CHAPTER 3

Materials and Methods

1. Subjects

Thirty four prepubertal boys volunteered to participate in the study. Subjects were 8 to 12 years old boys without cracking voice and axillary hair. All subjects were free of any known cardiovascular and respiratory diseases and reside in Chiang Mai for at least three years.

All subjects including parents were given oral and written information about the procedures and possible risks involved in the study. The signed informed consent was obtained from all subjects before their participation in the study. The experimental protocol was approved by the institutional ethic committee of the Faculty of Medicine, Chiang Mai University.

2. Preparation of subject prior to the experiment

All subjects do not smoke. Exercise and consumption of alcohol, caffeine and medicine were prohibited 1 day before the experiment. No food was consumed 1 hour before the experiment.

All subjects' body weight, height and skinfold thickness were determined before the sweating response was tested. Skinfold thickness was measured at two sites (triceps and Subscapularis muscle) using a skinfold caliper as illustrated in Figure 3.1 and 3.2 and mean skinfold thickness was then calculated. All subjects in the study must have a skinfold thickness in a range between 15.1–75.0 percentiles.

The skin temperature was measured with thermistor probes placed on the skin at two sites: forearm and thigh. The oral temperature was measured with thermistor probes. Skin and oral temperature were measured 5 minutes prior to sweating test and were

referred to as shell and core temperature, respectively. Subjects with core body temperature exceeding 37 °C were excluded from the study.

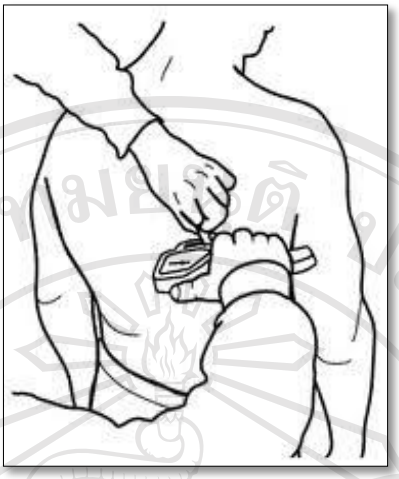

Figure 3.1 Subscapularis muscle site of skinfold thickness measured by caliper [Ratamess, 2014]

Figure 3.2 Triceps muscle site of skinfold thickness measured by caliper [Ratamess, 2014]

3. Determination of subject's physical fitness

Subjects in the high physical fitness groups have participated in endurance types of sport (football, basketball and cycling) for at least 2 years, approximately 2-3 h/day, 4-5 days/week, whereas the subjects in the sedentary group did not perform regular physical activity as determined by a questionnaire.

Maximal oxygen uptake (VO_{2max}) was estimated and used to separate subjects into high physical fitness group (VO_{2max} = 48–57 mL· kg^{-1}·min^{-1}) and sedentary group (VO_{2max} = 36–47 mL · kg^{-1}· min^{-1}) [Chaddock et al., 2010; Hoffman, 2006]. Subjects in the high physical fitness group had higher aerobic capacity than that of the subjects in the sedentary group. The maximal oxygen uptake was estimated for each subject at a submaximal level by pedaling on a bicycle ergometer (Aerobike 500U, Combi, Japan) at a constant frequency of 50–60 rpm for 5 min at 6 different exercise intensities. The heart rate was measured during the last minute of each exercise period. The VO_{2max} of each subject was estimated by extrapolating the relationship between the heart rate and the work rate to the estimated maximal heart rate for each subject [Alman, 2014].

4. Heat loss effector function test

The method used to determine heat loss effector responses was Quantitative sudomotor axon reflex test (QSART) [Illigens and Gibbons, 2009]. ACh-iontophoresis provided a basis for the comparison of pharmacologically induced sweating responses, whereas the volume of sweat output being an indicator of peripheral and central sudomotor activity. The QSART technique was applied to evaluate directly and indirectly mediated sweat gland activation by ACh at forearm and thigh site as illustrated in Figure3.3 and 3.4. All experiments were performed at the room temperature of 24–25°C and relative humidity of 70%.

The QSART sweat capsule with 2 concentric compartments is illustrated in Figure 3.5. ACh placed in the outer compartment is transported iontophoretically into the skin where it directly stimulates the underlying sweat glands (DIR), while the glands of the skin in the central compartment of the capsule are activated indirectly by an ACh-induced axon reflex (AXR) (Figure 3.6). The DIR response is induced by ACh directly acting on the muscarinic receptors of the sweat gland cells. In addition, ACh activates presynaptic nicotinic receptors of sudomotor efferent axons resulting in retrograde excitatory nerve impulses spreading across the distant sudomotor nerve fiber endings which accounts for the AXR [Bae et al., 2006].

During the period of iontophoresis, only the AXR response can be measured form the central compartment. After removal of the ACh solution from the outer compartment, the DIR of the skin underlying the outer compartment can then be determined.

In the current study, measurements of DIR, AXR and sweat gland density including determination of sweat gland output were performed as follows.

1. Two sets of sweat capsules were attached to the volar aspect of the forearm and thigh by means of elastic bands.
2. Nitrogen gas was passed through each capsule compartment at a constant flow rate of 0.3 L/min, and the change in relative humidity of the effluent gas was detected by means of a hygrometer. The sweating rates (mg $\cdot cm^{-2} \cdot min^{-1}$) were fed into a computer every 5 s [Illigens and Gibbons, 2009]. From the raw data, sweat onset time and sweat volumes were determined for the time period from 0 to 5 min after the start of ACh iontophoresis for AXR and after capsule exchange for the time period from 6 to 12 min for DIR.
3. The outer compartment of capsule 1 was filled with 10% ACh solution, while capsule 2 will be on standby.
4. Two milliamperes of direct current were applied for 5 min between an electrode in the ACh cell of capsule 1 (anode) and a flexible plate-electrode (cathode) attached to the forearm skin just proximal of the wrist joint and the thigh skin just proximal of the knee joint.
5. Capsule 2 was placed at capsule 1 in exchange at the end of the first 5 min for another 7 min.
6. The density of activated sweat glands and sweat gland output on the forearm and thigh were determined at the end of the ACh-iontophoresis period at 12 min from the start of the sweating test. The activated sweat glands density was determined at a site adjacent to the sweat capsule using the starch-iodide technique.
7. The sweat gland output at each site was calculated by dividing mean local sweat rate for 5 min by the number of activated sweat glands

Sweating data were acquired up to the 12th min for simultaneous observation of DIR and AXR sweating. Sweat production was determined with the capacitance hygrometer-ventilated capsule method [Illigens and Gibbons, 2009]. The full study protocol is summarized and shown in Figure 3.7.

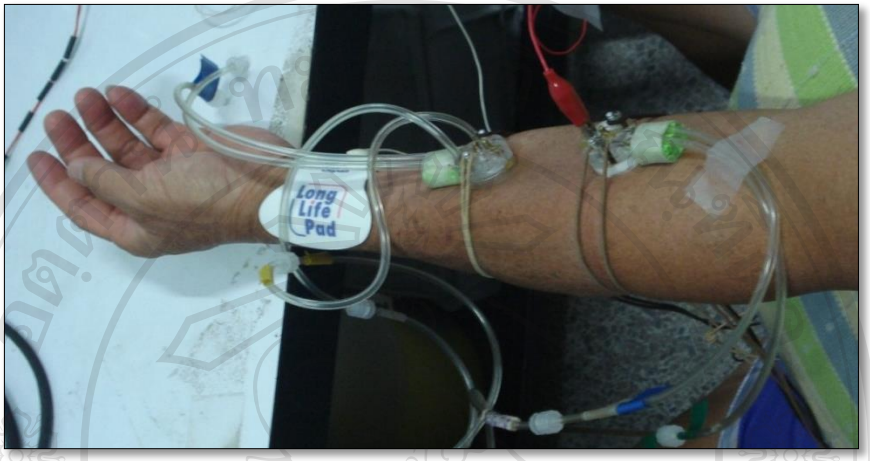

Figure 3.3 Forearm site of sweat gland responses measured

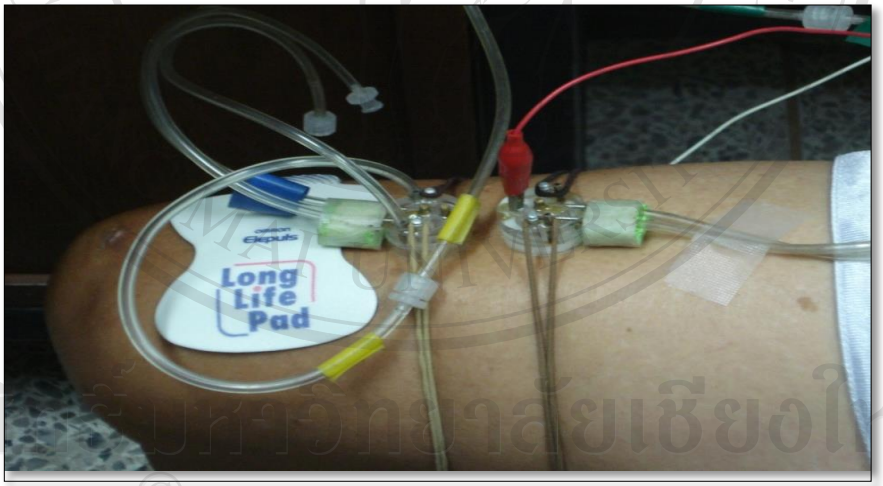

Figure 3.4 Thigh site of sweat gland responses measured

Figure 3.5 Hygrometer-ventilated capsule method was used to determined sweat gland function.

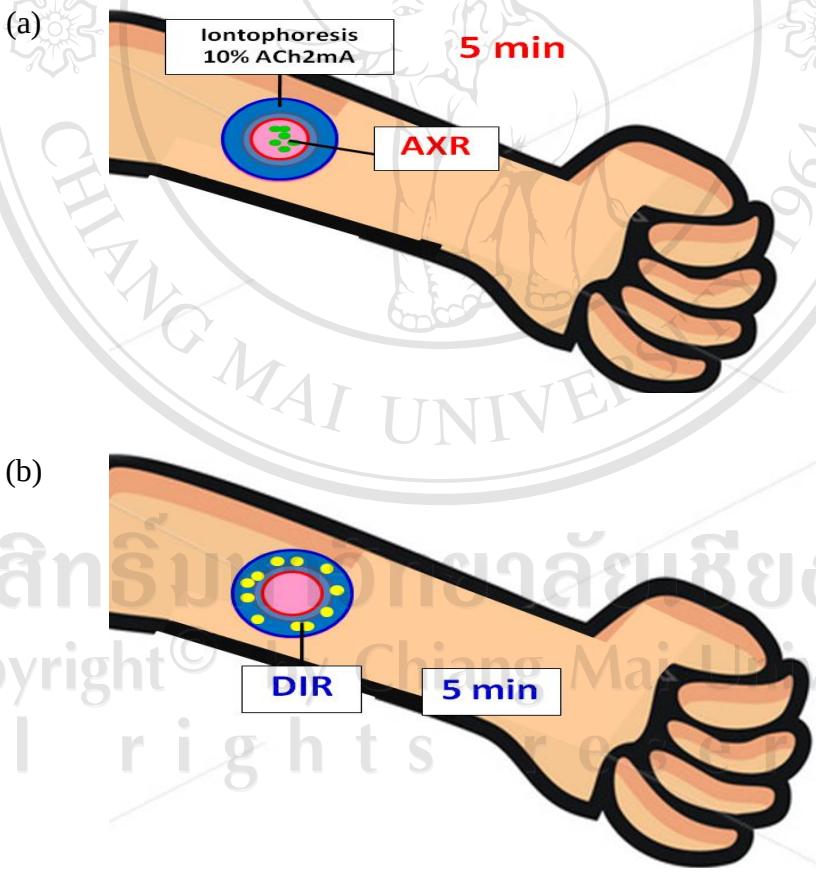

Figure 3.6 Quantitative sudomotor axon reflex test (a), directly stimulates sweat glands (b)

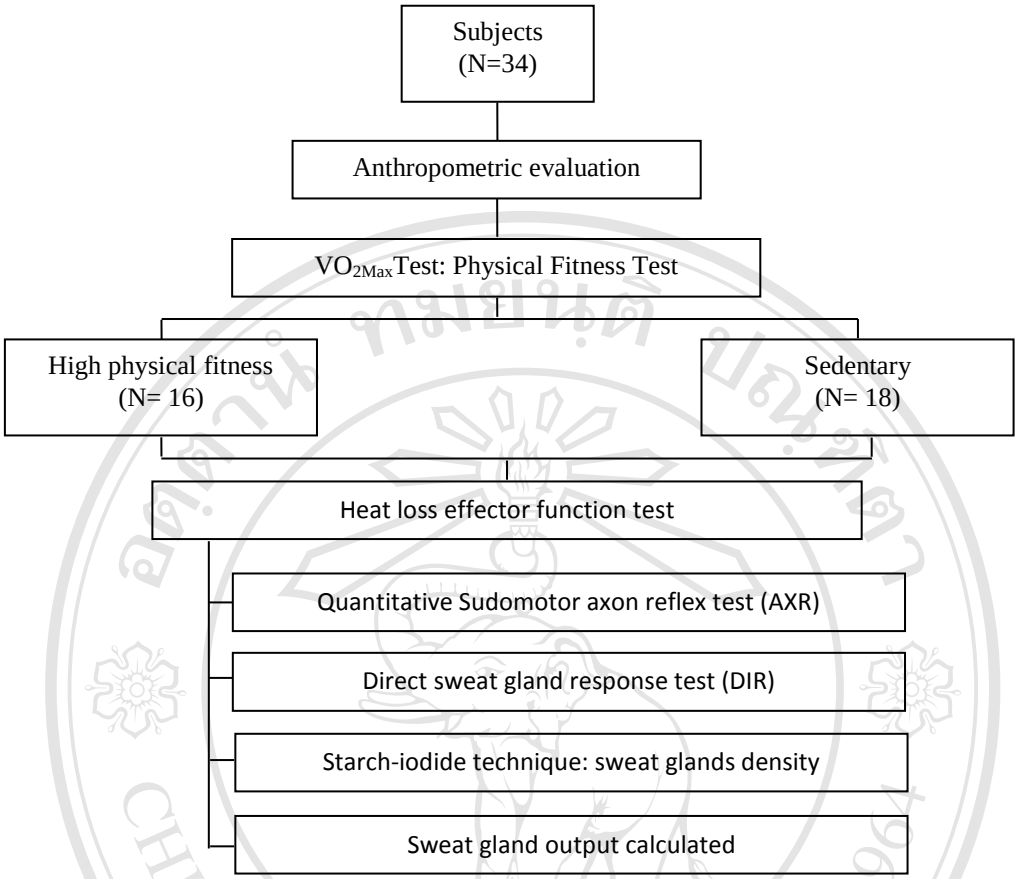

Figure 3.7 Schematic representation of experimental protocol

5. Statistical Analysis

The results are expressed as mean ± S.E.M. Student's t-test was performed to assess group difference. P-values of less than 0.05 were considered statistically significant.