CHAPTER 3

METHODS

3.1 Subjects

Since gender and menstrual cycle influence the degree of DOMS (Dannecker et al., 2003), therefore only male participants were recruited for the experiment in this thesis. Based on previous studies (Slater et al., 2003; Nie et al., 2005), to obtain the power analysis of 80% at the alpha level of 0.05, a total sample size estimation was approximately 25 subjects for study of DOMS characteristics. For the preventative study, based on a pilot study, the primarily clinical outcomes of DOMS (i.e., PPT, ROM-AE, pain-free GS and WES) were chosen to calculate the sample size. To obtain the power of 80% at the alpha level of 0.05 with the effect size of greater than 0.839, total sample size estimation would be approximately 14 subjects per group (Portney and Watkins, 2000) for the preventative studies (see appendix 1). All subjects were recruited by advertisement and personal contact. Each subject was required to complete an entry questionnaire that includes questions pertaining to personal data, history of subject's general health, sports involvement, arm dominant, upper extremity injury, and medical condition including causal diseases, and coffee, tobacco, alcohol, and prescription medicine use (Bajaj et al., 2001) (see appendix 2). They were included for the experiment according to the following criteria.

3.1.1 Inclusion criteria:

1. Healthy male age between 18-25 years old.
2. Subjects have normal range of vital signs and are not on any forms of medication at the time of the experiment.
3. Volunteers accept the procedures of the experiment and sign a consent form.

3.1.2 Exclusion criteria:

1. Persons who have past history of upper limb musculoskeletal disorders, neurological disorders, or any diseases that may affect on the study during the last 3 months (Nie et al, 2005).
2. Persons who have experience of arm resistance training at least 3 months before this experiment (Nie et al., 2006).

3.1.3 Discontinuation criteria:

1. Persons who take pain killers or anti-inflammatory drugs in the period of studying (Jamurtas et al., 2005).
2. The experiment would be stopped when the subject feels uncomfortable from symptoms such as acute pain, cramp or suffers an allergic reaction that may cause danger to our subjects.
3. Any subject who cannot follow the full experiment.

3.2 Study design

The experiment consists of two parts - an investigation of DOMS characteristics (part I) and DOMS prevention (part II). Part I uses a within subject, repeated measures design. Part II uses a mixed model of within subject, repeated

measures and between subjects with randomized control design. In addition, only one assessor performed the repeated measures of all outcome measures throughout the study.

3.3 Initial evaluations

Physical examination was performed to confirm that all subjects have full pain-free range of elbow and wrist motion, and no abnormal tenderness to palpation of the soft tissues over the extensor muscles of the forearm and wrist. Informed consent was obtained from each subject (see appendix 3). The study was approved by the institute ethics committee, certificate number 0515(012).1/RES153 (see appendix 4). Subjects were asked not to take coffee or alcohol 12 hours before the experiment, and were neither allowed to perform any vigorous physical activities or unaccustomed exercise (Nosaka and Sakamoto, 2001), nor take any anti-inflammatory drugs, and were reminded to maintain their usual nutritional and lifestyle habits. Throughout the study period subjects were asked to keep a logbook of their activities and diet (Jamurtas et al. 2005).

3.4 Study procedures

Part I (characteristic study): Subjects were requested to complete the 17 visits of experiment sessions. Before studying the data collection, subjects were familiarized with the purposes of the study and procedures. For baseline period, subjects were assessed twice with 24-hour interval for the dependent variables to confirm the baseline measurements before the DOMS induction. The reliability of all outcome measures was tested before the study.

Part II (preventative studies): Subjects were divided into 5 groups (control, PNF, massage, hot pack and sauna with 14 subjects per group), the control and experimental conditions were ordered using equally randomization with drawing lots. They were assessed for the baseline measurements of all sensitive parameters. The control group of this study received DOMS induction only. The experimental subjects got the assigned prevention before DOMS induction.

Subsequently for both parts (I & II), the outcome measures were evaluated immediately following DOMS induction. Then, the dependent variables were also be measured repeatedly at the same time of the day for a 14 day-period for part I, and an 8 day-period with the selective parameters for part II. Additionally, in the studies of part II, subjects' skin blood flow was measured before and after the application of intervention technique. For blood flow measurement, it was measured over muscle belly of ECRB by Laser Doppler Blood Flow Monitor (Moor instruments DRT4, UK) at an interval of 5 min before starting the experiment to serve as a baseline data, and immediately measured after the intervention by calculating its average and maximum values. Induction of DOMS was achieved only in non-dominant arm. In the second part of the study, the prevention methods including PNF technique, massage, hot pack, and sauna as previously mention in rationale section were evaluated in different individuals to avoid the repeated-bouts and carry-over effect (Cleary et al., 2002).

3.5 Criterion measures

The non-dominant arm was used in this study to avoid possible effects of daily activities on the measures. The criterion measures consisted of pain intensity, pain thresholds including thermal pain threshold [i.e., cold pain (CPT) and heat pain

(HPT)] and pressure pain threshold (PPT), vibration sense (VIB), range of motion in active wrist flexion (ROM-AF), active wrist extension (ROM-AE), passive wrist flexion (ROM-PF), and passive wrist extension (ROM-PE), joint position error sense (JPE), choice response time (CRT), grip strength (GS), wrist extension strength (WES) (see appendix 5). The order of measurements was pain intensity, CPT, HPT, VIB, PPT, ROM-AF, ROM-AE, ROM-PF, ROM-PE, JPE, and CRT. Muscle strengths were measured after these measurements with randomization between GS and WES. In part I for investigating characteristics of DOMS, subjects were requested to complete the 17 sessions including the preliminary study. In the preliminary session, subjects were familiarized with the purposes of the study and procedures. For baseline period, subjects were assessed twice with 24-hour interval for the dependent variables to confirm the baseline measurements before the DOMS, and then follow up the symptoms for 14 days after the induction (Figure 1). The same investigator conducted all measurements using the computerized system. Creatine kinase was voluntarily evaluated in 10 subjects to confirm the result of exercise-induced muscle damage. CK was assessed by using Kinetic Colorimetric Method before and the peak point at the 4th days after exercise (Clarkson et al., 1992; Zainuddin et al., 2005). These subjects were also asked to record logbook for their physical activities.

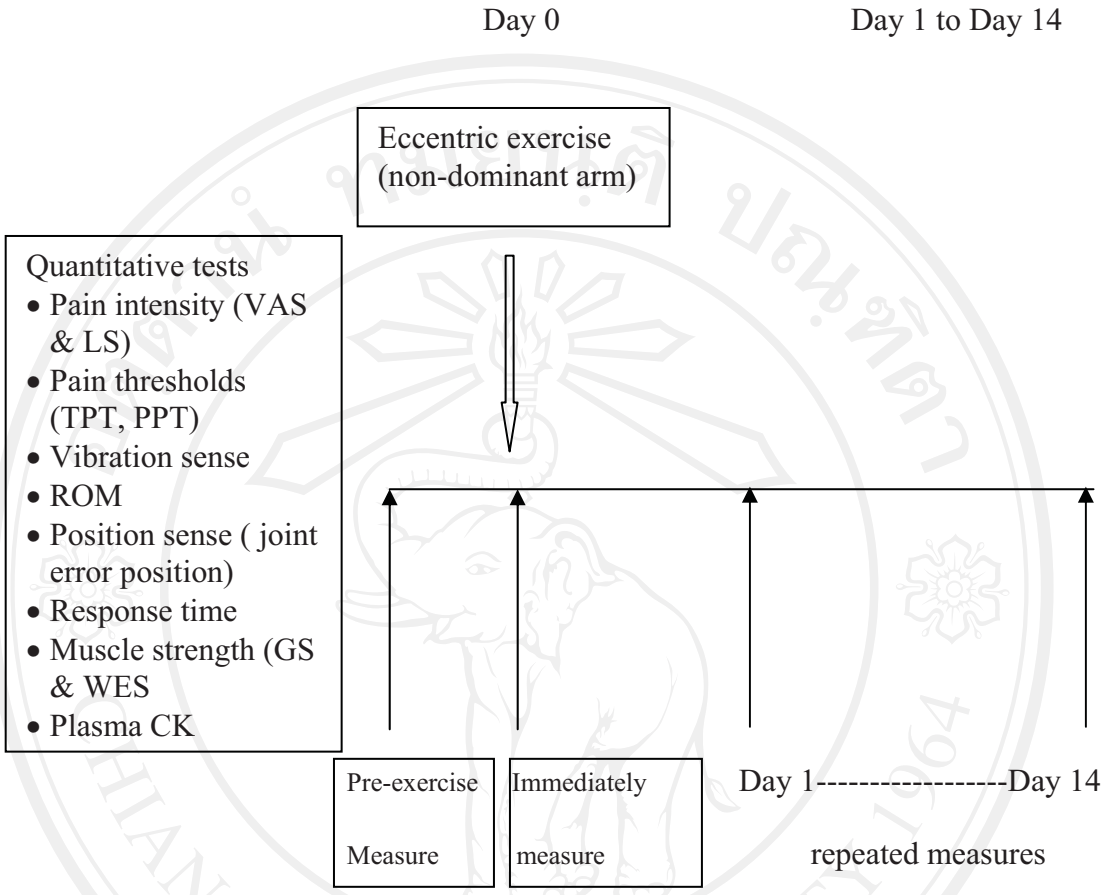

Figure 1 Schematic timeline representation of the experimental protocol on days 0 to 14. A series of quantitative tests were measured pre exercise Day 0, immediately after exercise, and at each day of Days 1-14.

In part II for evaluating the preventative effect of physical approaches, the outcome measures consisted of pain intensity of visual analogue scale (VAS) and modified Likert's scale (LS), pain thresholds including cold pain (CPT) and pressure pain threshold (PPT), range of motion in active wrist flexion (ROM-AF), active wrist extension (ROM-AE), passive wrist flexion (ROM-PF), and passive wrist extension (ROM-PE), grip strength (GS), wrist extension strength (WES). The HPT, VIB, JPE

and CRT were not included in the studies of part II, because these parameters were not reliable (Khamwong et al., 2010) or they were not the sensitive outcome measures from the study of part I. The order of measurements was pain intensity, CPT, PPT, ROM-AF, ROM-AE, ROM-PF, and ROM-PE. Muscle strengths were measured after these measurements with randomization between GS and WES. Subjects were requested to complete the 10 visits of experiment sessions including the preliminary session. At preliminary session, subjects were familiarized with the purposes of the study and procedures, and then followed up the symptoms for 8 days after the induction (Figure 2). The interval between different measures was at least 5 minutes, and the rest period between trials in the same measure was 30 to 60 s as indicated in each test protocol.

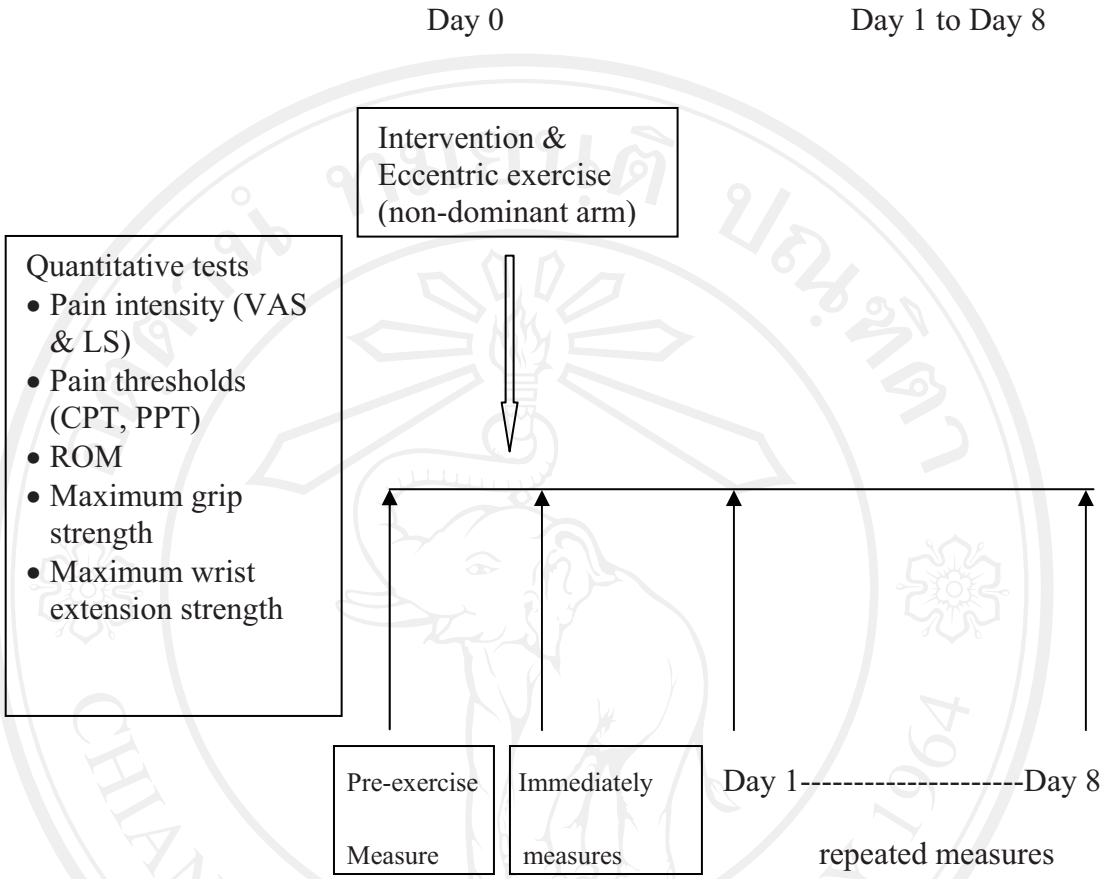

Figure 2 Schematic timeline representation of the experimental protocol on days 0 to 8. A series of quantitative tests were measured pre exercise Day 0, immediately after exercise, and at each day of Days 1-8.

3.6 Exercise induction

The eccentric-exercise protocol used the isokinetic mode of the Contrex dynamometer (CON-TREX Multijoint System, CMV AG manufacture, Switzerland). Subjects were seated on the chair of the dynamometer, and the trunk was fully supported and fixed by the double-cross chest belts. The forearm to be tested was placed on the handle bar, and the subjects were asked to maximally resist against the

dynamometer's movement from wrist extension to wrist flexion. The subjects were verbally encouraged to generate maximal force for the whole range of wrist extending motion, and the force output was displayed on a screen to motivate maximal effort. The exercise consisted of 5 sets of 60 maximal eccentric contractions of the wrist extensors at a velocity of $25^{o} \cdot s^{-1}$ with a 1-minute rest period between sets (Slater et al., 2005) (Figure 3). Peak torque and work of each contraction during the eccentric exercise were obtained by a software program of the dynamometer, and the average of each set (60 contractions) was used for further analysis.

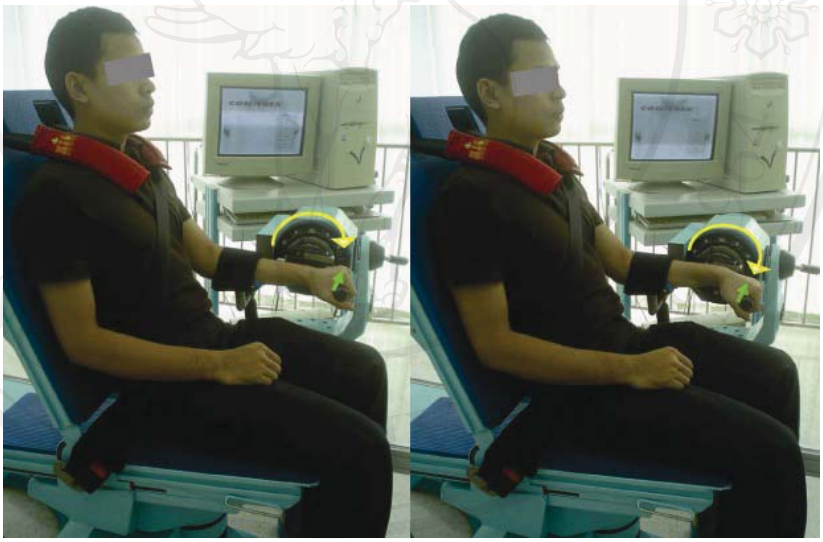

Figure 3 An eccentric-exercise induction was performed using isokinetic mode of the Contrex dynamometer.

3.7 Sensory perception

3.7.1 Pain intensity

The visual analogue scale (VAS) was used to rate the intensity of pain. The VAS consisted of a 10 cm line anchored with “no pain” on the left end and “extreme pain” on the right end (Figure 4). Subjects were asked to rate their perceived level of pain at rest.

Figure 4 The visual analogue scale (VAS).

A modified version of the Likert scale (LS) was also used to rate the level of muscle soreness as follow (Slater et al., 2003): 0 = a complete absence of soreness; 1 = a light soreness in the muscle felt only when touched/ a vague ache; 2 = a moderate soreness felt only when touched/ a slight persistent ache; 3 = a light muscle soreness when lifting objects or carrying objects; 4 = a light muscle soreness, stiffness or weakness when moving the wrist without gripping an object; 5 = a moderate muscle soreness, stiffness or weakness when moving the wrist; 6 = a severe muscle soreness, stiffness or weakness that limits my ability to move.

3.7.2 Thermal pain threshold (TPT)

Thermal pain threshold (TPT) was the level of temperature that induces initial pain, and was assessed using a Thermal Sensory Analyzer (Medoc Ltd., Neuro Sensory Analyzer Model TSA-II, Israel) for cold and heat pain threshold. The system was calibrated in accordance with the instruction manual, and a control resolution was 0.3°C. The measurement sites were the same as those for the vibration sense; the common extensor origin at the lateral epicondyle (O), and the belly of extensor group muscles located at the prominent point over the extensor carpi radialis brevis muscle (M). The temperature of the thermode (5 cm^2) was increased or decreased at a controlled rate ($2^o C \cdot s^{-1}$ for cold pain and $1^o C \cdot s^{-1}$ for heat pain). Each subject lay down on this back with arm by his side (0° elbow extension and 90° pronation), and the thermode was applied on the marked areas with Velcro strap. According to the previous standard protocol for evaluating TPT in the wrist extensors (Paungmali et al., 2003; Wright et al., 1992; Wright et al., 1994), the initial temperature for testing of cold pain threshold (CPT) was set at 32^o C, and the thermode temperature gradually decreased at a rate $2^o C \cdot s^{-1}$ to a minimum cut-off temperature of 0^o C. The subject held a control switch, and was instructed to press the button when he felt the sensation changing from cold to pain. For the heat pain threshold (HP), which was conducted 1 min after the cold pain threshold, the initial temperature was also set at 32^o C, and the temperature of the thermode was gradually increased at a rate of $1^o C \cdot s^{-1}$ up to a maximum cut-out temperature of 50^o C to avoid the heat injury on the skin. The subject was asked to press a control switch when he felt the sensation changing from hot to pain (Figure 5). The subject received a verbal instruction approximately 1-2 s before the initiation of each test, and each pain

threshold was assessed three times with a 30-s interval between trials. The mean value of the 3 trials was used for further analysis.

Figure 5 Thermal pain threshold (TPT) measurement.

3.7.3 Pressure pain threshold

Pressure pain threshold (PPT) was measured by an algometer (Somedic Production, Algometer type II, Sweden) with a probe of 1.0 cm^2. The algometer is factory calibrated to ± 3% of readout and is regularly recalibrated in the laboratory with a 100-kPa calibrating weight before experimentation. PPT was assessed at the two sites; the common extensor origin at the lateral epicondyle (O), and the belly of extensor group muscles located at the prominent point over the extensor carpi radialis brevis muscle (M), respectively. Each subject lay down on the back with his arm by the side (0° elbow extension and 90° pronation). The probe was placed at the reference site, and the pressure was increased at a rate of 30 $kPa \cdot s^{-1}$ until the subject felt the sensation changing from the pressure to pain, which was indicated by the subject's pressing a button (Figure 6). PPT was assessed 3 times for each site

with 30-s rest between trials, and the mean of the 3 trials was used for further analysis (Slater et al., 2005).

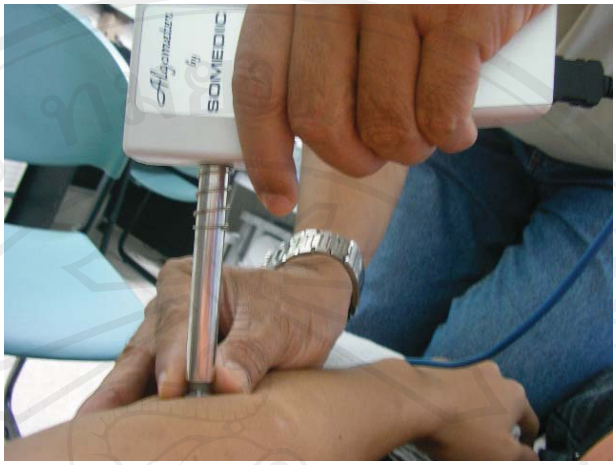

Figure 6 Pressure pain threshold measurement.

3.7.4 Vibration sense

Vibration sense (VIB) was assessed on 2 sites of the wrist extensor muscles; the common extensor origin at the lateral epicondyle (O), and the belly of extensor muscles located at the prominent point over the extensor carpi radialis brevis muscle (M) using a vibration neuro-sensory analyzer (Medoc Ltd., Neuro Sensory Analyzer Model TSA-II, Israel). The vibration is factory calibrated to ± 0.1 μm with a control resolution of ± 1%. Vibration stimulus was applied to the sites (i.e., origin site, muscle site) in progressive magnitude of 0.1 μm s^{-1} with a fixed frequency of 100 Hz. Each subject lay down on his back, and the elbow was flexed at 90° and rested his hand above the umbilicus for the test of the origin site, and his arm was placed by his side in 0° elbow extension and 90° pronation for the muscle site test. The subject held a control switch on the other hand, and was asked to press a control switch when he started to feel the vibration (Weerakkody et al., 2001) (Figure 7). The magnitude

of the vibration to be sensed (threshold) was assessed 3 times with a 30-s interval between trials. The mean value of the 3 trials was used for further analysis.

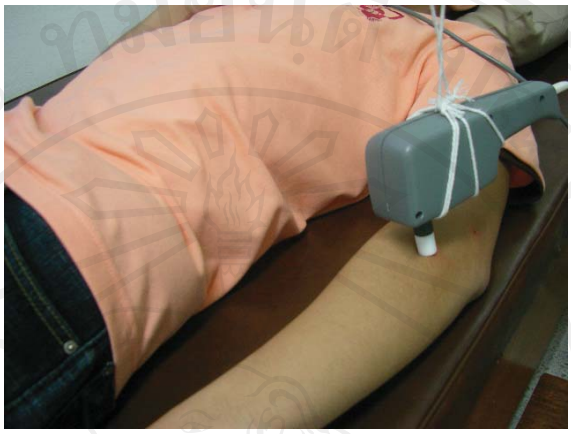

Figure 7 Vibration sense measurement.

3.7.5 Passive range of motion (PROM)

ROM was evaluated using an electrogoniometer (Biometrics, DLK 900, U.K.) with the resolution of within 1° for wrist extension and flexion to determine the pain-free active and passive range of motions. Subjects sat on an arm supporting chair, and were asked to rest the arm on the support. The bony prominences including triquetrum, olecranon and the fifth metacarpal head were marked with the permanent marker to identify the reference points clearly. The center of the goniometer was placed at the center of the axis of the wrist joint (triquetrum bone) with a stationary arm of the goniometer placed paralleled to the lateral midline of the ulna toward olecranon process, and a moveable arm of the goniometer was placed to the lateral midline of 5th metacarpal bone toward the metacarpal head (Reese and Bandy, 2002). The pain-free passive range of motion, the subject was asked to relax the hand during passive movement of the wrist joint into flexion and

extension directions by the investigator. The subject signaled the investigator for a position of the wrist when he felt initial perception of pain of the muscles or at the end range of motion (Figure 8). The measurements were taken 3 times with a 30 s interval. The mean of the 3 trials was used for further analysis.

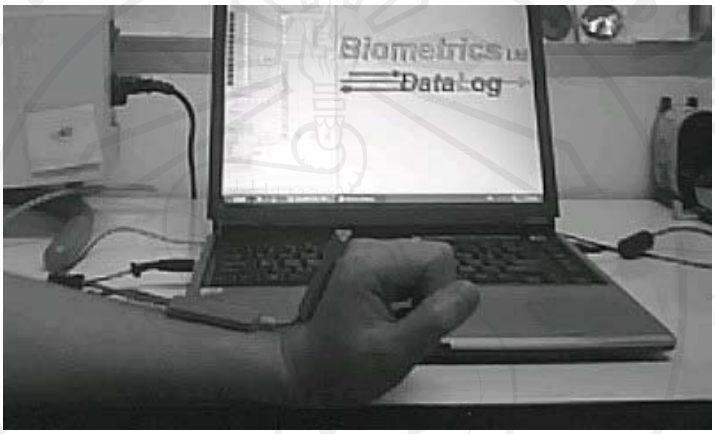

Figure 8 Range of motion measurement.

3.7.6 Joint position error

Joint position error (JPE) was measured using an electrogoniometer (Biometrics, DLK 900, U.K.) in the same setting as the ROM assessment. The target points were set between 45° wrist flexion and 45° wrist extension, which was about the middle range of the full wrist flexion and extension. Subjects were blindfolded to eliminate any visual cues and were told to concentrate on the position of the hand "in space." The investigator passively moved the subject's hand to a target angle as referencing by a universal goniometer and holds it for 3 s before returning the wrist to the neutral position. As soon as returning to the neutral position, subjects were asked to immediately re-position the hand back to the target angle and inform the investigator when they felt the position was achieved. At this time, the investigator

recorded the angle, and the absolute difference between the target angle and the recorded angle was determined (Dover & Powers, 2003) (Figure 9). The test was repeated 3 times with a 30-s rest between trials, and the mean of the 3 trials was used for the further analysis.

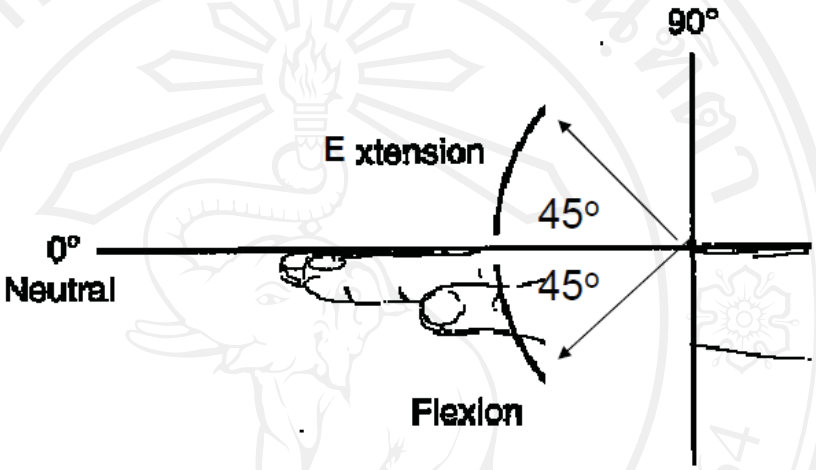

Figure 9 Joint position error test.

3.8 Motor functions

3.8.1 Active range of motion (AROM)

As the similar procedure as PROM, the pain-free active range of motion was performed by instructing the subject to move the wrist into flexion and extension directions actively. The subject was requested to stop the movement when initial perceiving pain.

3.8.2 Response time

In this experiment we used three choices response time (CRT) with 3 lighting stimuli. The response time was measured by a reaction timer (Thai Phan, Thailand) with the resolution of 1 ms. Each subject sat on a chair and placed the testing hand on a mark located on the center of a table, and faced to a box on which three buttons are located 30 cm between them. The distance from the mark on the table to the middle button was 20 cm. The subject was asked to reach out the button responding the light as soon as a randomly selected lighting stimulus was given in 0 to 3 s (Figure 10). Each standard test consisted of 12 trials with 30-s rest between trials, and six middle values of the 12 trials were averaged and used for further analysis (Hoegers, 1999; Bisset et al., 2006).

Figure 10 Response time measurement.

3.8.3 Grip strength

Grip strength (GS) was measured using an electronic digital hand dynamometer (Model MLT003/D, Power lab, Australia). The manufacturing resolution of the dynamometer was ± 0.6 N. Subjects sat on a chair with their arms supported by a platform, which was set at the same length as from the elbow to the wrist joint. The upper extremity was positioned according to the recommendations of the American Hand Society of Hand Therapist (Fess, 1992) such that the shoulder adducted and neutrally rotated, forearm in neutral position, and wrist slightly extended (20°). GS was measured with the elbow in 90° flexion and within the comfortable grip width of each subject. The subjects were requested to grip as strong as they could without pain (i.e., pain-free GS) and they were also instructed to perform a sustained maximal isometric contraction for 6 seconds (i.e., maximal GS) (Kamimura and Ikuta, 2001) (Figure 11). The measurement was performed 3 times with 1-min between trials, and the mean of 3 trials for peak values was used for further analysis.

Figure 11 Grip strength measurement.

3.8.4 Wrist extension strength

Wrist extension strength (WES) was recorded via a force transducer (Model MLT003/D, Power lab, Australia). The manufacturing resolution of the dynamometer was ± 0.6 N. A specifically designed pad hand attachment was connected to the underside of the force transducer. The transducer was mounted on a platform, which was located under the table. Each subject sat on a chair with his forearm in full pronation with 45^{o} elbow flexion supported on an armrest of the chair, and his wrist was set in 20^{o} extension with the 3^{rd} knuckle placed to the centre of the force transducer. The subjects were requested to extend the wrist by pushing the dorsal surface of the hand on to the padded surface of the hand attachment as strong as they could without pain (i.e., pain-free WES) and they were also instructed to maximally extend the wrist against the dynamometer and sustained a maximal isometric contraction for 6 seconds (i.e., maximal WES) (Kamimura and Ikuta, 2001) (Figure 12). Subjects performed the contraction 3 times with a 1-min rest between the trials. Peak value was determined for each trial, and the mean of the 3 trials was used for further analysis.

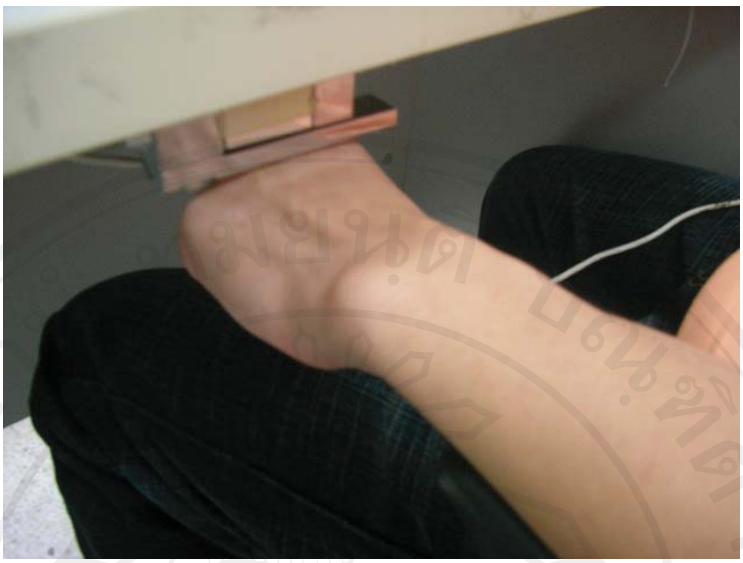

Figure 12 Wrist extensor strength measurement.

3.8.5 Additional outcome measures

Skin temperature over the prominent point of extensor carpi radialis brevis muscle was measured by a thermal sensor (Biofeedback, Myomed 432, Enraf Nonius, USA) before, during, and after the intervention. Although it was ideal to measure muscle temperature, it was not possible to include in the thesis study because of the invasive nature of the muscle temperature measure (e.g. inserting a needle probe to the muscle) that might affect the outcome measures. Skin blood flow was measured by a laser doppler blood flow monitor (Moor instruments DRT4, UK) at muscle belly of the extensor carpi radialis brevis for the duration of 5 min before and immediately after the preventative application.

3.9 Reliability section

The reliability assessments were quantified in twenty-five healthy young men (20.6 ± 1.3 years), and based on the measures between two occasions at the same time of the day with a 24-hour interval. The subjects were advised to use the tested limb at minimal level between the testing sessions. The same investigator performed all measurements and was blinded from the previous scores.

3.10 Prevention methods

The intervention methods including PNF technique, massage, hot pack, and sauna were the potential approaches being evaluation in this thesis as previously described in the rationale part.

3.10.1 PNF technique

The PNF technique (hold-relax with agonist contraction) was performed for warm up stretching maneuvers as a preventative method of DOMS. Each subject was asked to sit on a chair with supporting arm, and then move the hand beyond the edge of the supporting surface. To standardize stretching method for the stretching group, the investigator passively stretched the wrist extensor muscles of the testing arm until each subject reported a mild stretch sensation and held that position for 10 seconds. Next, each subject was required to (isometrically contract the wrist extensor muscles to its maximum capacity) for 7 seconds by attempting to push his wrist back against the resistance of the investigator. After the contraction, each subject was allowed to relax for 5 seconds. Each subject was then asked to actively stretch the muscle, thus adding to the stretch force until a new point of mild stretch

sensation was reached. The stretch was held for another 20 seconds (Figure 13). This sequence was repeated 10 times by each subject in the experimental group (Spernoga et at., 2001; Baechle and Earle, 2000).

Figure 13 The PNF stretching technique (hold-relax with agonist contraction)

3.10.2 Massage

Subjects seated on a comfortable chair with adjustable height, and arm was rested on the table to give comfortable support elbow, forearm and hand. A Sports Massage was given on the testing arm over the wrist extensor muscles for 15 min, consisting of 2.5 min muscles pressed and shaken, 6 min effleurage, 0.5 min tapotement, 5 min petrissage and 1 min effleurage (Rodenburg et al., 1994) (Figure 14). No powder or oil was used during the massage.

Figure 14 Massage.

3.10.3 Hot pack

The application of hot pack was in accordance with the clinical application procedure (Cameron, 2003). A standard size (25 x 30 cm) hot pack (Tropic Pac®, Chattanoga, USA) was used after being stored in hot water (75°C) of a hydrocollator for at least 2 hours. Each subject sat on a chair and relaxed his arms, and the exercise arm was supported on a table. The hot pack was placed to cover the whole wrist extensor muscles and their common origin, and wrapped by 8 layers of a 0.2 mm thickness dry towel for 20 min. (Figure 15)

Figure 15 Hot pack.

3.10.4 Sauna

Standard sauna method was used by the recommendation of American College of Sports Medicine (ACSM). Each subject was asked to shower before entering a sauna room, size of 3 m^2. The subject was seated and rested on the bench with a towel. The sauna temperature was kept between 170-180 ^oF (76.67-82.22^oC) at a comfortable level of humidity for 15 min (Hannuksela and Ellahham, 2001; Peterson and Tharrett, 1997) (Figure 16).

Figure 16 Sauna.

3.11 Statistical analysis

For the reliability section, the reliability test of intraclass correlation coefficients (ICC), coefficient of variation (CV), and standard error of measurements (SEMs) were used to analyze. The data were presented as a comparison of mean, standard deviation, standard error of mean and the percentage change over time. CK

level was analyzed by Pair T-test. The data have been normalized. The outcomes measures such as pain intensity, TPT, PPT, vibration sense, ROM, joint error position, response time, grip strength and wrist extension strength were normalized and further analyzed by repeated-measures ANOVA. The mean of the experimental and control group were also compared using a mixed model of ANOVA tests to determine effective of prevention methods. If the ANOVA showed a significant difference between conditions for interaction or main effects, a Tukey HSD was applied as a post-hoc test to detect differences between groups for each time point. When a significant time effect was found in the ANOVA, a one–way repeated measures ANOVA was utilized to compare the values between the time points after exercise and the pre-exercise for each group separately. Statistical significance was set at $p \leq 0.05$ for all analyses. The data was analyzed by Statistical Package for the Social Sciences (SPSS) for Windows version 16.1.

3.12 Setting

Data collection was taken place at the Neuro-musculoskeletal and Pain Research Unit, Department of Physical Therapy, Faculty of Associated Medical Sciences, Chiang Mai University.