CHAPTER III

MATERIALS AND METHODS

A. MATERIALS

1. A weighing scale (Yamato DP-6100GP, Japan)
2. A scale for height
3. Cardiotachmeter (Polar Sport Tester; Polar Electro Oy FIN-90440, Finland)
4. Oxygen and carbon dioxide gas analyzer (Quinton Metabolic Cart, QMC, USA)
5. Cardiac stress testing equipment (Quinton Instrument CO, Q 4500, USA)
6. Bicycle ergometer (CORIVAL 400)
7. Stop watch
8. L-Lactate analyzer (Yellow Springs Instrument CO, YSI 1500 Sport, USA)
   - Capillary injecter (25 ul)
   - Capillary tube package, 25 ul (100 ea)
   - Cell Lysing Agent (Lactate, 8 x 500 ml pks)
   - L-Lactate standard, 5.0 mM (125 ml)
   - L-Lactate membrane kit (pkg of 4)
   - Buffer kit (8 x 500 ml envelops)

B. METHODS

1. Subjects

The study was conducted on 57 male athletes (15 basketball, 11 football, 5 rugby, 15 athletic, 7 teakwondo, 3 swimming, 1 polo).

1.1. The inclusion criteria

1.1.1. Thai male athletes using the aerobic and anaerobic such as basketball, football, rugby, athletic, teakwondo, swimming, and polo.

1.1.2. Age between18-28 years old.

1.1.3. Continually experienced training for at least 2 years and a training period of at least 4 days a week.

1.1.4. Signed informed consent

1.2. The exclusion criteria

1.2.1. Regular medicine taken is needed (depending on drug).

1.2.2. Muscle injury surrounding legs or knee.

1.2.3. Regular drinker of coffee or alcohol.

1.2.4. Cardiac arrhythmia

1.2.5. Hypertension

1.2.6. Do not have enough sleep on the testing day.

1.2.7. Have physical or mental illness during the test.

2. General Procedure

2.1 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. All subjects gave written consent after being informed about the nature and purpose of the study. The study procedure was approved by the local ethics committee Faculty of Medicine, Chulalongkorn University.

2.2 Vital sign at rest

The subjects were instructed to standardize their diets for 24 hours and their training for 48 hours before each test. No more than light training was permitted 24 hours before each test. Each subject reported to laboratory in the afternoon. Heart rate, lactic acid concentration were measured at rest with heart rate monitor (Polar Sport Tester; Polar Electro Oy FIN-90440, Finland) and lactate analyzer (YSI 1500 Sport, USA).

2.3 Anthropometric Measurement

The measurements of weight and height were carried out by the same investigator. Body weight was measured to the nearest 0.02 kg on digital platform scale (Yamato DP-6100GP, Japan).

3. Experimental Design

Each subject performed an incremental exercise test on an electromagnetically braked cycle ergometer (CORIVAL 400), in an air-conditioned laboratory in order to maintain a constant degree of temperature and humidity throughout the experiment. The heart rate (HR) was recorded by a telemetry system (Polar Sport Tester; Polar Electro Oy FIN-90440, Finland) (Figure 3.1).

Before the test, a Teflon catheter No.22 was placed in an antecubital vein to enable serial blood sampling throughout post-exercise recovery period, and clotting was prevented by back flushing the catheter with 0.9% normal saline solution (Figure 3.2).

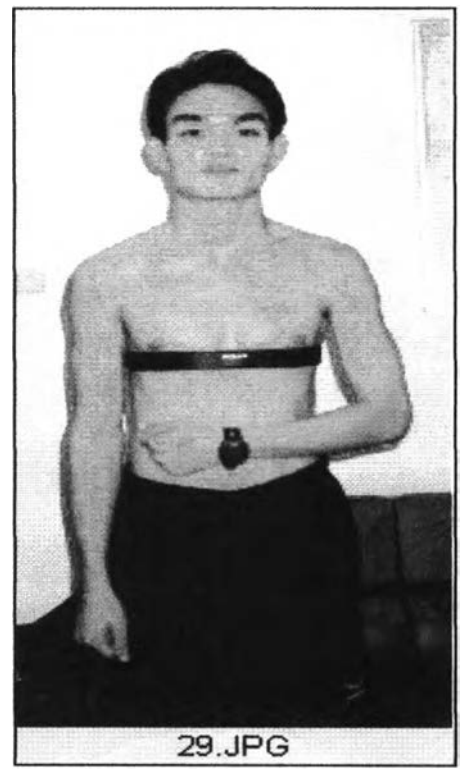


Figure 3.1. Subject is wearing Polar Sport Tester in order to monitor heart rate.

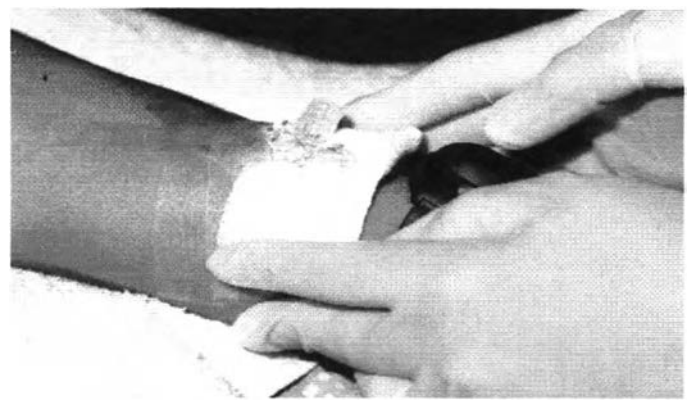


Figure 3.2. On a Teflon catheter No.22 was placed in an antecubital vein of subjects before test.

At first, the volunteers were subjected to a system of graded exercises of the lower limbs on a bicycle ergometer until exhaustion in order to determine the level of VO_2max. The initial load was adjusted to 1 watt/kg of body weight and increased every 2 min by 0.5 watt/kg of body weight and at 80 rpm of pedalling. Respiratory gas exchange was analyzed continuously and computed every 30 sec during each stage of the exercise session using an Oxygen and carbon dioxide gas analyzer (Quinton Metabolic Cart, QMC) (Figure 3.3).

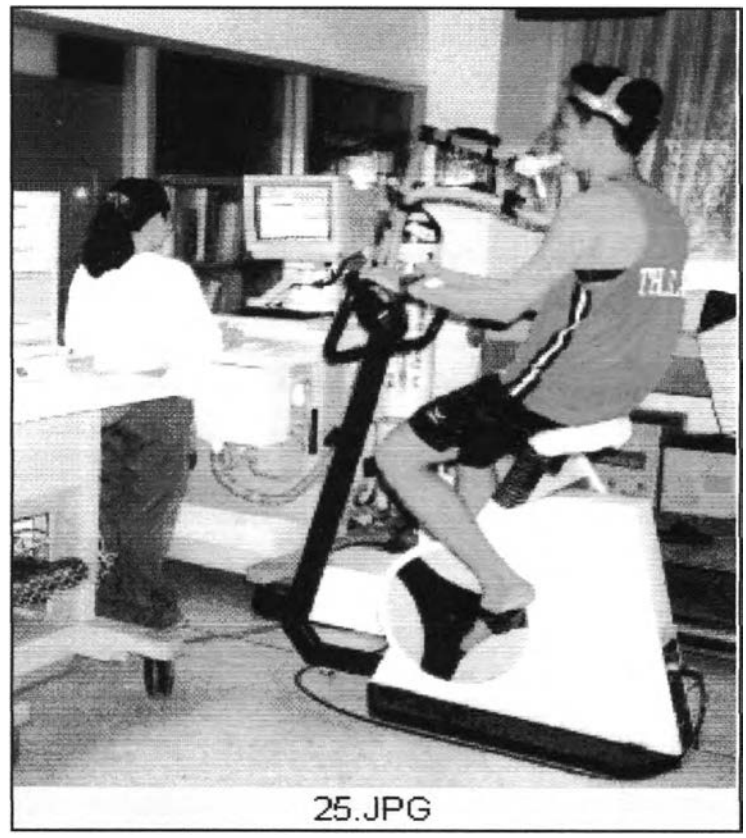


Figure 3.3. Measurement of oxygen uptake using oxygen and carbon dioxide gas analyzer (QMC).

In the next phase, supramaximal exercise was executed by the volunteers in sessions of 1 min of exercise with a pedalling rate of 80 rpm followed by 15 sec recovery periods untill exhaustion. As a whole, the volunteers were able to tolerate about 3 to 5 sessions of such exercise. After maximal exercise three types of recovery modes were studied in different group. The recovery modes were as follows-resting in a relaxed sitting position (passive recovery or PR) for 30 min, pedalling on a bicycle ergometer at 30%VO_2max and at 60 rpm of pedalling (active recovery or AR) for 30 min , and the application of massage to the extremities (massage recovery or MR) for 30 min. The massage, consisting of Traditional Thai Massage (APPENDIX E) , was provided by a certified physiotherapist and only the lower limbs received massage (Figure 3.4).

Figure 3.4. The application of massage to the lower extremities in MR group.

During the recovery period blood samples were collected from the pre-warmed anticubital vein at forearm - just after the exercise (0 min or peak), 5, 10, 15, 20, and 30 min after the completion of exercise.

The samples were analyzed by using a lactate analyzer (YSI 1500 Sport) (Figure 3.5). The gas exchange (VO_2 and VCO_2) and heart rate (HR) were also recorded every 30 sec until the end of 30 min of recovery.

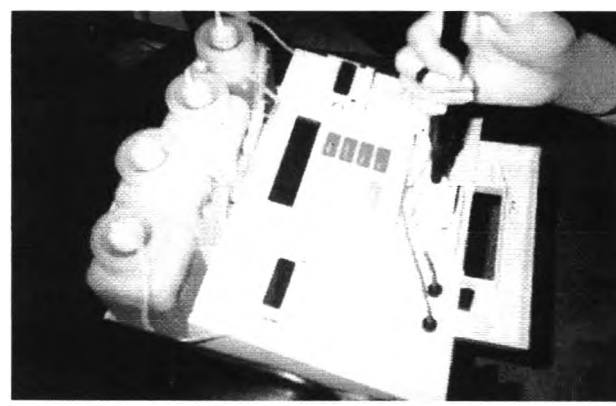
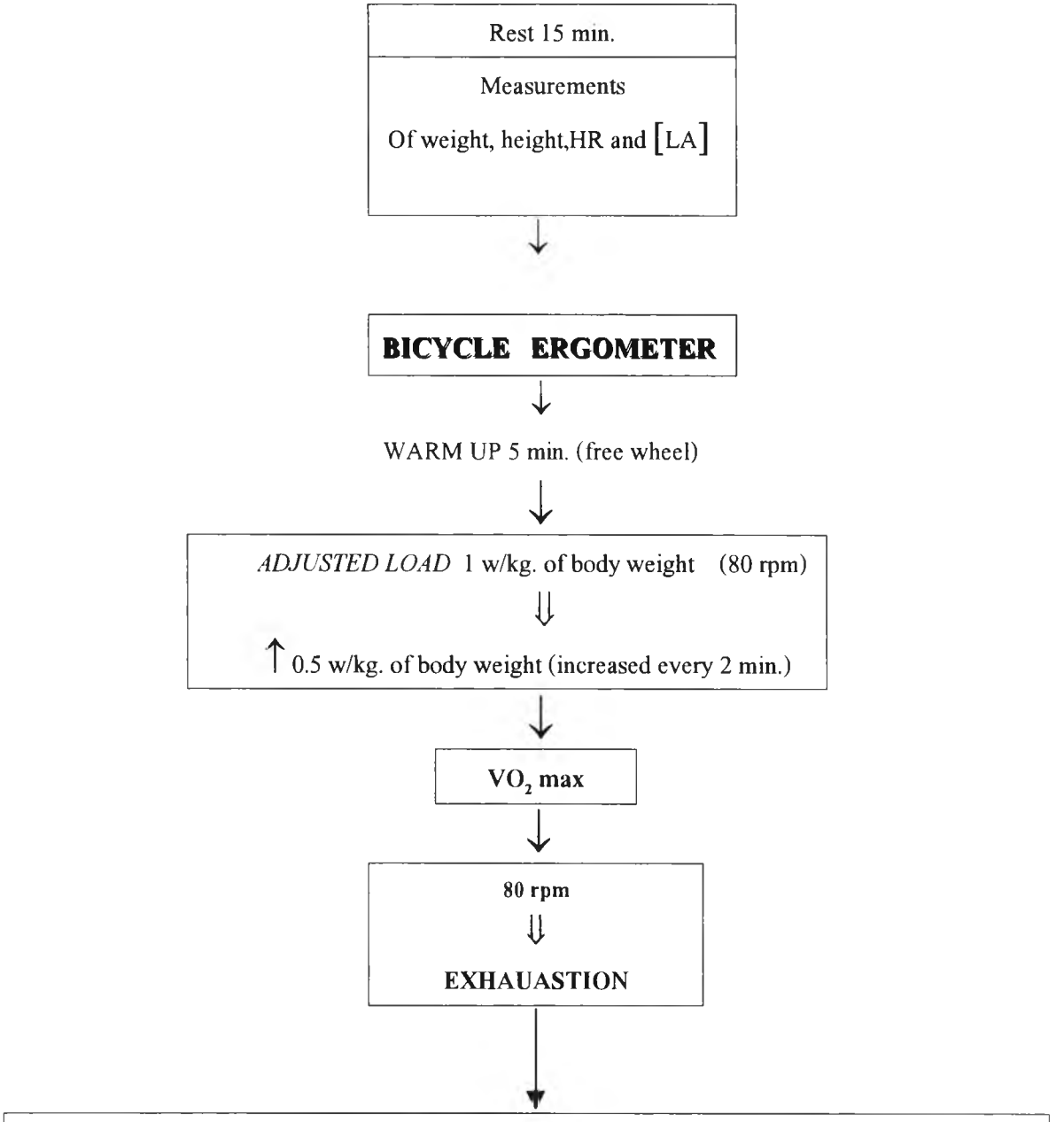


Figure 3.5. The samples were analyzed by using a lactate analyzer (YSI 1500 Sport).

Figure 3.6. Experimental Design



- Lactate concentration [LA] measurement at rest, 0 min or peak, 5, 10, 15, 20, and 30 min after exercise.
- Gas analysis measurement throughout the testing.

Respiratory Exchange Measurements

Respiratory exchange measurements were determined by means of opencircuit, oxygen gas analyzer (Quinton Metabolic Cart; Instrumentarium Oy, Datex Division, Helsinki, Finland). The exercise test was conducted in an air-conditioned laboratory with atmosphere temperature of 24-26°C, barometric pressure of 722-755 Torr, and relative humidity of 50-70 per cent.

The instrument was calibrated before each test with standardized gas mixture (Low gas; oxygen 10.74 ± 0.2 per cent and carbon dioxide 0.00 per cent with balanced nitrogen, High gas; oxygen 25.7 ± 0.2 per cent and carbon dioxide 5.14 ± 0.1 per cent with balanced nitrogen), and the system was cleared of room air before starting each measurement. During the calibration procedure VO_2 was measured by using a mouthpiece connected to a two – way respiratory valve to determine pulmonary ventilation. The expired air was measured and analyzed breath-by-breath by an automated system. The diagram of respiratory exchange measurement system is shown in figure 3.7.

A computer was interfaced with at least three instruments: a system to continually sample the subject's expired air, a flow meter or turbine device to record the volume of air breath, and oxygen and carbon dioxide analyzers to measure the fractional composition of the expired gas mixture. A printed or graphic display of the data was provided throughout the measurement period.

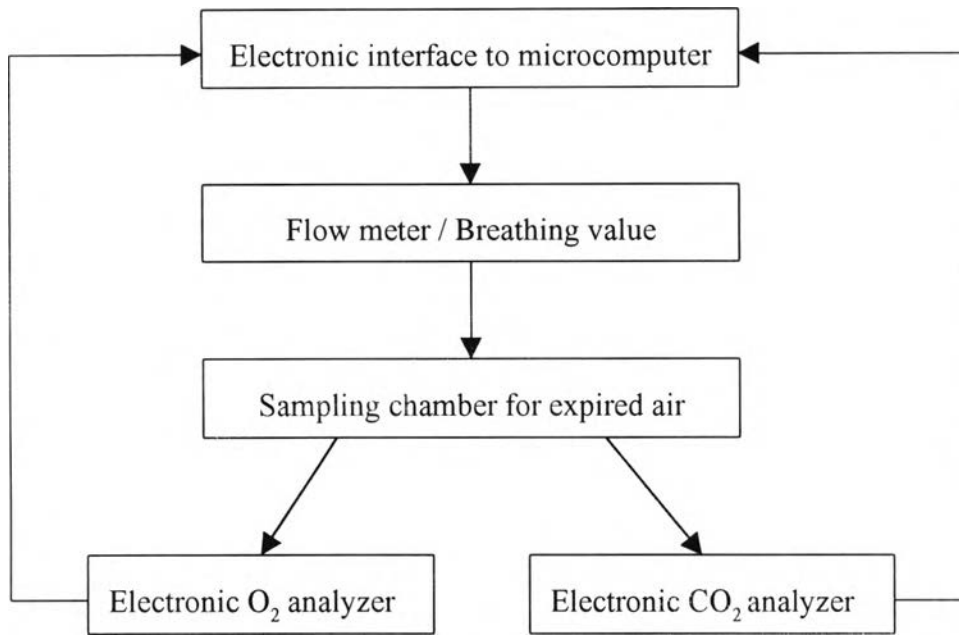


Figure 3.7. Diagram of respiratory exchange measurement system

VO_2max Determination

The VO_2max was considered when at least two of the following criteria were achieved :

1) identification of a plateau of O_2 uptake
2) respiratory exchange ratio (RER) higher than 1.10 (RER = VCO_2/VO_2)
3) heart rate closed to 220-the individual's age-related maximum in year (220-age) $\pm$10 beats/min.
4) signs of exertion intolerance (fatigue) and an inability to maintain the required work load.

Statistical Analysis

The data in this study were statistically analyzed by using the SPSS for Windows Version 9.0. The data are expressed as mean $\pm$ SD. In order to estimate the differences among the mean values, repeated measurement of one-way ANOVA were applied with post hoc - Bonferroni test. The significance level was taken at $p < 0.05$.