CHAPTER III
METERIALS AND METHODS

This Chapter describes the methodology in this study. It also provides details of ethical approval, volunteer recruitment, protocol and statistical analysis.

1. Study design

The present research is an experimental study.

2. Subjects

2.1 Ethical approval

Before participation in this study, all subjects were informed of the experimental protocol and possible risks involved. They were informed verbally and in writing before signing the consent form to participate in an experiment. Then the consent forms were signed by the investigator involved. A consent form is approved by the Ethical Committee of Khon Kaen University in accordance with the 1964 Declaration of Helsinki (HE510731).

2.2 Volunteer Recruitment

All subjects were informed about the nature and risks of the experimental procedures, before their inform consent to participate are obtained.

Before participation in this study, all subjects participate in a routine medical examination in which a medical history by completion of a health-risk questionnaire, ECG, anthropometric measurements (body mass, height, body composition from skinfold thickness) and physical measurement (BP and HR). A blood sample for routine blood chemistry was also collected.

2.3 Study population

Fifty subjects, 34 men and 16 women aged between 30 and 75 years were recruited. All patients with suspected CAD performed exercise testing during

March 2009 to February 2010 at Queen Sirikit Heart Center of the Northeast Hospital, Khon Kaen University. All subjects were recruited by the following criteria;

Inclusion criteria

1. Men or women aged between 30 and 75 years
2. Thai patients who were clinically suspected of having CAD

Exclusion criteria (Gibbons et al., 2002)

1. Acute myocardial infarction (within 2 days)
2. High-risk unstable angina
3. Uncontrolled cardiac arrythmias causing symptoms or hemodynamic compromise
4. Symptomatic severe aortic stenosis
5. Uncontrolled symptomatic heart failure
6. Acute pulmonary embolus or pulmonary infarction
7. Acute myocarditis or pericarditis
8. Acute aortic dissection

2.4 Sample size

The sample size according to calculation by one sample comparison of mean to hypothesized value by WINPEPI PROGRAMS DESCRIBE MANUAL (Version 1.92) © J.H. Abramson (Revised October 14, 2008)

3. Experimental protocol

All subjects were measured anthropometry (height, weight, waist and hip circumference) and body composition (fat mass and fat free mass), blood lipid profiles (HDL, LDL, TG, TC). Then they performed an EST on a treadmill (TM55 Ultradrive digital treadmill) according to the Bruce protocol. The 12 lead ECG, HR and BP were measured throughout this study. All subjects were measured $\dot{V}O_{2,max}$ during the exercise period. Expired gas was continuously collected and analyzed during the EST by using gas analysis system (AD instrument, ML206, Australia). The highest oxygen uptake value ($\dot{V}O_{2,max}$) of the subject were determined when any of

because of some symptom e.g. chest pain and fatigue or 2) the HR reached 85% of maximal HR (220-age). $\dot{V}O_{2,max}$ of this study were calculated from expired gas and Bruce protocol formula. After the EST if subject is diagnosed as positive EST, they were confirmed by other tests. Finally, In Queen Sirikit heart center of the Northeast, Coronary angiography (CAG) was used to determine CAD. CAG was performed in the patients who had an EST positive for evaluate coronary artery anatomy and coronary artery stenosis. Therefore, the test was considered positive when an epicardial coronary artery stenosis greater than 50% was detected (Oliveira et al., 2007).

4. Outcome

4.1 Anthropometry

Height (cm)

Subjects will be in a free-standing position with the feet together and heels, buttocks and upper parts of the back touching the scale of balance scale. The head was not touch the scale.

Body mass (kg)

Subjects in minimal clothing were stand still on spring balance without support, with the weight evenly on both feet over the centre of the scales.

Height and body mass were then used to calculate body mass index (BMI) using the formula shown below;

$$BMI = \text{Body mass (kg)} / \text{Height (m)}^2$$

Waist and hip circumferences (cm)

Waist circumference (W) was measured at the narrowest part of the torso and hip circumference (H) was measured around the buttocks at the level of maximal extension in a free-standing position. Waist and hip circumferences were used to calculate waist to hip ratio (waist:hip ratio) using the formula shown below;

$$\text{Waist:hip ratio (W/H ratio)} = \text{W (cm)} / \text{H (cm)}$$

4.2 Body composition

Percentage of body fat (%BF)

Procedure

Fat mass was measured indirectly by skinfold thickness. Subjects were sit on a chair for the arm and shoulder (subscapular) measurements and stand for the suprailiac measurement. Measurements were made on the right side of the body. The sites that were measured in both male and female subjects were as follow.

1. Biceps: over the mid-point of the muscle belly with the arm resting supinated on the subject's thigh.

2. Triceps: over the mid-point of the muscle belly, midway between the olecranon process and the tip of the acromion, with the upper arm hanging vertically.

3. Subscapular: just above the tip of the inferior angle of the scapula, at an angle of about 45° to the vertical.

4. Suprailiac: just above the iliac crest in the mid-axillary line

At each site, the skinfold was pinched up firmly between the thumb and forefinger and pulled away slightly from the underlying tissues before applying the calipers for the measurement.

The instrument used was the Harpenden skinfold calipers (British Indicators Ltd, St Albans, Herts.), which exert a constant pressure at the varying openings of the jaws. The width of the opening was read off on a scale incorporated in the apparatus.

The sum of the four sites were then used, in combination with age and sex to obtain % BF from the table provided by the British Indicators Ltd (Durnin and Womersley, 1974).

Many body composition equations derive their measure of percent body fat from first determining body density. Once body density is determined, %BF can be calculated using the Siri equation below (Siri, 1961);

$$\%BF = [(4.95/\text{body density}) - 4.5] \times 100$$

4.3 BP

Subject's BP (mmHg) was measured in a supine on a bed at rest, every 3 min while exercise period until stop protocol with the cuff wrapped around the left upper arm by automatic sphygmomanometer. Systolic (SBP) and diastolic (DBP) blood pressure were used to calculate as mean arterial pressure (MAP) following the formula shown below:

$$MAP = DBP + 1/3 (SBP - DBP)$$

4.4 HR

HR was measured and monitored throughout the exercise periods by an oscilloscope monitor.

4.5 ECG

Cardiac function was assessed by a 12-lead surface ECG (Q-Stress exercise test v 3.5).

4.6 The EST

Procedure

The EST was carried out on a Quinton® treadmill system (TM55 Ultradrive digital treadmill) according to the Bruce protocol. The EST on a treadmill was monitored HR, BP and 12-lead ECG during the test. The subject's BP is usually recorded during the second minute of each stage. HR and 12-lead ECG were measured throughout exercise periods. However, BP may be recorded more frequently if the readings are too high or too low. The physician was determined to stop protocol when the HR reached 85% of maximal HR (220-age), or following the indication shown below (Ellestad, 2003; Gibbons et al., 2002).

Absolute indications for termination of EST

1. Drop in SBP of greater than 10 mmHg from baseline BP, despite an increase in workload, when accompanied by other evidence of ischemia

2. Moderate to severe angina

3. Increasing nervous system symptoms (for example; ataxia, dizziness, near-syncope)

4. Signs of poor perfusion (cyanosis or pallor)

5. Technical difficulties in monitoring ECG tracings or SBP

6. Subject desire to stop

7. Sustained ventricular tachycardia

8. ST elevation (>1 mm) in leads without diagnostic Q waves (other than V_1 or aVR)

Relative indications for terminating EST (The following indications may be superseded if done so far good clinical reasons)

1. Drop in SBP greater than or equal to 10 mmHg from baseline BP, despite an increase in workload, in the absence of other evidence of ischemia

2. ST or QRS changes such as excessive ST depression (>2 mm of horizontal or down-sloping ST segment depression) or marked axis shift.

3. Arrhythmias other than sustained ventricular tachycardia including multifocal premature ventricular contractions (PVCs), triplets of PVCs, supraventricular tachycardia, heart block, or bradyarrhythmias

4. Fatigue, shortness of breath, wheezing, leg cramps, or claudication

5. Development of bundle branch block or intraventricular conduction delay that cannot be distinguished from ventricular tachycardia

6. Increasing chest pain

7. Hypertensive response

Clinical symptoms during the test are determined "positive" when they meet the following criteria: 1) the ischemic ECG changes develop in the first 3 minutes of exercise or persist 5 minutes after exercise has stopped, 2) the magnitude of the ST segment depression is $\geq$ 2 mm, 3) the SBP decreases during exercise, 4) high grade ventricular arrhythmia develop, or 5) the subjects cannot exercise for at least 2 minutes because of cardiopulmonary limitations. Subjects with no symptom,

leg fatigue, musculoskeletal pain or shortness of breath were determined "negative" (Lipton et al., 2008). If results of the EST represent to symptom of positive, Subjects were visit again. The physician may recommend more tests to predict severity of CAD.

4.7 $\dot{V}O_{2,max}$ calculated from expired gas

Each subject was performed EST on a treadmill (TM55 Ultradrive digital treadmill) according to the Bruce protocol. Expired-air, ECG, BP, and HR were recorded at rest and during the test. The highest oxygen uptake value ($\dot{V}O_{2,max}$) of the subject were determined when any of the following criteria is achieved: 1) the subjects can not perform the test because of some symptom e.g. chest pain and fatigue or 2) the HR reached 85% of maximal HR (220-age). After the test $\dot{V}O_2$ and HR at each time point were used to determine $\dot{V}O_{2,max}$. The $\dot{V}O_2$ and HR equation at 85% of maximal HR (220-age) to estimate $\dot{V}O_{2,max}$ from expired gas. After the subjects reached to the criteria they rested, and were measured their HR and BP until returned to normal.

4.8 $\dot{V}O_{2,max}$ calculated from Bruce protocol formula

In Queen Sirikit heart center of the Northeast, Bruce protocol was used to determine CAD. $\dot{V}O_{2,max}$ was calculated from the Bruce protocol formula (Bruce et al., 1973) (shown below).

For Men;

$$\dot{V}O_{2,max} = 14.8 - (1.379 \times T) + (0.451 \times T^2) - (0.012 \times T^3)$$

For Women;

$$\dot{V}O_{2,max} = 4.38 \times T - 3.9$$

T = Total time on the treadmill measured as a fraction of a minute

4.9 Levels of aerobic capacity

Levels of aerobic capacity were divided using the $\dot{V}O_{2,max}$ norms in Thai population. The $\dot{V}O_{2,max}$ norms in Thai population was then used, in combination with age and sex (Sport science institute, 2000).

Table 1 Level of aerobic capacity determined by $\dot{V}O_{2,max}$ values (ml/kg/min) in men

Level of aerobic capacity	**Men (years)**					
	17-19	**20-29**	**30-39**	**40-49**	**50-59**	**60-72**
Excellent	>55.5	>51.6	>43.3	>37.4	.>33.9	>30.7
Good	50.6-55.4	47.1-51.5	39.4-43.2	34.1-37.3	30.7-33.8	27.9-30.6
Fair	40.7-50.5	38.0-47.0	31.5-39.3	27.4-34.0	24.2-30.6	22.2-27.8
Poor	35.8-40.6	33.5-37.9	27.6-31.4	24.1-27.3	21.0-24.1	19.4-22.1
Very poor	<35.7	<33.4	<27.5	<24.0	<20.9	<19.3

Table 2 Level of aerobic capacity determined by $\dot{V}O_{2,max}$ values (ml/kg/min) in women

Level of aerobic capacity	**Women (years)**					
	17-19	**20-29**	**30-39**	**40-49**	**50-59**	**60-72**
Excellent	>48.0	>45.8	>40.2	>35.8	>30.9	>30.8
Good	43.9-47.9	41.9-45.7	36.9-40.1	32.4-35.7	28.3-30.8	27.8-30.7
Fair	35.6-43.8	34.0-41.8	28.7-36.8	25.5-32.3	23.0-28.2	21.7-27.7
Poor	31.5-35.5	30.1-33.9	24.9-28.6	22.1-25.4	20.4-22.9	18.7-21.6
Very poor	<31.4	<30.0	<24.8	<22.0	<20.3	<18.6

4.10 Percentage of heart rate reserve (% heart rate reserve)

Peak HR, resting HR, maximal HR (220-age) were used to calculate heart rate reserve using the formula shown below (Wilkoff and Miller, 1992);

$$\% \text{ heart rate reserve} = (\text{peak HR} - \text{resting HR}) / (\text{maximal HR} - \text{resting HR}) \times 100$$

4.11 HRR at 1 minute

Immediately following $\dot{V}O_{2,max}$ test subjects were placed in the sitting position. HRR was defined as the difference between peak HR during exercise and one minute after the cessation of test, following the formula shown below;

$$\text{HRR} = \text{peak HR} - \text{HR at 1 minute after the cessation of the test}$$

4.12 Blood chemistry

Blood samples were collected after overnight fasting for 12 hours to measure lipid profiles (HDL, LDL, TG, TC). Blood samples were obtained from an antecubital vein with the subject in a seated position after overnight fasting. These samples were measured using the standard automated laboratory methods by the Queen Sirikit heart center of the Northeast, Khon Kaen University.

5. Statistical analysis

All data were presented as the mean and the standard deviation (mean ± SD). The baseline of subject characteristics was used to a descriptive statistics test. Data were analyzed using unpaired Student's t-test as appropriate. For correlation studies, Pearson's correlation test was used to between parameters. Furthermore, Mann-Whitney test when data showed departure from normality was used. If the statistical probability (p-value) was less than 0.05, the differences were considered to be statistical significance.

6. Research place

Queen Sirikit heart center of the Northeast, Khon Kaen University and Department of Physiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.