CHAPTER III
MATERIALS AND METHODS

This study aims to determine the effects of moderate exercise training and moderate exercise training combined with vitamin C supplement on physiological changes and rhinitis symptoms in allergic rhinitis patients. The present study included two studies with are study I and study II. Study I, demonstrated the effects of acute exhaustive and moderate intensities exercise on physiological changes and rhinitis symptoms in allergic rhinitis patients. Study II demonstrated the effects of moderate exercise training and moderate exercise training combined with vitamin C supplementation on physiological changes and rhinitis symptoms in allergic rhinitis patients. All protocol and procedures employed in this study were reviewed and approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University, Bangkok, COA No. 481/2011.

Sample group

The sample group of this study included healthy subjects and patients with allergic rhinitis who are students and official personnel in Chulalongkorn University. ranging in age from 18 to 45 years old.

Study I: The effect of acute exhaustive and moderate intensity exercise on physiological changes and symptoms in allergic rhinitis patients.

The study was conducted in a group of 14 healthy volunteers and 13 allergic rhinitis patients.

Inclusion criteria

1. The healthy subjects had no chronic disease and negative skin prick test to house dust mite *(D.pteronyssinus)*.

2. The rhinitis patients were the persistent allergic rhinitis who had rhinitis symptoms more than 4 days a week and positive skin prick test to house dust mite (D.pteronyssinus).

- They had no complications with allergic rhinitis and sinusitis, ear tube malfunctions and asthma, and patients without kidney disease and kidney stones.

- All volunteers stopped taking all medicine before the study such as antihistamine for at least 3 days, oral steroid and nasal steroid for at least 2 weeks and luekotriene receptor antagonist for at least a week prior to the study, but the patients could take pseudo ephedrine.

3. The participants had no exercise training program. They were non-smoker and without any food supplementation.

4. Volunteers signed the consent form to become subjects.

Exclusion criteria

1. The participants were sick or injured.

2. The participants were not voluntarily continued participating in the experiment.

Study II: The effects of moderate exercise training combined with vitamin C supplementation on physiological changes and symptoms in allergic rhinitis patients.

Inclusion criteria

1. The study was composed of 19 patients with allergic rhinitis (8 control group, 9 exercise group and 10 exercise combined vitamin C supplementation group)

2. The rhinitis patients were the persistent allergic rhinitis who had rhinitis symptoms more than 4 days a week and positive skin prick test to house dust mite (D.pteronyssinus).

- They had no complications with allergic rhinitis and sinusitis, ear tube malfunctions and asthma, and patients without kidney disease and kidney stones.

- All volunteers stopped taking all medicine before the study such as antihistamine for at least 3 days, oral steroid and nasal steroid for at least 2 weeks and luekotriene receptor antagonist for at least a week prior to the study, but the patients could take pseudo ephedrine.

3. The participants had no exercise training program. They were non-smoker and without any food supplementation.

4. Volunteers signed the consent form to become subjects.

Exclusion criteria

1. The participants were sick or injured.

2. The participants were not voluntarily continued participating in the experiment.

3. The participants who participate less than 80% of training program.

Data collection

All allergic rhinitis patient volunteers were diagnosed by the co-advisor (Asst. Prof. Jettanong Klaewsongkram, M.D.), a medical professor of allergy and clinical immunology division, King Chulalongkorn Memorial hospital. The research working was carried out in the Faculty of Sports Science, Chulalongkorn University, Bangkok, Thailand.

Instruments

Instrument used in the selection of the sample

1. The Patient / Participant Information Sheet
2. The Informed Consent Form
3. The Physical Activity Readiness Questionnaire (PAR-Q)
4. The general health history questionnaire

Instrument for exercise training protocol

1. Treadmill (Landice, UK.)
2. Heart rate monitor (Polar, Finland)

Instrument for measuring physiological data variables

1. Body composition analyze (Inbody, Korea)
2. Digital blood pressure (Omron, Japan)
3. Heart rate monitor (Polar, Finland)

Instrument of measuring blood chemical data variebles

1. Centrifugator

2. Freezer - 80°C

3. Flow cytometer (BD FACSCalibur Flow Cytometer, USA)

Instruments for measuring symptoms variables of allergic rhinitis

1. Laser Doppler flowmeter (DRT4 MoorLAB, Moor instrument, England)

2. Peak nasal inspiratory flow meter (Clement Clark International model IN-CHECK ORAL, UK.)

3. Rhinitis symptoms score

Instruments for measuring physical fitness variables

1. Spirotouch (Spacelabs Burdick, Inc., Deerfield, Wisconsin USA.)

2. Cardiopulmonary gas exchange system (Cortex, Metamax 3X): Breath by breath, Germany)

Methodology

Study I: The effect of acute exhaustive and moderate intensity exercise on physiological changes and symptoms in allergic rhinitis patients. (Figure 3.1)

1. The volunteers have been aware of the details to perform the testing and data collection and signed in the Informed Consent Form.

2. The participants were divided into 2 groups which are

Group I: 14 Healthy subjects.

Group II: 13 Allergic rhinitis patients.

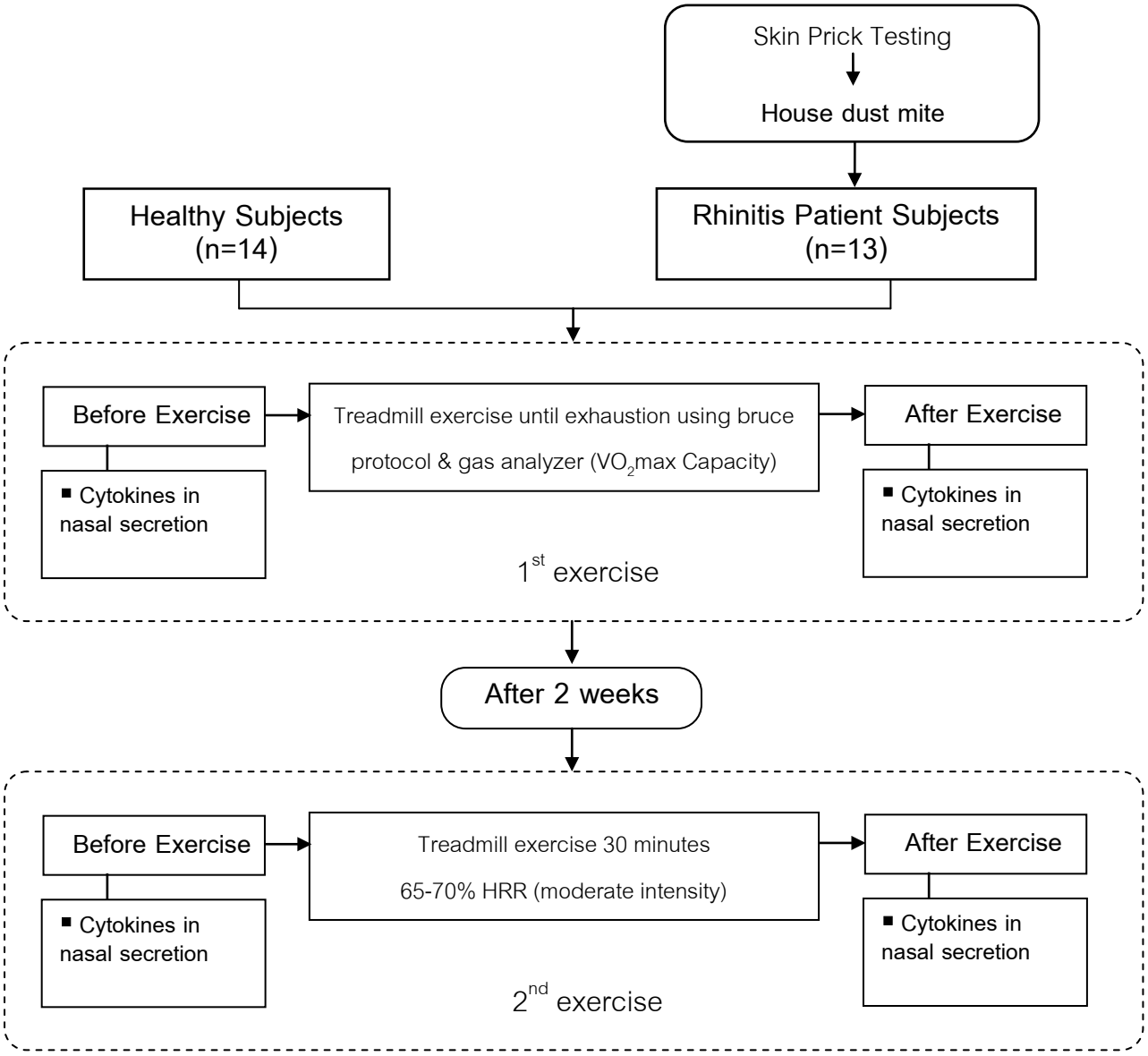

Figure 3.1 Study I procedure

3. Two exercise protocols were set in this study. Exhaustive exercise was performed using the Bruce treadmill protocol (Souza MS. et al., 2004). Subjects were asked to run on a treadmill (Landice, USA) in which the grade and intensity were increased every 3 minutes until exhaustion. Heart rate, oxygen consumption (VO_2) and carbondioxide production (VCO_2) was measured throughout the test using a breath-by-breath gas analysis system (Cortex Metamax 3X, Germany). Hereafter, we refer to this VO_2max test as the exhaustive exercise protocol. After 2 weeks, each subject

performed a moderate-intensity exercise bout, which comprised of running on the treadmill at an intensity corresponding to 65-70% heart rate reserve for 30 minutes.

4. The data collection procedure that is defined (Figure 3.1). Subjects in all group have been tested parameters before and after experimental such as; physiological characteristics, pulmonary function, blood collection and nasal secretion collection.

Study II: The effects of moderate exercise training combined with vitamin C supplementation on physiological changes and symptoms in allergic rhinitis patients. (Figure 3.2)

1. The volunteers have been aware of the details to perform the testing and data collection and signed the Informed Consent Form.

2. The sample account for 3 groups by using immunoglobulin E (IgE) data.

Group I: Subjects could do daily life as usual but no exercise program, 8 persons.

Group II: Subjects were aerobic exercise by walking - running on a treadmill at a moderate intensity or about 65-70% of heart rate reserve (HRR) combined with placebo, 9 persons. The target heart rate used this following formula;

$$\text{Target HR} = (HR_{max} - HR_{rest}) \times \%\text{intensity} + HR_{rest}$$

Group III: Subjects were aerobic exercise by walking - running on a treadmill at a moderate intensity or about 65-70% of heart rate reserve (HRR) combined with vitamin C supplementation, 10 persons.

Exercise training protocol

Subjects got exercise training for 30 minutes per session three times a week for 8 weeks at the Faculty of Sports Science and they were took care by the researchers and staff. Subjects wear heart rate monitor (Polar, Finland) for control heart rate, warm up and stretching about 5 minutes, then walking - running on a treadmill (Landice, USA) at intensity of 65-70% HRR. The speed start at about 5 km

/ hour and the slope at about 0-2% level for 30 minutes, after that cool down for 5 minutes, so take the time to exercise a total of 40 minutes.

Vitamin C supplementation

The participants were vitamin C supplemented daily with an oral dose of 2,000 mg 2 times/day (1,000 mg in the morning and evening) for 2 months. (Ascorbic acid, The Government Pharmaceutical Organization, Thailand)

3. The data collection procedure that is defined (Figure 3.2). Subjects all group have been tested parameters before and after experiment. The test is divided into the following 2 days. The first day, subjects were collected physiological characteristics variables, physical fitness variables and blood chemical variables. The second day, they were collected nasal blood flow, peak nasal inspiratory flow, cytokine in nasal secretion, rhinitis symptom scores and nasal challenge by house dust mite (*D.pteronyssinus*).

Parameter Assessment

Physiological characteristics

3.1 Physiological characteristics testing

Body composition assessment

Direct segmental multi-frequency bioelectrical impedance analysis method is used to measure percentage body fat, a body composition analysis device (InBody 220, Biospace, Korea). Subjects take off their shoes and socks before measured.

Resting heart rate

The participant was sitting and had an adequate rest period of at least 5 minutes prior to the measurement. Adequate rest was indicated when the heart rate had stabilized at a low rate. The resting heart rate was measured with heart rate monitor (Polar, Finland).

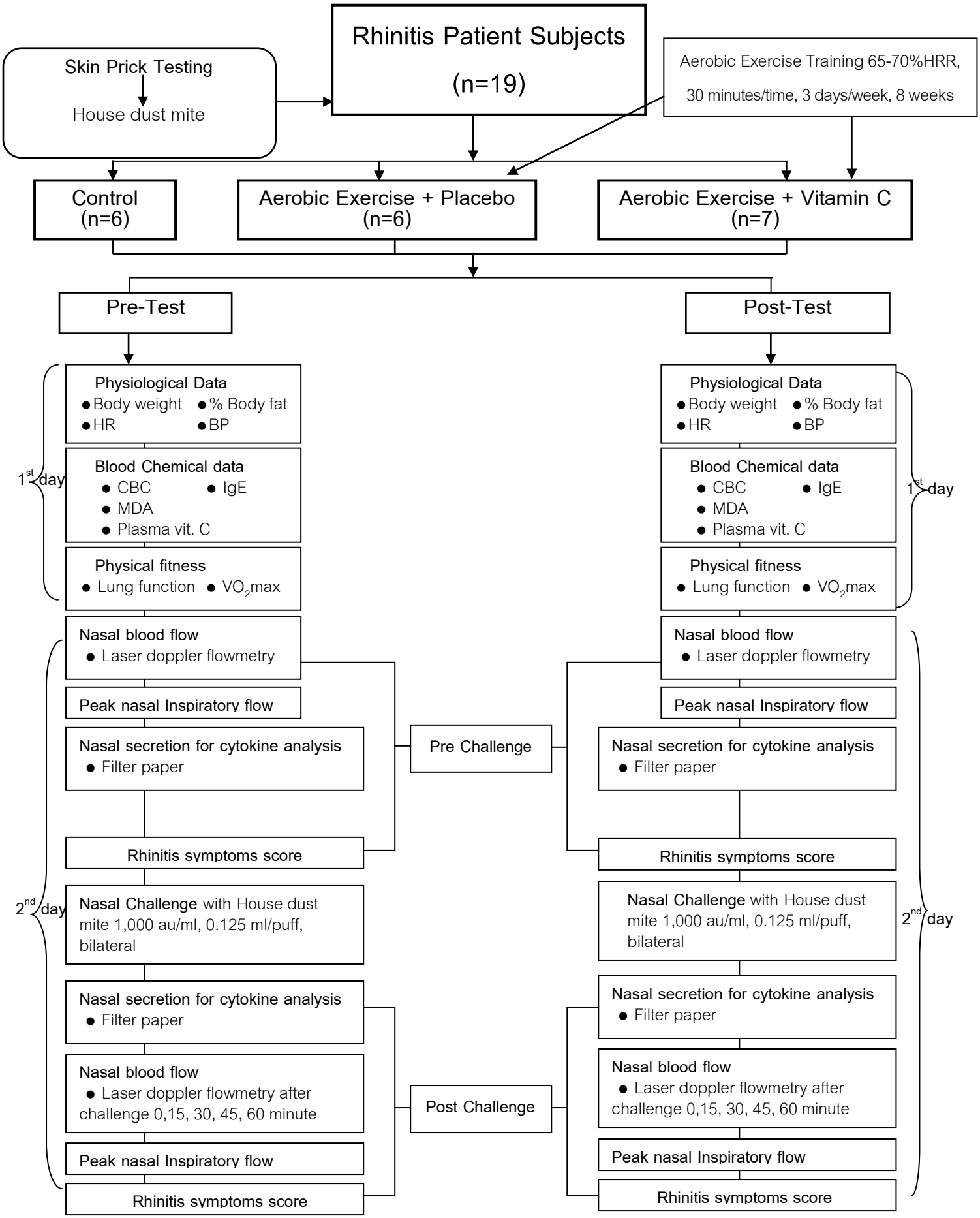

Figure 3.2 Study II procedure

Resting blood pressure

The participants were sitting upright in a straight backed chair. Both feet were flat on the floor, and the left arm was resting on the table with the elbow flexed. Subject was relaxed for a few minutes in this position. Conversation was discouraged. The blood pressure was measured with digital blood pressure (Omron, Japan). The phase systolic pressure and diastolic pressure were recorded in millimeters of mercury (mmHg).

Physical fitness testing

Pulmonary function

Pulmonary function (FVC and FEV_1) were conducted on all subjects using a calibrated computerized pneumotachograph spirometer (Spirotouch; Burdick, Inc., Deerfield, Wisconsin USA.) according to American Thoracic Society (ATS) recommendations (Laszlo G., 2006).

Cardiorespiratory fitness (VO_2max) testing

VO_2max was performed using bruce treadmill protocol. Subjects were asked to run on a treadmill (Landice, USA) in which the grade and intensity were increased every 3 minutes until exhaustion.

Blood collection and analysis

Blood samples were obtained from an antecubital vein. A portion of blood was collected into a tube containing ethylenediamine tetraacetic acid and was place immediately on ice for the determination of hematological parameters and vitamin C level. Another portion of blood was collected in plain tubes, left on ice for 30 min to clot and centrifuged at 1200 g for 20 min at 4 ºC for serum separation. Lipid profiles including total cholesterol, triglyceride, high density lipoprotein (HDL-C), and low density lipoprotein (LDL-C) were analyzed using the homogenous enzymatic colorimetric method. The rest serum was transferred in tubes and was stored at -70 ºC until analysis (Suksom D. et al., 2011).

Nasal secretion collection and handing

Nasal secretions collection were performed bilaterally with filter paper strips (7x30 mm Whatman No.42, Whatman, Clifton, NJ). Three filter paper strips were sequentially placed on each anterior portion of the inferior turbinate for 10 min. This filter paper strips were collected into appropriate tubes and centrifuged at 3,000 rpm for 5 min at 4 °C and immediately frozen at -70 °C until later analysis.

Cytokines analysis

The cytokines IL-2, IL-4, IL-13, and tumor necrosis factor (TNF)-α in blood and nasal secretion were determined by using the flow cytometry technique (Scavuzzo MC. et al., 2003). Data were acquired using the Flow cytometer (BD FACSCalibur Flow Cytometer, USA) and analyzed by Flowcytomix™ Pro software (eBioscience, USA.).

Rhinitis symptom score

Nasal symptoms were recorded using rhinitis symptom scores questionnaires. The patients were asked to score symptoms of persistent allergic rhinitis; nasal congestion, itching, sneezing, and rhinorrhea before and after each exercise protocol. The score ranged from 0 to 3 points (0 = none, 1 = mild, 2 = moderate, 3 = severe).

Nasal blood flow

Nasal mucosa blood flow was measured by laser doppler flowmetry (DRT4 moor instrument, UK.). A side delivery endoscopic probe with flexible nylon sleeve diameter 1.34 mm. was place on anterior surface of the nose. The nasal blood flow values before and after exercise in each protocol were measured.

Peak nasal inspiratory flow

Peak nasal inspiratory flow (PNIF) measured by using a Peak nasal inspiratory flow meter (Clement Clark International model IN-CHECK ORAL, UK.) attached to an anesthesia mask. During this procedure, subjects places the mask over the nose and mouth and inspires forcefully through the nose, with lips tightly closed. The measurement is carried out in a scale which varied between 30-370 liters/minute. PNIF was measured before and after exercise in each protocol.

Nasal secretion collection and handing

Nasal secretion collection was performed bilaterally with filter paper strips (7x30 mm Whatman No.42, Whatman, Clifton, NJ). Three filter paper strips were sequentially placed on each anterior portion of the inferior turbinate for 10 min. This filter paper strips were collected into appropriate tubes and centrifuged 3,000 rpm for 5 min at 4 °C and immediately frozen at -70 °C until later analysis.

Nasal challenge

Participants were encouraged to nasal challenge by house dust mite allergen. Bilateral nasal provocation used a nasal spray (metered-dose bottle) delivering a fixed volume of 0.125 mL/puff, 1 puff in each nostril of 1000 AU/ml of *D. pteronyssinus* (Chusakul S. et al., 2010).

Data analysis

All data were presented as means and standard errors of mean (SEM). Pair t-test for analyzed between pre and post-test were used. For comparison among groups, one way analysis of variance (one-way ANOVA) was used. One-way repeated measure ANOVA for analyze between each time after nasal challenge. An alpha level of 0.05 was used to determine statistical significance. All statistical analyses were performed using SPSS version 17 for Windows statistical software (SPSS Inc., Chicago, USA).