and acknowledge data collection software (Biopac Systems, USA). Each signal was sampled at 1000 Hz throughout all testing. This program allowed for instantaneous analog to digital conversion of the EKG with recording stored for off-line analysis using a Lap chart 7 (ADInsruments PowerLab, Australia). In this study, HRV was measured by both frequency domain and time domain analysis. Files were imported to Sigma stat software program version 3.1 for descriptive analyses of HRV variables based on current recommendations. All resting HRV variables were calculated from the last 5 minutes of resting period.

Time domain analysis measures the changes in heart rate over time or the intervals between successive normal cardiac cycles (Camm, 1996). Standard deviation of all NN intervals (SDNN) and the square root of the mean of the sun of the squares of differences between adjacent NN intervals (RMSSD) represent total power and parasympathetic activity, respectively. Power spectral density was quantified in total power (the energy in the heart period power spectrum between 0-0.4 Hz); very low frequency (VLF) (the energy of the spectrum power below 0.04), which its physiological significance is obscure; low frequency (LF) (the energy of the spectrum power between 0.04-0.15 Hz), which indicates primarily sympathetic nervous system with minor influence from parasympathetic activity; high frequency (HF) (the energy of the spectrum power between 0.15-0.4 Hz), which reflects solely parasympathetic activity of cardiac function (Camm, 1996). The LF to HF ratio reflects relative sympathovagal balance. VLF, LF and HF were measured in normalized units [n.u. = (LF or HF)/(total power-VLF)]. The representation of LF and HF in n.u. emphasizes the controlled and balanced behavior of the sympathetic (SNS) and parasympathetic (PNS) branches of the ANS. Moreover, normalization tends to minimize the effect on the values of LF and HF components of the changes in total power. Nevertheless, as it is recommended that n.u. should always be quoted with absolute values of LF and HF power in order to describe in total the distribution of power in spectral components, this study, therefore present LF and HF in n.u.

Blood Chemistry

Blood samples were collected to analyze lipid profiles, fasting blood glucose (FBG). Blood chemistry was analyzed by Clinical Laboratory, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University.

Oxidative stress markers

Assay of plasma malondialdehyde

Plasma malondialdehyde (MDA) concentration was quantified as thiobarbituric acid reactive substance by a spectophotometric method as previously described (Khontong, 2012) with some modifications (Draper and Hadley, 1990). In brief, 150 µl plasma samples were reacted with 10% trichloroacetic acid, 5 µM EDTA, 8% sodium dodecyl sulfate and 0.5 μgml^{-1} of butylated hydroxytoluene. The mixture was incubated for 10 min at room temperature, then 0.6% thiobarbituric acid was added, and the mixture was boiled in a water bath for 30 min. After cooling to room temperature, the mixture was centrifuged at 10000 g for 5 min. The absorbance of the supernatant was measured at 532 nm by spectrophotometer. A standard curve was generated with appropriate concentrations of 1, 1, 3, 3-tetraethoxypropane (0.3–10 $\mu moll^{-1}$).

Assay of glutathione

Assay of total glutathione in the whole blood was performed as previously described (Somparn *et al.*, 2007) (Tietze, 1969) and glutathione disulfide was assayed by a previously described method with some modifications (Saowanee Nakmareong and 2012) Briefly, 100 µl of whole blood was immediately reacted with 33 mM 1-methyl-2 vinyl-pyridinum trifate or distilled water and subsequently treated with 5% cold metaphosphoric acid. Optical density at 412 nm was read 10 times at 15s intervals by using a spectrophotometer (Biochrom, Cambridge, UK). A standard curve was generated by using appropriate concentrations of standard GSH. The redox ratio was calculated as GSH/glutathione disulfide (GSH/GSSG).

Statistical Analyses

Data were expressed as means ± SD. Unpaired t-test was used to compare differences in characteristics and all parameters between male and female. Two-sample Wilcoxon rank-sum (Mann-Whitney) test was used when data deviate from normality. A value of $p<0.05$ was taken to be the threshold of statistical significance.

Results

Baseline value

The characteristics of all subjects are shown in table 1. There was no significant difference in age, BMI, blood glucose, fasting blood glucose, lipid profiles and blood pressure between male and female. Body weight in male was higher than those of in female *(P<0.05)*.

Table 1 Characteristics, anthropometric and the blood biochemistry profiles of all participants**.**

Parameters	Male (N= 9)	Female (N=11)	P *value*
Age (years)	27.22 ± 6.02	32.78 ± 6.65	0.082
Body weight (kg)	56.90 ± 7.34	64.73 ± 5.65	0.022*
BMI (kg/m^2)	21.64 ± 1.48	22.59 ± 2.12	0.281
FBG (mg/dL)	88.875 ± 8.35	91.67 ± 6.52	0.452
TC (mg/dL)	185.77 ± 22.75	209.44 ± 35.26	0.110
LDL-C (mg/dL)	114.44 ± 25.46	140.33 ± 51.93	0.198
HDL-C mg/dL)	60.77 ± 15.04	67.33 ± 13.40	0.344
TG (mg/dL)	83.88 ± 39.05	93.78 ± 45.93	0.629
SBP (mmHg)	118 ± 7.87	115 ± 11.20	0.546
DBP (mmHg)	71.22 ± 7.34	75.67 ± 8.45	0.254
PP (mmHg)	46.7 ± 8.30	39.33 ± 7.30	0.060
MAP (mmHg)	86.81 ± 6.48	88.78 ± 8.87	0.698

All data are presented as mean ± SD. BMI, body mass index; FBG, fasting blood glucose; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein; TG, triglyceride; SBP systolic pressure; DBP, diastolic pressure; MAP, mean arterial pressure; PP, pulse pressure**.**

Heart rate variability

In supine position, there was no significant difference of time domain between genders. However, frequency domain, LF and LF/HF ratio were significantly high in male comparing to female (p<0.05), HR and HRV time domain in (tilting position) did not differ between genders (table 2).

Table 2 Comparison of HRV parameters between male and female during supine and tilt positions.

Position		male(N= 9)	Female(N= 11)	P value
Supine	HR (bpm)	66.44 ± 7.6	71.20 ± 5.43	0.14
	Time domain			
	SDNN (ms)	53.10 ± 21.62	46.17 ± 15.81	0.44
	RMSSD(ms)	40.64 ± 19.98	44.49 ± 25.99	0.72
	Frequency domain			
	LF (n.u.)	46.82 ± 11.89	31.06 ± 14.25	0.02*
	HF (n.u.)	46.48 ±1 4.42	56.22 ± 8.15	0.09
	LF/HF ratio	1.08 ± 0.56	0.58 ± 0.30	0.03*
Tilt	HR (bpm)	85.08 ± 7.36	86.82 ± 8.70	0.77
	Time domain			
	SDNN (ms)	40.92 ± 14.10	38.61 ± 21.40	0.33
	RMSSD(ms)	28.17 ± 22.68	32.22 ± 33.92	0.93
	Frequency domain			
	LF (n.u.)	63.48 ± 21.17	48.78 ± 27.29	0.22
	HF (n.u.)	31.13 ± 16.02	37.15 ± 21.58	0.512
	LF/HF ratio	3.88 ± 4.77	2.16 ± 2.06	0.770

Data are expressed as mean ± SD; SDNN, standard deviation of all NN intervals; RMSSD, square root of the mean of the sum of the squares of differences between adjacent NN interval; low frequency power (LF); high frequency power (HF); LF/HF ratio. *p<0.05 male versus female

Oxidative stress markers

There were no significant difference of the levels of plasma MDA, GSH and GSH/GSSG in whole blood between male and female as shown in table 3.

Table 3 Oxidative stress markers in male and female.

Parameter	Male(N= 9)	Female(N= 11)	P value
MDA	4.48 ± 0.54	6.23 ± 1.28	0.139
GSH	633.7 ± 60.8	664.1 ± 57.1	0.72
GSH/GSSG	66.1 ± 15.9	51.2 ± 21.6	0.58

Data are expressed as mean ± SD; MDA, plasma malondialdehyde; GSH, glutathione; GSH/GSSG ratio

Discussion

Heart rate variability

HRV frequency domain parameters, LF and LF/HF ratio were significantly high in male indicating higher cardiac sympathetic activity in male. The results were consistent with the other previous studies that female had low HRV comparing to those of in male. It was found that total power, very low-frequency power, LF and LF/HF ratio were lower in female comparing to male (Aronson and Burger, 2000; Jensen-Urstad *et al.*, 1997). They suggested that genders may have differences of HRV under normal physiological condition. This might be related to the tentative of CVD development among genders. It has been reported that the increase of sympathetic nerve activity in men at rest was related to the increase in sympathetic nerve activity in muscle and the high level of plasma norepinephrine in men (Barnett *et al.*, 1999; Ng *et al.*, 1993).

Oxidative stress markers

Plasma MDA

MDA is one of the most frequently used indicators of lipid peroxidation. Previous study reported that plasma MDA in men and women healthy Thai volunteers, 70 volunteers aged ranging 40-70 years are 2.20 ± 0.012 µM and 2.42 ± 1.1 µM, respectively (Khontong, 2012). MDA level found in this study was quite high comparing to the previous

study. It is probably due to the difference in numbers and age of study subjects.

Glutathione

Glutathione is an antioxidant which presented in the cells in both the reduced (GSH) and oxidized (GSSG) forms. Numerous studies have suggested that GSH is a critical factor in protecting organisms against toxicity and disease (Richie *et al.*, 1996; Russo *et al.*, 1986). GSH/GSSG ratio is a major determinant of oxidative stress (Kloek *et al.*, 2010). Pathophysiologic conditions causing oxidative stress would result a decreased GSH/GSSG ratios (Shaik and Mehvar, 2006). The levels of GSH and GSH/GSSG in male and female were comparable and within normal ranges in this study.

Conclusions

In healthy Thai subject aged 20-50 years, there were no significant difference of HR, HRV (time domain) while LF and LF/HF ratio in supine position was significantly high in male, indicating the dominant of sympathetic nerve function in male. Furthermore, the oxidative stress status did not differ between genders. All parameters observed in this present study were in normal ranges. Further study in a larger number of subjects is needed. Moreover, the effect of the aerobic exercise on HRV and oxidative stress markers will be of interest for evaluation.

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