CHAPTER III

MATERIALS AND METHODS

1. Research design

The design of the study can be classified as observational study with cross-sectional analytical method. The local ethics committee, Faculty of Medicine Chulalongkorn University, approved the study protocol.

2. Subject

This study comprised three groups of young healthy male volunteers, namely endurance trained athletes (n=20, age 20-25 years), resistance trained athletes, (n=20, age 20-25 years) and sedentary subject (n=20, age 20-25 years). None of these subjects had any diseases or was taking any medication. The subjects and their guardians provided in-formed consent. Endurance trained athletes (ETA) were selected from long-distance runners in The Amateur Athletic Association of Thailand and long-distance runner in the Ramkhumheang University who had trained (15-30 km.d, 6d.wk) for at least 3 years and participated in national field competitions. Resistance trained athletes (RTA) were selected from weightlifter in The Amateur Weightlifter Association of Thailand who had trained (2-4 h.d, 6 d.wk) for at least 3 years and participated in national field competitions. Sedentary subjects (SS) were selected from students in the Chulalongkorn University and none of them had been engaged in any type of regular long-term physical training for last 3 years before participation in this study.

Exclusion criteria included current smoking, habitual consumption of caffeine and alcohol, hypertension, long-term use of any medication, obesity, psychiatric diagnose, any metabolic or cardiopulmonary disease that could affect the autonomic nervous function.

2. Equipments

The following equipments were used for determination of physical fitness characteristics, which included body height, weight, percent body fat, systolic and diastolic arterial blood pressure, maximum oxygen consumption, cardiac stress test and for determination of cardiac autonomic control.

2.1 Physical healthy fitness

- Stethoscope and sphygmomanometer (AII-K2,Japan)
- Weight balance (Yamato DP-6100GP,Japan)
- A scale for height
- Skinfold caliper (Lang, USA)
- Oxygen and carbondioxide gas analyzer (Quinton Metabolic Cart, QMC, USA)
- Cardiac stress testing equipment (Quinton instrument CO, Q4500, USA)
- Cycle ergometer (Corival 400, USA)

2.2 Cardiac autonomic control testing

- Electrocardiogram amplifier module (ECG100A)
- Universal interface module (UIM100)
- Computer (Atec PC)
- Software analysis (BIOPAC system Acknowledge 3.2)
- Cycle ergometer (Cateye ergociser, Japan)

3. General Procedure

3.1 Health history and informed consent

Each subject was informed about the purpose and the possible risks and benefit of this study prior to giving his consent. General history of each subject was obtained from questionnaire in which medical history, injury, daily activities, physical training and past illness were included.

3.2 Vital sign at rest

Each subject reported to laboratory in the morning. Heart rate, Systolic and diastolic arterial pressure were measured at rest with stethoscope and sphygmomanometer.

3.3 Antropometric measurement

Body weight and height of subject were measured by using a weight balance (Yamato DP6100GP,Japan). Percent body fat was determined as previously described by Watson. The thickness of skin fold at the following sites of the body was measured by using a skinfold caliper (Lange, USA):

- Biceps: over the mid point of the muscle belly with arm resting, supinated, and vertically relax.
- Triceps: over the mid point of the muscle belly between the olecranon process, with the arm hang vertically.
- Subscapular: just below the tip of the inferior angle of the scapular at the angle of about 45 degree to the vertical.
- Suprailiac: just above the iliac crest in the mid axillary line.

The thickness measurement at each site was repeated three times per site and the average value was used.

Body density and percent body fat were calculated according to the equations of Durnin and Rhamana

For subjects age > 20 years

Body density = 1.1631 – 0.0632 log S where S in the sum of four skin fold reading (in mm.)

Percent body fat was calculated from the body density using Siri's equation.

Body fat (%) = (4.95/D – 4.5) x 100

Where D is body density

3.4 Heart rate monitors and electrocardiogram (ECG) recording

3.4.1 Resting 12 – Lead ECG

A resting 12 – lead electrocardiogram (ECG) was measured during the supine rest before the exercise test to detect resting arrhythmias, heart block, or evidence of myocardial ischemia or infarction. To measure the standard 12 leads ECG, 10 electrodes were used. The electrodes for the three limb leads (I, II, and III), three augmented unipolar leads were placed on the right clavicle, left clavicle, and left iliac crest on midaxillary line. A ground electrode was placed on the right iliac crest on midaxillary line.

The six chest electrodes (V1 to V6) measure the voltage across a specific area of the chest, with the average voltage across the other three leads. Figure 8 illustrates electrode placement for the chest leads, V1: on right sternal border in 4^{th} intercostals space, V2: on left sternal border in 4^{th} intercostals space, V3: at midpoint of a stright line between V2 and V4, V4: on midclavicular line in 5^{th} intercostals space, V5: on anterior axillary line and horizontal to V4 and V5, and V6: on midaxillary line and horizontal to V4 and V5. During the resting ECG recording, the client should lie quietly in a supine position on a table. The electrode sites should be shaved, if hair is present, and cleaned with alcohol. Remove the superficial layer of skin at each site by rubbing it with fine grain emery paper or a gauze pad. Disposable electrodes contain electrode gel and adhesive disks. After applying the electrode, tap it firmly to test for noisy leads. Calibrate has been performed the ECG recorder prior to use by recording the standard 1mV deflection per centimeter. To standardize the time base for the ECG, set the paper speed was set to 25 mm per second.

3.4.2 Twelve-lead exercise electrocardiogram (ECG)

A twelve-lead exercise electrocardiogram (ECG) was measured during the incremental exercise test to detect myocardial ishemia and infarction.

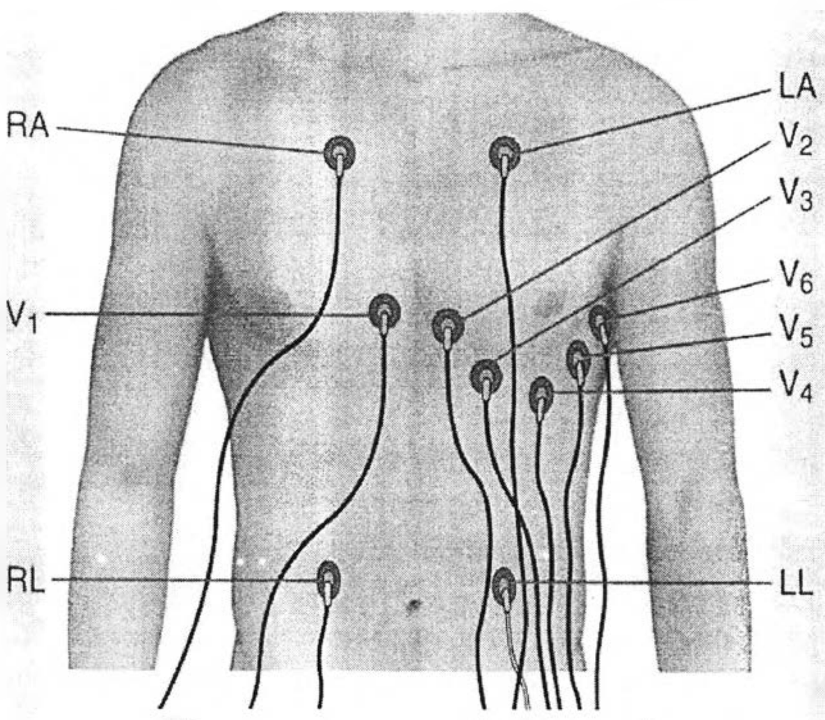

Fig 8 illustrates electrode placement for the chest leads

3.5 Incremental exercise test

Incremental exercise test was performed with each subject on a calibrated cycle ergometer (Corival 400, USA) to determine maximum oxygen consumption rate (VO_2max). The height of the saddle of the ergometer was adjusted to be appropriate for individual subjects. The subject was requested to maintain a pedaling frequency of 60 pedal revolutions per minute. VO_2max test was started with 3 min of load less pedaling. Thereafter the power output was subsequently increased by 25 watts every minute until the subject could not maintain pedaling at frequency of 60 rpm or reached volition fatigue.

3.5.1 Gas exchange measurement

Maximum oxygen consumption (VO_2max) was measured during the incremental exercise test. Oxygen consumption (VO_2), CO_2 production (VCO_2), minute ventilation (VE), and other derived parameters were continuously monitored breath-by-breath using a computerized system (Quinton Metabolic Cart, QMC, USA). Data on VO_2 and VCO_2 were expressed in a standard condition of standard temperature pressure dry (STPD) and VE in the condition of body temperature pressure saturated with water vapor (BTPS).

3.5.2 Criteria of obtaining VO_2max

One or more of the following defined the level of exercise intensity at which VO2 reaches maximum.

1) a plateau of oxygen consumption (an increment of less than 150 ml. O_2 min $^{-1}$ with a further increase in work rate)
2) a heart rate close to the individuals age-related maximum (220-age) $\pm$10 beats/min.
3) a respiratory gas exchange ratio (R) value greater than 1.1

4) sign of exertional intolerance (fatigue) and an inability to maintain the required pedal rhythm.

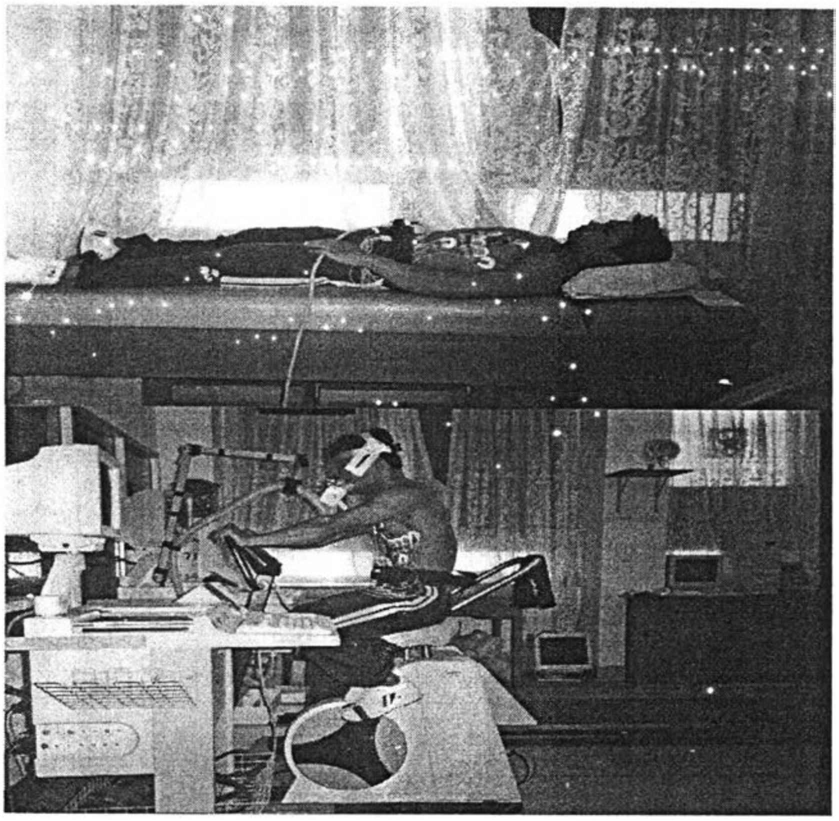

Figure 9 illustrates heart rate monitors and electrocardiogram (ECG) recording

3.6 Cardiac autonomic control test

Cardiac autonomic control tests were performed at least two-day after the incremental exercise test. Each subject reported to laboratory in the morning (9.30 am.) at least 2 hours after breakfast. The subjects were instructed to perform no strenuous exercise and abstinence from caffeine on the day of the experiment. Continuous ECG recordings were obtained during the following physiological maneuvers: 5 min sitting rest state and 5 min steady state exercise at 50% maximum oxygen consumption. Three electrodes were firmly attached to the right clavicle, left clavicle, and left iliac crest on midaxillary line. Care being taken to ensure adequate R wave signals for data processing while avoiding DC interference and minimizing motion artifacts. Subjects were first asked to rest comfortably in chair for 30 min. The room was quiet, in semi-darkness, and maintained at a constant temperature of 23-24'C. Care was taken to avoid repetitive external auditory or visual stimuli at frequencies below 1 Hz. Subjects were asked to remain awake and relaxed during this steady state phase (5 min) of the study. No attempt was made to influence the pattern, depth, or rate of respiration during any recording session.

Subject then performed steady state exercise at 50% of maximum oxygen consumption while sitting upright on a mechanically braked cycle ergometer (Cateye ergociser, Japan). Steady state exercise was achieved when heart rate varied less than five beats.min^{-1} throughout the 5 min exercise. It was only then (approximately 5 min from the start of exercise) that continuous ECG signals were recorded for power spectrum analysis. The total duration of this steady state portion of exercise was about 10 min.

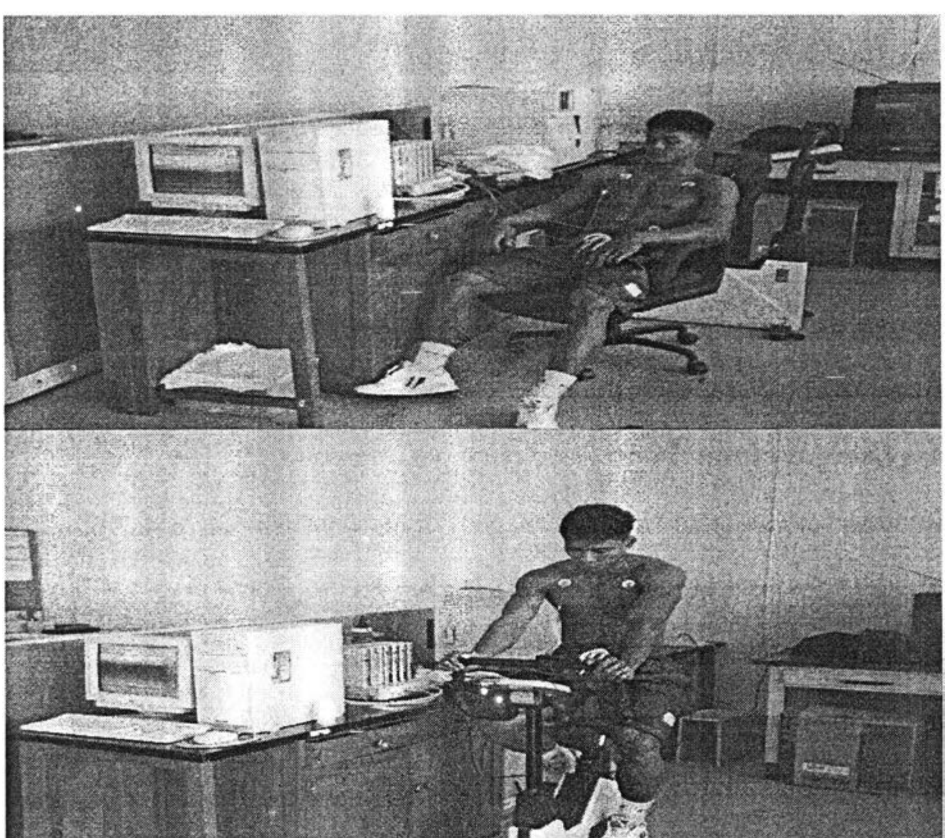

Fig 10 illustrates cardiac autonomic control test

Signal processing and power spectral analysis

The ECG signals were digitized using a 16-bit analogue to digital convert at 1 kHz. A peak detection algorithm for locating the R wave implemented in the software as follows. If the amplitudes of the ECG R wave exceeded a preset threshold, the presence of the peak was indicated. Then the amplitudes of the successive ECG samples were serially compared, to locate precisely in time a voltage maximum in the QRS complex, ie, R wave peak. The threshold was set so as to exceed the noise level and the peak amplitudes of the P,S and T wave in the ECG waveform. Thus an R-R interval series was formed from the continuous samples of the ECG data. Figure 16 shows a schematic example of the simultaneous analysis of R-R interval variability. From the ECG signal a derivative/threshold algorithm, using as a fiducial point the peak value of the R wave. Provided the continuous series of R-R interval (tachogram). To improve the accuracy of R peak detection, a parabolic interpolation was incorporated in the program.

Heart rate variability is a point event series. However, power spectral analysis requires as follows. A beat-to-beat heart rate series computed from the successive R-R intervals and the resulting heart rate was resample using linear interpolation to obtain an equally sampling time series. Computations were performed on 512 sample points, each separated by 0.5 s, thus yielding individual records of 5 min duration. These records were selected for power spectrum analysis. Because the mean value in the time domain series possesses no intrinsic oscillating properties and because of the small variance in R-R interval, the mean value for the R-R interval series was removed. The power spectrum of R-R interval variability was using a fast Fourier transform (FFT) and then calculated the power in the frequency band of 0.04-0.05 Hz, to determine low frequency (LF) power, represent sympathetic and parasympathetic activity, while 0.15 to 0.40 Hz, to determine high frequency (HF) power, a measure of parasympathetic modulation of sinus node depolarization.

4. Statistical analysis

SPSS for window program was used for calculation in this study. The data were expressed as means and standard deviation (SD). Characteristics of subjects, HR, BP, Wt, Ht, and % fat and time and frequency domain were compared using one-way analysis of variance (ANOVA) Posthoc test. All values were reported significantly different at $P<0.05$ levels.

5. Observational design