# รายงานวิจัยฉบับสมบูรณ์

## โครงการ ผลของการฝึกแอโรบิกแบบหนักสลับเบาที่มีต่อการเสื่อมของเซลล์บุผนังหลอดเลือดตามอายุ ในกลุ่มคนอายุน้อยและผู้สูงอายุ

Effects of Interval Aerobic Training on Age-Associated Endothelial Function in the Youngs and Elderlies

**โดย ผู้ช่วยศาสตราจารย์ ดร.วิทิต มิตรานันท์ และคณะ**

พฤษภาคม 2563

สัญญาเลขที่ MRG5980163

# รายงานวิจัยฉบับสมบูรณ์

## โครงการ ผลของการฝึกแอโรบิกแบบหนักสลับเบาที่มีต่อการเสื่อมของเซลล์บุผนังหลอดเลือดตามอายุในกลุ่มคนอายุน้อยและผู้สูงอายุ

## Effects of Interval Aerobic Training on Age-Associated Endothelial Function in the Youngs and Elderlies

| คณะผู้วิจัย | สังกัด |
|---|---|
| 1. ผู้ช่วยศาสตราจารย์ ดร.วิทิต มิตรานันท์ | มหาวิทยาลัยศรีนครินทรวิโรฒ |
| 2. ศาสตราจารย์ ดร.ดรุณวรรณ สุขสม | จุฬาลงกรณ์มหาวิทยาลัย |

สนับสนุนโดยสำนักงานคณะกรรมการอุดมศึกษา และสำนักงานกองทุนสนับสนุนการวิจัย

**Project title: Effects of Interval Aerobic Training on Age-Associated Endothelial Function in the Youngs and Elderlies**

**Principal investigator:** Witid Mitranun, Department of Sport Science, Faculty of Physical Education, Srinakhariwirot University, Thailand

## Abstract:

Aging is one of the major causes for the pathogenesis of cardiovascular disease. The mechanism of disease is through the endothelial dysfunction by the increment of oxidative stress, decreased flow-mediated dilatation (FMD) and increased pulse wave velocity (PWV). Exercise training, a non-pharmacological therapeutic option, has shown to improve endothelial function. Habitual exercise shows the significant enhancement of vascular function. However, the effects of exercise on age-related endothelial function in the youngs and elderlies are still unclear. In this study, we selected the interval aerobic exercise (INT) program and measure various vascular parameters before the training, 12 weeks after training, 4 weeks of detraining, and 6 weeks of detraining. Therefore, subjects were separated into four groups: SED-elderly = Sedentary elderly subjects, INT-elderly = Elderly subjects perform the interval aerobic training, SED-young = Sedentary young subjects, and INT-young = Elderly subjects perform the interval aerobic training.

Both SED-elderly and INT-elderly groups showed similar results in all parameters at baseline. And also, the similarity was observed at baseline in both SED-young and INT-young groups. However the elderly group (SED-elderly and INT-elderly) showed the significant difference in all parameters at baseline as compared to the young group (SED-young and INT-young). After 12 weeks of interventions, both the INT-elderly and the INT-young groups showed the decrement of body fat, systolic blood pressure, diastolic blood pressure, mean arterial pressure, brachial-ankle pulse wave velocity (baPWV), carotid intima-media thickness (IMT), and malondialdehyde, in the similar way as the increment of maximal $O_2$ consumption, Knee flexion muscular strength, knee flexion muscular strength, flow-mediated dilatation and nitric oxide. But only INT-elderly group had a significant decrease in body mass, and body mass index. Interestingly, both the INT-elderly and the SED-young group demonstrated the equality of body mass index, systolic blood pressure, diastolic blood pressure, mean arterial pressure, maximal $O_2$ consumption, baPWV and malondialdehyde at 12 weeks of the exercise program.

At the detraining phase, the INT-elderly showed the improvement of flow-mediated dilatation, baPWV, and nitric oxide at 12 weeks and a month of detraining and the value of these parameters return to the baseline at 6 weeks of detraining. Only the enhancement of baPWV value in the INT-elderly could maintain even after 6 weeks of detraining. In the INT-young group, flow-mediated dilatation, baPWV, and nitric oxide return to baseline after 4 weeks of detraining. In addition, the percent change of flow-mediated dilation, nitric oxide, baPWV, and malondialdehyde were higher in the INT-elderly group.

We conclude that interval aerobic training is an effective program for improving health-related physical fitness and age-associated endothelial function in the youngs and elderlies. The potential of the training is to reverse in some parameters of endothelial dysfunction in older age to same level as young age individuals and can maintain endothelial function after detraining. This study will be beneficial to recommend interval aerobic exercise program as a treatment for the improvement the endothelial function in the process of aging.

Keyword: Flow mediated dilatation, Oxidative stress, Pulse wave velocity, Interval aerobic training, Aging, Endothelial dysfunction

Research area: Sport Science

# Executive Summary

โครงการ ผลของการฝึกแอโรบิกแบบหนักสลับเบาที่มีต่อการเสื่อมของเซลล์บุผนังหลอดเลือดตามอายุในกลุ่มคนอายุน้อยและผู้สูงอายุ เป็นการศึกษาผลของการฝึกแอโรบิกแบบหนักสลับเบาที่มีต่อหน้าที่การทำงานของเซลล์บุผนังหลอดเลือดในกลุ่มคนอายุน้อยและผู้สูงอายุ การศึกษาในครั้งนี้เป็นการแก้ปัญหาการเสื่อมของเซลล์บุผนังหลอดเลือดตามอายุโดยใช้โปรแกรมการออกกำลังกายที่ผู้วิจัยได้ออกแบบขึ้นมา นอกจากนี้ยังศึกษาผลของการคงอยู่ภายหลังจากการหยุดออกกำลังกายที่ส่งผลต่อสมรรถภาพร่างกายและหน้าที่การทำงานของเซลล์บุผนังหลอดเลือด

การศึกษาครั้งนี้เก็บข้อมูลในกลุ่มวัยรุ่นและกลุ่มผู้สูงอายุ โดยแบ่งการการทดลองออกเป็น 4 กลุ่ม ดังนี้

1. กลุ่มคนอายุน้อยที่เป็นกลุ่มควบคุม
2. กลุ่มคนอายุน้อยที่นำมาฝึกแอโรบิกแบบหนักสลับเบา
3. กลุ่มผู้สูงอายุที่เป็นกลุ่มควบคุม
4. กลุ่มผู้สูงอายุนำมาฝึกแอโรบิกแบบหนักสลับเบา

ภายหลังจากการเข้าร่วมโปรแกรมการฝึกแอโรบิกแบบหนักสลับเบาเป็นระยะเวลา 12 สัปดาห์ พบว่า สมรรถภาพทางกายในด้านต่างๆ และหน้าที่การทำงานของเซลล์บุผนังหลอดเลือดดีขึ้นทั้งในกลุ่มคนอายุน้อยและกลุ่มผู้สูงอายุ นอกจากนี้ยังพบว่า ผลจากการออกกำลังกายนี้ทำให้บางตัวแปรสมรรถภาพทางกายและหน้าที่การทำงานของเซลล์บุผนังหลอดเลือดของผู้สูงอายุได้แก่ ดัชนีมวลกาย, ความดันโลหิตตัวบน ความดันโลหิตตัวล่าง ความดันโลหิตเฉลี่ย ค่าการใช้ออกซิเจนสูงสุด ค่าการวัดความแข็งตัวของหลอดเลือด (Brachial-ankle pulse wave velocity; baPWV) และมาลอนไดอัลดีไฮด์ สามารถพัฒนาจนกระทั่งมีค่าเทียบเท่ากลุ่มคนอายุน้อยอีกด้วย

ภายหลังจากหยุดออกกำลังกายไป 4 สัปดาห์ หรือ 6 สัปดาห์ พบว่า บางตัวแปรของการวัดหน้าที่การทำงานของหลอดเลือดได้แก่ ไนตริกออกไซด์ และค่าการไหลของเลือดผ่านการขยายตัวของหลอดเลือด (Flow-mediated dilatation; FMD) สามารถคงสภาพของการพัฒนาได้ 4 สัปดาห์ และบางตัวแปรของการวัดหน้าที่การทำงานของหลอดเลือด ได้แก่ ค่าการวัดความแข็งตัวของหลอดเลือ สามารถคงสภาพของการพัฒนาได้ถึง 6 สัปดาห์ และเป็นที่น่าสนใจว่าเปอร์เซ็นการพัฒนาของตัวแปรด้านหลอดเลือดในกลุ่มผู้สูงอายุอันเป็นผลมาจากการออกกำลังกายจะมีค่าที่สูงกว่ากลุ่มคนอายุน้อย

ประโยชน์ที่ได้จากงานวิจัยในครั้งนี้คือ การเข้าใจกลไกการเปลี่ยนแปลงของสมรรถภาพทางร่างกายและหน้าที่ของเซลล์บุผนังหลอดเลือดในช่วงวัยที่แตกต่างกันอันเป็นผลมาจากการฝึกแอโรบิกแบบหนักสลับเบา ทั้งนี้เราสามารถนำโปรแกรมการออกกำลังกายไปแนะนำให้กลุ่มผู้สูงอายุหรือผู้มีอายุน้อยให้ปฏิบัติ เพื่อพัฒนาหรือฟื้นฟูสมรรถภาพทางร่างกายและหน้าที่การทำงานของเซลล์บุผนังหลอดเลือดได้

## Introduction

Vascular endothelium is a simple squamous monolayer cells that lined inside the lumen of blood vessel and lymphatic vessel in the mosaic pattern. The crucial roles of endothelial cells are controlling of vascular tone and systemic blood pressure. Flow-mediated dilation (FMD) test is one of the noninvasive standard for assessing in vivo of endothelial-dependent vasodilation (Stoner et al., 2011), through the response to shear stress and cause the releasing of nitric oxide (NO), prostacyclin (PC) and endothelial-derived hyperpolarizing factor (EDHF) (Clifford and Hellsten, 2004). FMD can be measured by the changes of arterial diameter which monitored by high-resolution external vascular ultrasound in response to an increase in blood flow causing shear-stress during reactive hyperemia (sphygmomanometer cuff inflation and then deflation).

Endothelial dysfunction can be induced by mechanical denudation, hemodynamic forces, immune complex deposition, irradiation, and chemicals (Betik et al., 2004) . Reactive oxygen species (ROS) are chemicals that can induce endothelial injury while antioxidants can inhibit oxidative stress to the endothelial cells. Endothelial dysfunction is an early event of the atherosclerotic process which associated with increased pulse wave velocity (PWV), an indicator of arterial stiffness (van Popele et al., 2001) and low magnitude of FMD (Betik et al., 2004) . Clinically, PWV and FMD can be used as indicators for cardiovascular diseases, cerebrovascular diseases, congestive heart failure and their mortality risks (Brunner et al., 2005; Laurent et al., 2001; Mitchell et al., 2010).

Aging is one of the major factors for the development of endothelial dysfunction (Lakatta and Levy, 2003) . Old age individuals have vascular stiffness, loss of vascular elasticity and having vascular endothelial dysfunction (Santos-Parker et al., 2014). The increment of PWV and the decrement of FMD can be observed in advancing age even in adults without any significant cardiovascular disease (Eskurza et al., 2004; Lakatta and Levy, 2003). The mechanism of aging in vascular changes can be explained by oxidative stress of increased superoxides resulted in collagen deposition, fragmentation of elastin, protein oxidation and formation of advanced glycation end products (Santos-Parker et al., 2014)

Aerobic exercise training, a non-pharmacological therapeutic option, has shown to improve endothelial function and inhibited the pathologic change of arterial stiffness (Fleenor et al., 2013; Santos-Parker et al., 2014). In old age persons, aerobic exercise showed the superior arterial compliance over the sedentary persons in the age and sex matched individuals (Santos-Parker et al., 2014). A recent cross sectional study of an endurance exercise training in the middle age and old age subjects have also shown the results of the lower PWV and the higher FMD (Santos-Parker et al., 2014). Considering potential of aerobic exercise, it is stated that habitual moderate intensity can improve the age-related vascular function (Santos-Parker et al., 2014; Xia et al., 2012). Interval aerobic exercise training, intermittent bout of high intensity, have emerged for recent years and have shown the notable results on endothelial function compared to continuous of moderated aerobic exercise training in patients with heart failure (Wisloff et al., 2007), metabolic syndrome (Tjonna et al., 2008), and type 2 diabetes (Mitranun et al., 2014). However, there is no known significant scientific study of the effects of interval aerobic exercise on age-related endothelial function. This is the interesting question to study the endothelial effects on different age groups; young and older. We decided to select our previous exercise program which is an unique interval aerobic exercise training program (Mitranun et al., 2014) to apply in the present study of age-related endothelial function. The purposes of our study are for avoiding endothelial dysfunction in acute exercise and the progressive step up process of exercise to achieve the most effective outcome. Acutely high exercise intensity normally contribute to a decrement of FMD (Cosio-Lima et al., 2006; Harris et al., 2008). Thus, our program was designed to perform in moderate intensity in the first phase duration to avoid the acute effects of endothelial dysfunction. However, in some previous studies did not confirm this acute effect (Tjonna et al., 2008; Wisloff et al., 2007). In the later phases of this study, progressive program of the intensity and volume in second and third phases were added after vascular adaptation occurred in the first phase of exercise. Accordingly, the purpose of the present study was to investigate the effects of interval aerobic exercise training on age-related endothelial function. We will also study the endothelial effects of detraining after completion of exercise program. We hypothesize that interval aerobic training is a potent program sufficient for reversing the endothelial function in older age to same level as young age and might maintain endothelial function after detraining. This study will be beneficial to suggest interval aerobic exercise program as treatment of the endothelial aging process.

Conceptual Framework

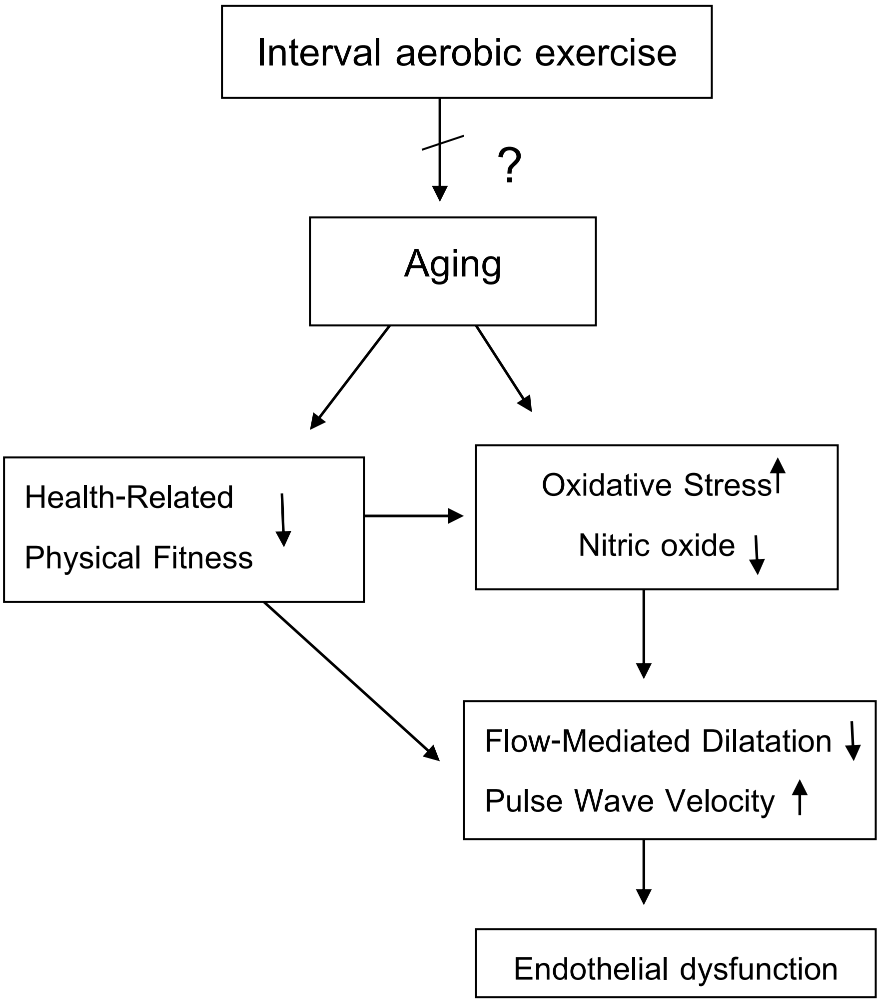



Figure 1 Conceptual framework

## Objective

The aims of this study are to investigate 1) the effects of interval aerobic exercise training on various vascular parameters in different age group participants. 2) the potential effect of interval aerobic exercise training on maintaining vascular function in detraining period.

## Methodology

### -Participants

The participants consist of untrained and nonsmoking males from Srinakharinwirot University, Nakhon Nayok, Thailand. The inclusion criteria are including healthy males, ages 20-30 years and 60-70 years and no previous exercise training in the past 6 months. All participants were free from any recent injuries, and had no history of cardiovascular and cerebrovascular diseases. The present study will be submitted for approving by the Ethics Committee of Srinakharinwirot University, Thailand and will be conducted according to the Helsinki Declaration. An informed consent will be obtained from all participants.

There are 60 male participants in this study which consist of 30 participants, age ranged 20-30 years and 30 participants, age ranged 60-70 years. The eligible participants in each age ranged group will be randomly allocated in equal numbers into 2 groups: control (sedentary) group which will not perform any exercise training and interval aerobic training (INT) group. Therefore, subjects were separated into four groups:

1. SED-elderly = Sedentary elderly subjects
2. INT-elderly = Elderly subjects perform the interval aerobic training
3. SED-young = Sedentary young subjects
4. INT-young = Elderly subjects perform the interval aerobic training

The sampling is assigning subjects into control groups and exercise groups. The random assignment of subjects in control groups and exercise groups help to ensure that among all four groups will be in the homogeneous populations, (Figure 2).

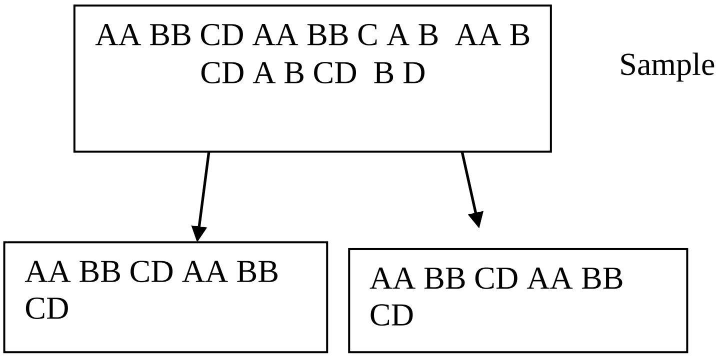


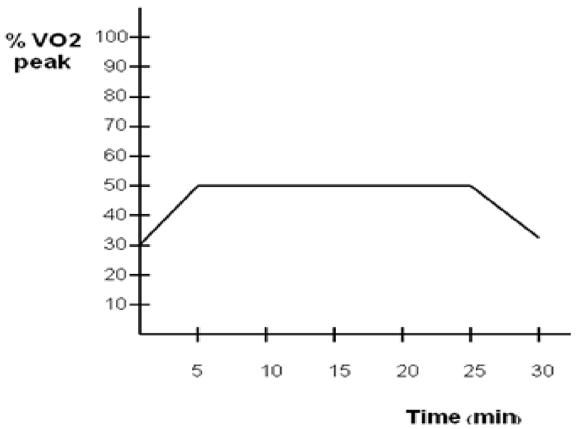

Figure 2 Random assignment

-Exercise training programs

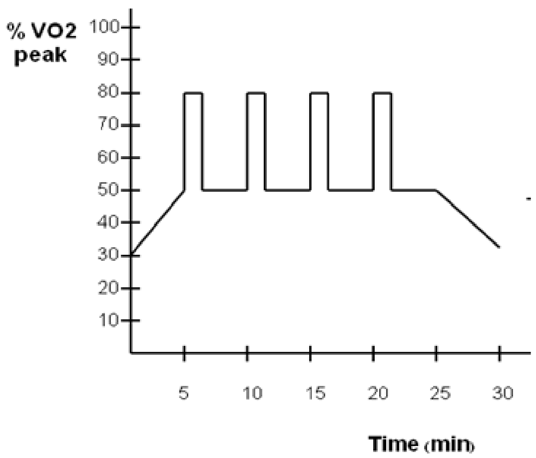
The exercised participants undergo a 12-week training period, which involved exercising 3 days per week. Interval aerobic training (INT) program is assigned in each participants group of exercise.

The interval exercise training (INT) program: In the Phase 1, the participants warmed up gradually to achieve a 50% of the peak oxygen consumption ($VO_2$ peak) within 5 min, maintained this intensity for 20 min, and 5 min for cooling down, giving a total time of 30 min. In the Phase 2, following the same warm-up to reach the 50% of $VO_2$ peak within 5 min, the participants performed the interval of four 1-min high-intensity exercises at 80% of $VO_2$ peak with a 4-min low-intensity exercise at 50% $VO_2$ peak. The exercise session was concluded with a 5-min cool-down period, giving a total session time of 30 min. In the Phase 3, all participants performed warming up to achieve a 60% of $VO_2$ peak within 5 minutes, then performed the interval of six 1-min high-intensity exercise at 85% $VO_2$ peak with a 4-min low-intensity exercise at 60% $VO_2$ peak and a 5-min cool-down period, giving a total session time of 40 min.

Phase 1 (weeks 1-2)




Phase 2 (weeks 3-6)




Phase 2 (weeks 7-12)

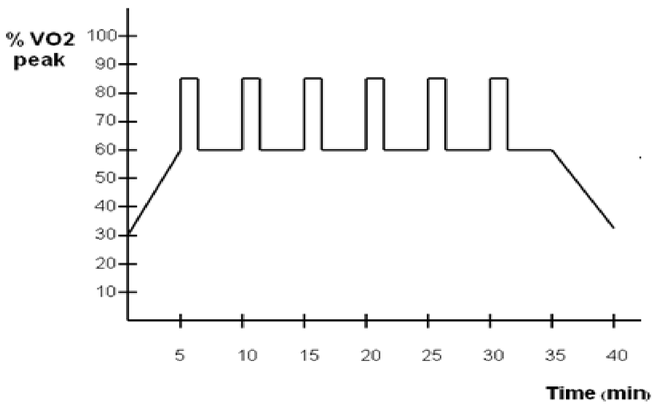



Figure 3 Interval training program

### -Measurements

Two hours after having breakfast, all participants are asked to measure the biological data, blood chemistry, brachial artery characteristics data, pulse wave velocity data and blood pressure data. Before and after 12-week interval aerobic exercise training period, all participants will be asked to measure these parameters and repeat all measurements in detraining period (4 weeks and 6 weeks after performing a 12-week interval aerobic exercise training) (Figure 3).

**Biological data;**

The participants are put in the supine position for at least 5 minutes as a resting period prior to the measurement. The blood pressure and heart rate will be measured with digital blood pressure (Omron M2, Omron Healthcare Europe B.V., Hoofddorp, Netherland). The mean arterial pressure (MAP) is calculated using the formula MAP=1/3 x [systolic blood pressure – diastolic blood pressure] + diastolic blood pressure.

**Health-related physical fitness assessment;**

Fat mass, body fat and muscle mass are performed by using DEXA. Muscle strength is performed with weight machine (Leg extension machine, Leg curl machine, Chest press machine and Lat machine, Nautilus, USA), using one repetition maximum method (1RM). The participants performed to lift the heaviest weigth only 1 time. Sit and reach box was used to assess body flexibility. Cardiovascular and respiratory fitness are assessed by Modified Bruce protocol in which the grade and intensity were increased every 3 minutes until exhaustion.

**Blood biochemistries;**

Blood samples are collected and centrifuged at 3500 rpm for 10 min at 4 °C for separation of erythrocytes and plasma. Malondialdehyde (MDA) is measured in erythrocytes with thiobarbituric acid reactive substances (TBAR) method. Nitric oxide (NO), as estimated by the total nitrite and nitrate concentrations is measured in plasma with assay kit (Colorimetric nitric oxide assay kit).

**Brachial artery characteristics data;**

Brachial artery characteristics data evaluate with the ultrasound equipment using the brachial artery occlusion on the right forearm. All participants are asked to rest in the supine position for 20 min and blood pressure cuff is placed around the right forearm throughout the measurement. The brachial artery characteristics are recorded longitudinally to antecubital fossa at 1 min baseline, 5 min occlusion and 3 min deflation (Corretti et al., 2002; Dhindsa et al., 2008). At the occlusion period, the cuff is inflated to 50 mmHg above systolic blood pressure (Mitranun et al., 2014). In order to minimized the investigator bias in image analyses, computer-bases analysis program (Brachial Analyzer, Medical Imaging Applications, Coralville, IA, USA) is used for analyzing changes on vascular diameter. Shear stress presented as shear rate is calculated by blood velocity/vascular diameter (Pyke et al., 2008). FMD is calculated using the equation FMD = (d2-d1) x 100/d1 when d1 is the average brachial artery diameter at baseline, d2 is the average brachial artery diameter post occlusion (Naidu et al., 2011). Brachial vascular conductance is calculated as brachial blood flow/mean arterial pressure. The brachial vascular resistance is a reverse ratio of brachial vascular conductance.

**Pulse velocity data** (Yang et al., 2013);

Pulse wave velocity (PWV) method will be measured using a volume-plethysmographic device with four cuffs matched with oscillometric sensors, placed around the upper arms and ankles. Deflation phase, the pulse volume records of the bilateral brachial and tibial arteries are monitored. Oscillometric method is used to calculate the blood pressure of each lesion. Electrodes are placed on both wrists and a microphone is placed on the left edge of the sternum. Transit time ($\Delta T_{ba}$) is monitored by a time delay between the feet of the wave at the two sites. The distance between the two sites of PWV is calculated by using the height of the paticipants and $\Delta T_{ba}$.

$L_b$ = 0.2195 × height of the paicipants (cm) - 2.0734

$L_a$ = 0.8129 × height of the participants (cm) + 12.328.

$PWV = (L_a – L_b) / \Delta T_{ba}$

## Scope of research

There are 60 male participants in this study which consist of 30 participants, age ranged 20-30 years and 30 participants, age ranged 50-60 years. The participants in each age ranged are randomly allocated in equal numbers into 2 groups;

-Sedentary control (SED) group which is not assign to perform any exercise training; n=15

-Interval aerobic training (INT) group; n=15

8.2 The variables used in the study are included;

-Independent variable is interval aerobic training program.

-Dependent variables are biological variables, health-related physical fitness variables, blood biochemistry variables, brachial artery characteristics variables, and pulse wave velocity variables.

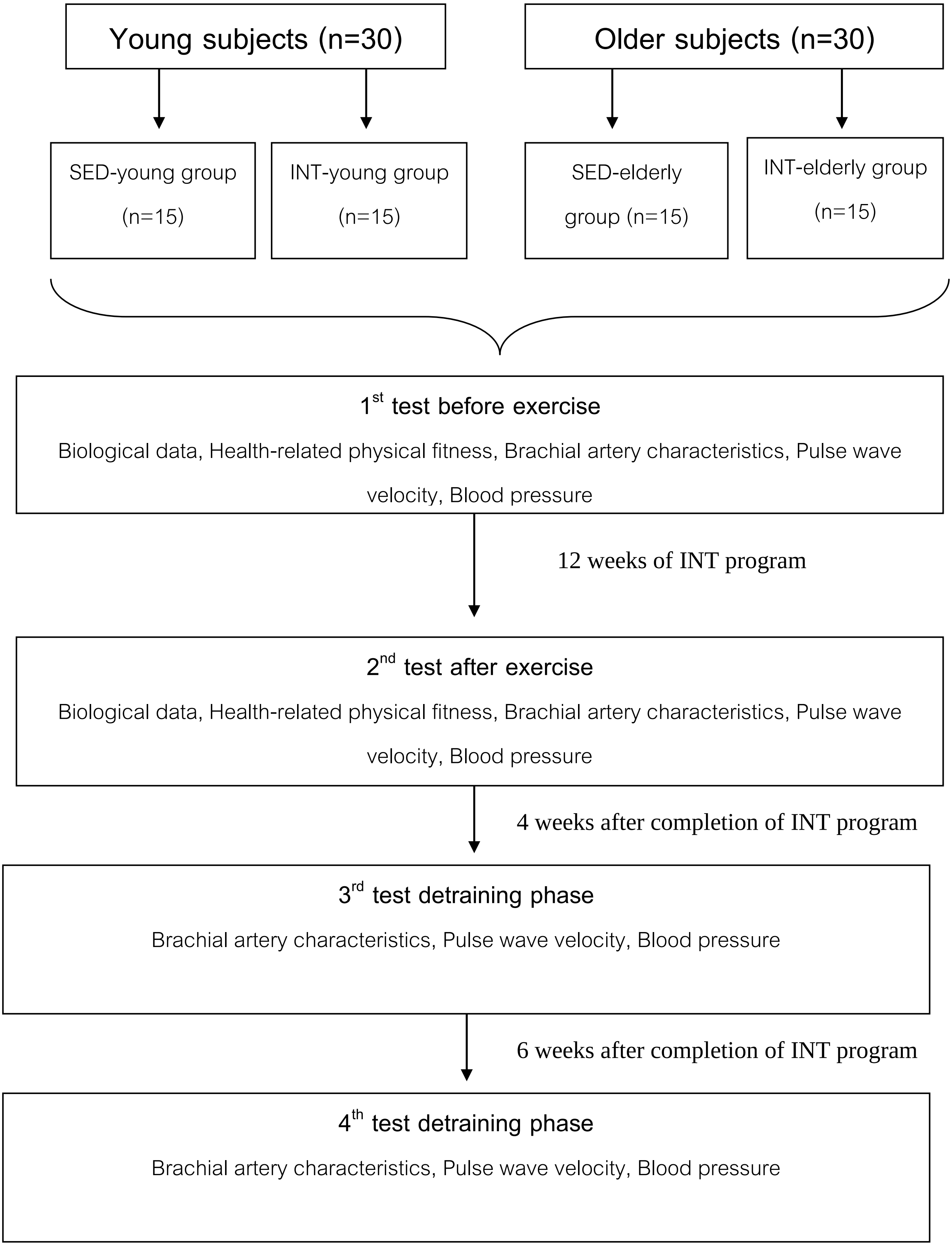



Figure 4 Procedures

## Equipment needed for the project

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 program

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

Instrument for measuring biological data variables

1. DEXA
2. Digital blood pressure
3. Heart rate monitor

Instrument for measuring blood chemical data variables

1. Centrifuge
2. Freezer -40 $^{o}$C

Instrument for measuring physical fitness variables

1. Cardiopulmonary gas exchange system
2. Treadmill
3. Leg extension machine
4. Leg curl machine
5. Chest press machine
6. Lat machine
7. Sit and reach box

Instrument for measuring brachial characteristics variables

1. Ultrasound equipment
2. Brachial artery analyzer program

Intrument for measuring pulse wave velocity variables

1. Volume-plethysmographic device

## Statistic methods

The results were expressed as mean+/-standard deviation. All the data were first checked with the tests of normality. Two-way (group × time) analysis of variance with repeated measures, followed by Tukey's multiple comparison, was used to determine the significant differences among groups. A level of $P<0.05$ was considered to be a significant difference

## Results

Biological data at baseline of participants are shown in Table 1. Age, Body mass, Body mass index, body fat, heart rate at rest, and blood pressure were higher in elderly group (n=26) as compare to young group (n=24). No significant difference of height was observed between groups

**Table 1. Biological data at baseline of participant characteristics.**

| | Young group | Elderly group |
|---|---|---|
| Number (n) | 24 | 26 |
| Age (y) | 22.6±0.7 | 67±4.8* |
| Height (cm) | 174±3.7 | 169±4.6 |
| Body mass (kg) | 73.2±5.4 | 76.4±6.5* |
| Body mass index ($kg/m^2$) | 24.2±1.1 | 26.7±0.9* |
| Body fat (%) | 19.6±1.1 | 27.4±1.4* |
| Heart rate at rest | 70.6±6.4 | 80.5±4.9* |
| Systolic blood pressure (mmHg) | 121.6±4.2 | 130.0±5.1* |
| Diastolic blood pressure (mmHg) | 77.5±3.2 | 86.0±4.3* |
| Mean arterial pressure (mmHg) | 92.5±2.7 | 98.5±3.4* |

Data are mean ± SD. * $P<0.05$ vs. Young group

Table 2. Biological and health-related physical fitness data.

| | SED-eldery (n=13) | | INT-elderly (n=13) | | SED-young (n=12) | | INT-young (n=12) | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Body mass (kg) | 76.9±6.2 | 77.2±7.1 | 75.9±6.8 | 71.3±7.0*† | 73.4±5.4†‡ | 73.0±6.1†‡ | 73.0±5.3†‡ | 72.8±6.5†‡ |
| Body mass index ($kg/m^2$) | 26.5±0.9 | 26.9±1.4 | 26.9±1.0 | 24.8±1.7*† | 24.4±1.2†‡ | 24.0±1.0† | 24.0±1.3†‡ | 23.8±1.2† |
| Body fat (%) | 27.0±1.3 | 28.2±1.0 | 27.8±1.6 | 23.5±0.9*† | 19.4±1.1†‡ | 18.9±1.0†‡ | 19.8±1.0†‡ | 16.1±1.4*†‡# |
| Heart rate at rest (bpm) | 79.3±4.8 | 80.4±5.1 | 81.7±5.0 | 80.2±6.7 | 70.3±6.5†‡ | 72.6±6.2†‡ | 70.9±5.9†‡ | 68.9±5.6†‡ |
| Systolic blood pressure (mmHg) | 128.6±4.8 | 129.7±8.4 | 131.4±5.9 | 122.3±8.2*† | 120.3±4.0†‡ | 121.9±5.2† | 122.9±5.3†‡ | 116.9±6.1*†‡# |
| Diastolic blood pressure (mmHg) | 88.3±4.0 | 87.9±5.6 | 83.7±4.7 | 76.9±6.2*† | 76.9±3.0†‡ | 78.8±5.6† | 78.1±4.0†‡ | 70.9±5.0*†‡# |
| Mean arterial pressure (mmHg) | 98.8±3.0 | 101.8±6.1 | 98.2±4.1 | 92.6±5.0*† | 92.6±2.7†‡ | 93.4±4.3† | 92.4±2.6†‡ | 86.2±3.3*† |
| Maximal $O_2$ consumption ($VO_2$ peak) (mL/kg/min) | 24.5±3.1 | 25.2±3.6 | 23.9±3.7 | 30.7±4.0*† | 33.2±4.3†‡ | 34.1±4.0† | 33.9±3.3†‡ | 39.0±4.4*†‡# |
| Trunk flexibility (cm) | -2.3±0.9 | -2.0±0.5 | -1.8±0.6 | -1.7±0.5 | 3.4±1.3†‡ | 3.8±2.7†‡ | 4.0±1.3†‡ | 4.1±1.7†‡ |
| Leg extension muscular strength (kg) | 50.1±2.6 | 52.6±3.3 | 53.8±3.4 | 60.7±4.9*† | 74.3±4.8†‡ | 76.6±5.5†‡ | 75.5±5.8†‡ | 83.6±6.5*†‡# |
| Knee flexion muscular strength (kg) | 36.5±4.5 | 38.1±5.5 | 35.9±5.3 | 45.4±5.0*† | 50.4±6.6†‡ | 51.0±6.0†‡ | 52.4±5.6†‡ | 65.4±6.3*†‡# |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Chest press muscular strength (kg) | 56.2±4.6 | 57.6±6.9 | 57.0±5.4 | 56.8±6.4 | 65.2±7.4†‡ | 63.2±6.2†‡ | 64.2±4.4†‡ | 62.2±4.2†‡ |
| Lat pulldown muscular strength (kg) | 58.2±5.6 | 59.6±3.9 | 57.2±4.4 | 58.9±6.0 | 67.8±4.4†‡ | 65.2±6.2†‡ | 66.2±5.4†‡ | 64.9±3.2†‡ |

Data are mean ± SD.

SED-elderly = Sedentary elderly subjects; INT-elderly = Elderly subjects performed interval training; SED-young = Sedentary young subjects; INT-young = Elderly subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* P<0.05 vs. Pre

† P<0.05 vs. SED-elderly at the same timeline (Pre or Post)

‡ P<0.05 vs. INT-elderly at the same timeline (Pre or Post)

# P<0.05 vs. SED-young at the same timeline (Pre or Post)

Biological and health-related physical fitness data are shown in Table 2. Both SED-elderly and INT-elderly groups showed similar results in all parameters at Pre (baseline). And also, the similarity was observed at Pre in both SED-young and INT-young groups. However the elderly group (SED-elderly and INT-elderly) showed the significant difference in all parameters at Pre as compared to young group (SED-young and INT-young). In both the INT-elderly and the INT-young groups, the decrement of body fat, systolic blood pressure, diastolic blood pressure, and mean arterial pressure were observed, in the similar way as the increment of maximal $O_2$ consumption, Knee flexion muscular strength, and knee flexion muscular strength. But only INT-elderly group had a significant decrease in body mass, and body mass index. After 12 weeks of interventions, both the INT-elderly and the SED-young group demonstrated the equality of body mass index, systolic blood pressure, diastolic blood pressure, mean arterial pressure, and maximal $O_2$ consumption

As shown in Table 3, both SED-elderly and INT-elderly groups showed similar results in all parameters at Pre (baseline). And also, the similarity was observed at Pre in both SED-young and INT-young groups. However the elderly group (SED-elderly and INT-elderly) showed the significant difference in all parameters at Pre as compared to young group (SED-young and INT-young). In both the INT-elderly and the INT-young groups, the decrement of baPWV, carotid IMT, and malondialdehyde were observed, in the similar way as the increment of flow-mediated dilatation and nitric oxide. After 12 weeks of interventions, both the INT-elderly and the SED-young group demonstrated the equality of baPWV and malondialdehyde.

Table 3. Vascular reactivity and blood chemistry data.

| | SED-eldery (n=13) | | INT-elderly (n=13) | | SED-young (n=12) | | INT-young (n=12) | |
|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| Flow-mediate dilatation (%) | 6.5±1.2 | 6.7±1.3 | 6.4±0.9 | 7.9±1.2*† | 11.4±1.8†‡ | 11.9±1.0†‡ | 11.8±1.5†‡ | 13.0±1.1*†‡# |
| baPWV (cm/sec) | 1445±154 | 1423±143 | 1501±173 | 1205±172*† | 1173±48†‡ | 1185±54† | 1157±68†‡ | 1065.0±47*†‡# |
| Carotid IMT (mm) | 0.57±0.01 | 0.58±0.01 | 0.57±0.01 | 0.55±0.01*† | 0.40±0.01†‡ | 0.39±0.01†‡ | 0.39±0.01†‡ | 0.37.1±0.01*†‡# |
| Nitric oxide (μmol/L) | 4.3±0.4 | 4.4±0.3 | 4.2±0.5 | 5.0±0.7*† | 7.9±1.3†‡ | 7.6±0.9†‡ | 7.7±1.4†‡ | 8.2±1.5*†‡# |
| Malondialdehyde (μmol/L) | 1.09±0.22 | 1.2±0.12 | 1.09±0.01 | 0.65±0.10*† | 0.64±0.04†‡ | 0.60±0.07† | 0.62±0.04†‡ | 0.55±0.07*†‡# |

Data are mean ± SD.

SED-elderly = Sedentary elderly subjects; INT-elderly = Elderly subjects performed interval training; SED-young = Sedentary young subjects; INT-young = Young subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* P<0.05 vs. Pre

† P<0.05 vs. SED-elderly at the same timeline (Pre or Post)

‡ P<0.05 vs. INT-elderly at the same timeline (Pre or Post)

# P<0.05 vs. SED-young at the same timeline (Pre or Post

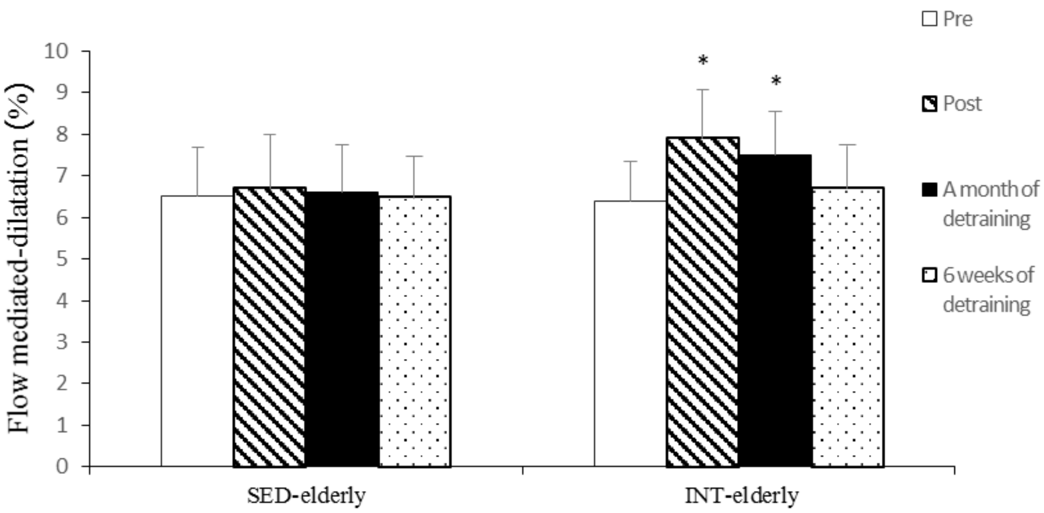



Figure 5 Flow-mediated dilatation in the elderly group

Data are mean ± SD.

SED-elderly = Sedentary elderly subjects; INT-elderly = Elderly subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* P<0.05 vs. Pre

The flow-mediated dilatation data in the elderly group at the Pre, Post, a month of detraining, and 6 weeks of detraining were shown in Figure 5. The SED-elderly group showed no significant change in all timelines, however the INT-elderly showed significant improvement of flow-mediated dilatation at Post and a month of detraining (P<0.05) as compared to Pre.

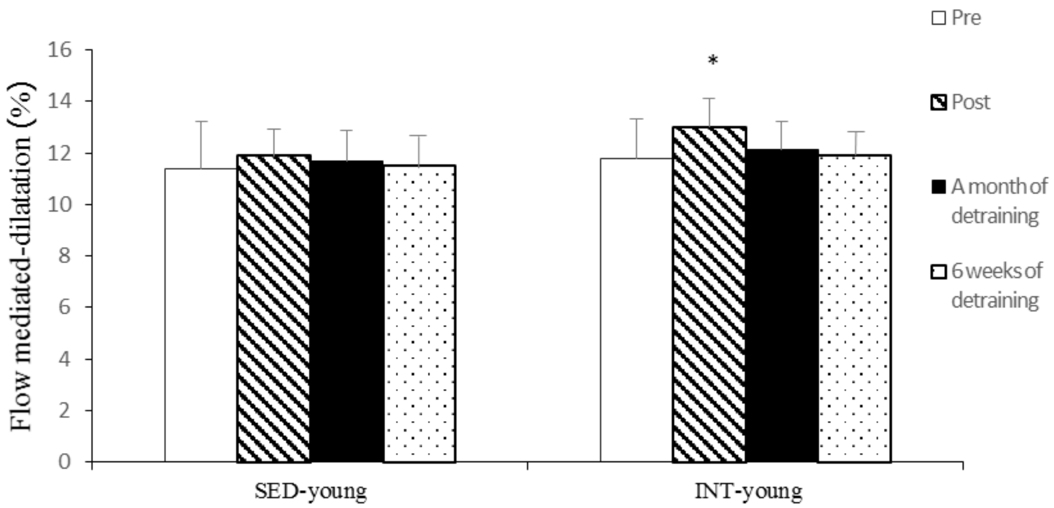



Figure 6 Flow-mediated dilatation in the young groups

Data are mean ± SD.

SED-young = Sedentary young subjects; INT-young = Elderly subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* P<0.05 vs. Pre

The flow-mediated dilatation data in the young group at the Pre, Post, a month of detraining, and 6 weeks of detraining were shown in Figure 6. The SED-young group showed no significant change in all timelines, however the INT-young showed significant improvement of flow-mediated dilatation at Post (P<0.05) as compared to Pre.

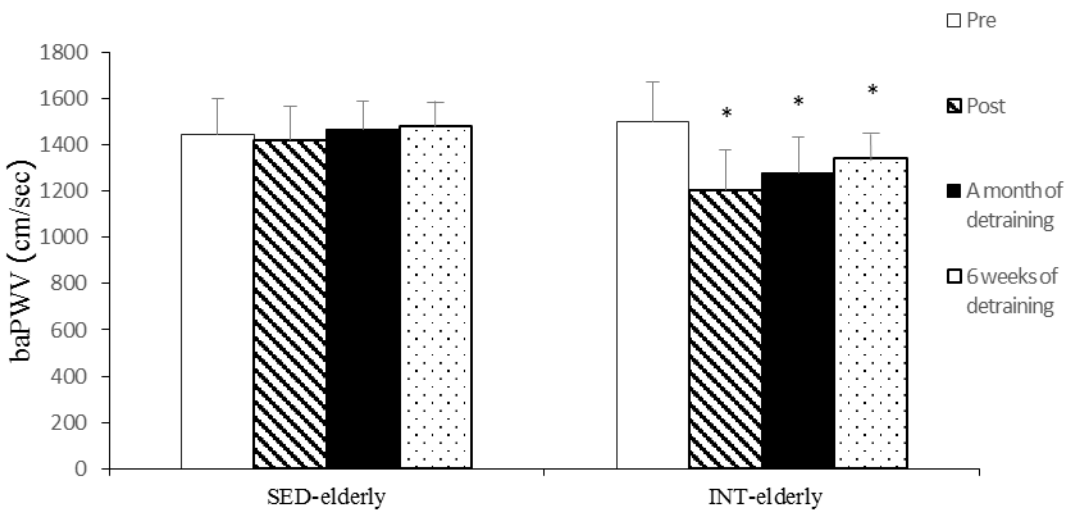



Figure 7 baPWV in the elderly groups

Data are mean ± SD.

SED-elderly = Sedentary elderly subjects; INT-elderly = Elderly subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* $P<0.05$ vs. Pre

The baPWV data in the elderly group at the Pre, Post, a month of detraining, and 6 weeks of detraining were shown in Figure 7. The SED-elderly group showed no significant change in all timelines, however the INT-elderly showed significant improvement of baPWV at Post, a month of detraining, and 6 weeks of detraining ($P<0.05$) as compared to Pre.

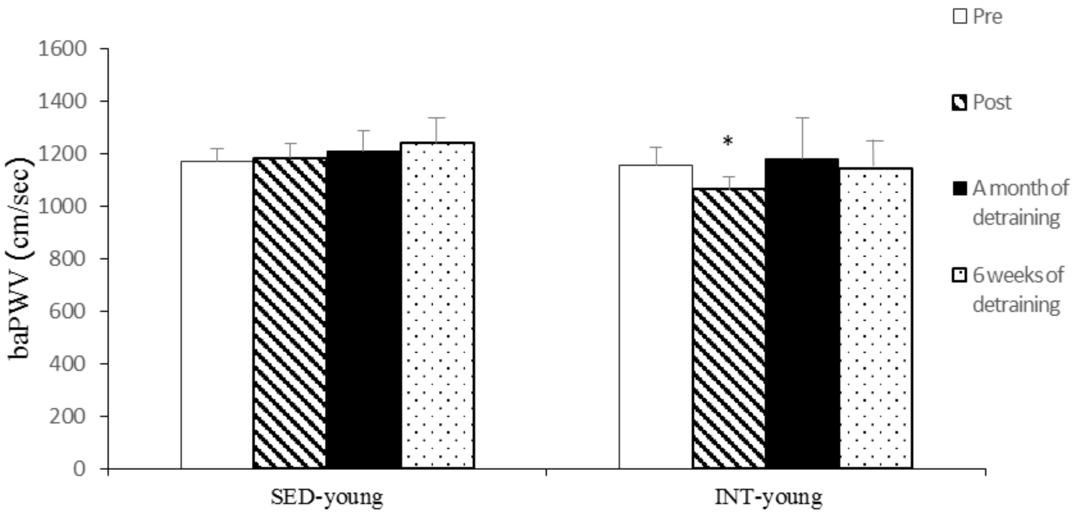



Figure 8 baPWV in the young groups

Data are mean ± SD.

SED-young = Sedentary young subjects; INT-young = Elderly subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* $P<0.05$ vs. Pre

The baPWV data in the young group at the Pre, Post, a month of detraining, and 6 weeks of detraining were shown in Figure 8. The SED-young group showed no significant change in all timelines, however the INT-young showed significant improvement of baPWV at Post ($P<0.05$) as compared to Pre.

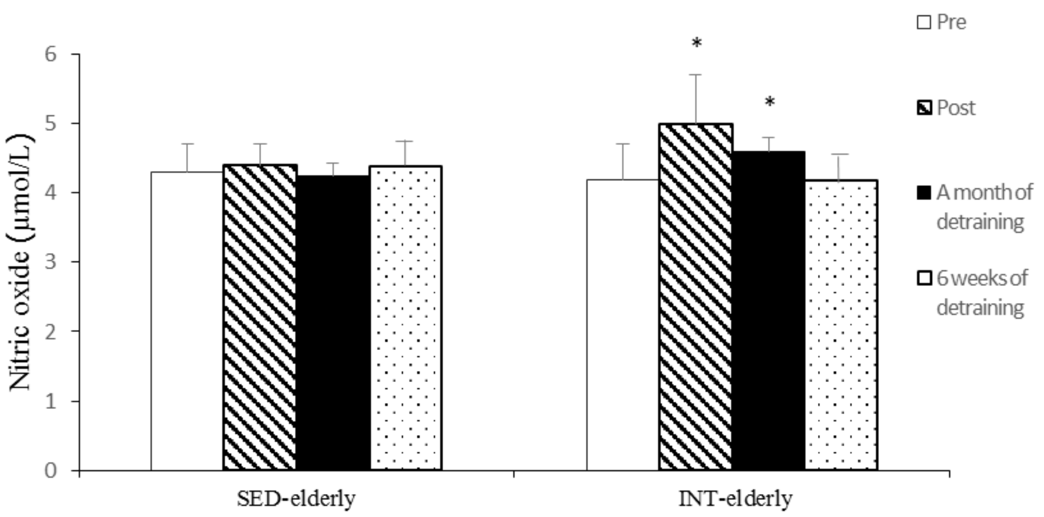



Figure 9 Nitric oxide in the elderly groups

Data are mean ± SD.

SED-elderly = Sedentary elderly subjects; INT-elderly = Elderly subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* $P<0.05$ vs. Pre

The nitric oxide data in the elderly group at the Pre, Post, a month of detraining, and 6 weeks of detraining were shown in Figure 9. The SED-elderly group showed no significant change in all timelines, however the INT-elderly showed significant improvement of nitric oxide at Post and a month of detraining ($P<0.05$) as compared to Pre.

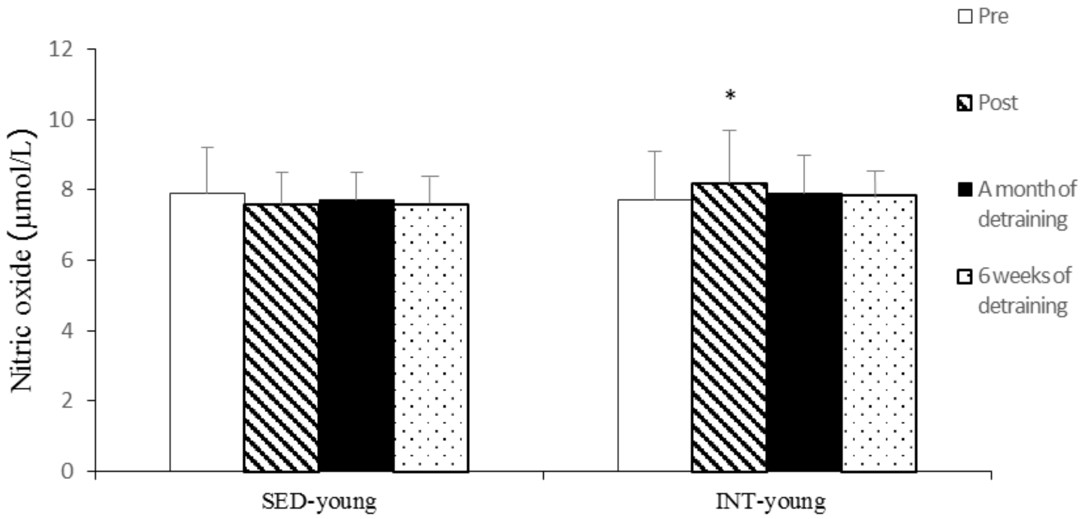



Figure 10 Nitric oxide in the young groups

Data are mean ± SD.

SED-young = Sedentary young subjects; INT-young = young subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions)

* $P<0.05$ vs. Pre

The nitric oxide data in the young group at the Pre, Post, a month of detraining, and 6 weeks of detraining were shown in Figure 10. The SED-young group showed no significant change in all timelines, however the INT-young showed significant improvement of nitric oxide at Post ($P<0.05$) as compared to Pre.

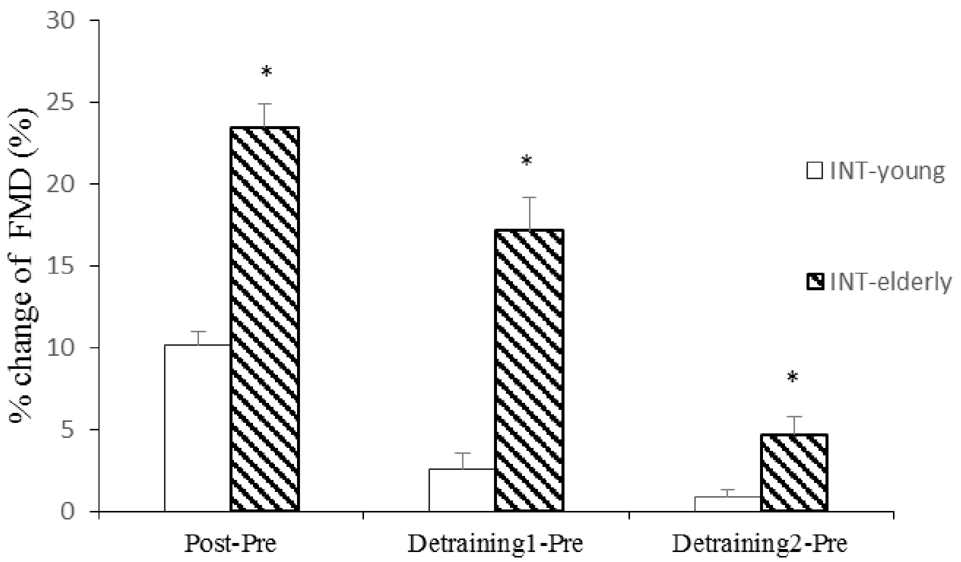



Figure 11 % change of Flow-mediated dilatation (FMD) in INT-young and INT-elderly

Data are mean ± SD.

SED-young = Sedentary young subjects; INT-young = young subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions); Detraining1 = a month of detraining; Detraining2 = 6 weeks of detraining

* P<0.05 vs. Pre

The % change of Flow-mediated dilatation (FMD) in INT-young and INT-elderly groups were shown in Figure 11. The INT-elderly group showed significant higher at Post-Pre, Detraining1-Pre, and Detraining2-Pre (P<0.05) as compared to the INT-young group.

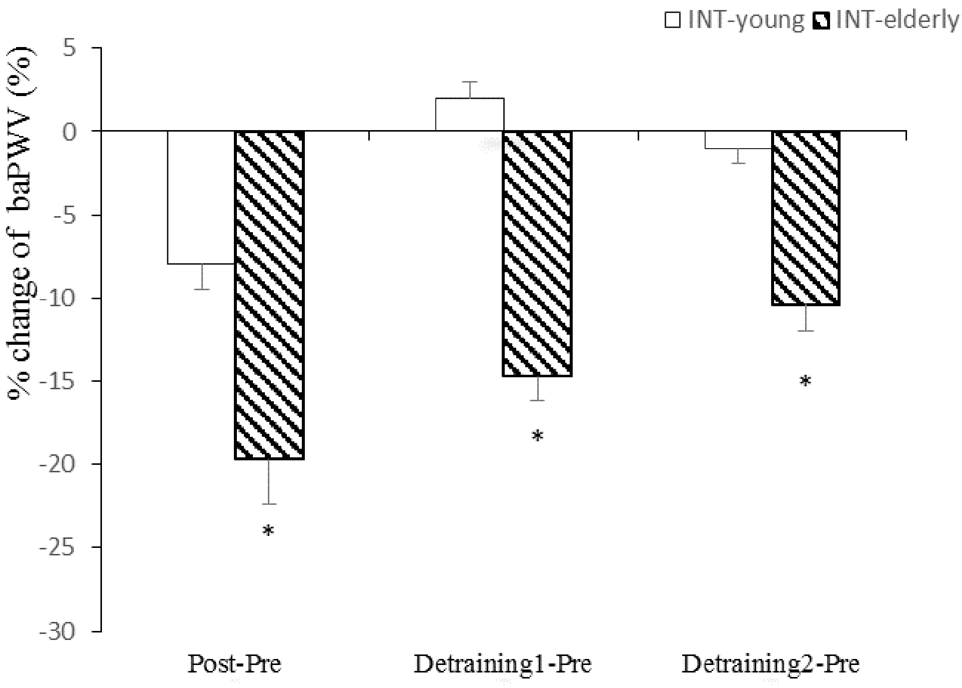



Figure 12 % change of baPWV in INT-young and INT-elderly

Data are mean ± SD.

SED-young = Sedentary young subjects; INT-young = young subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions); Detraining1 = a month of detraining; Detraining2 = 6 weeks of detraining

* $P<0.05$ vs. Pre

The % change baPWV in INT-young and INT-elderly group were shown in Figure 12. The INT-elderly group showed significant difference at Post-Pre, Detraining1-Pre, and Detraining2-Pre ($P<0.05$) as compared to the INT-young group.

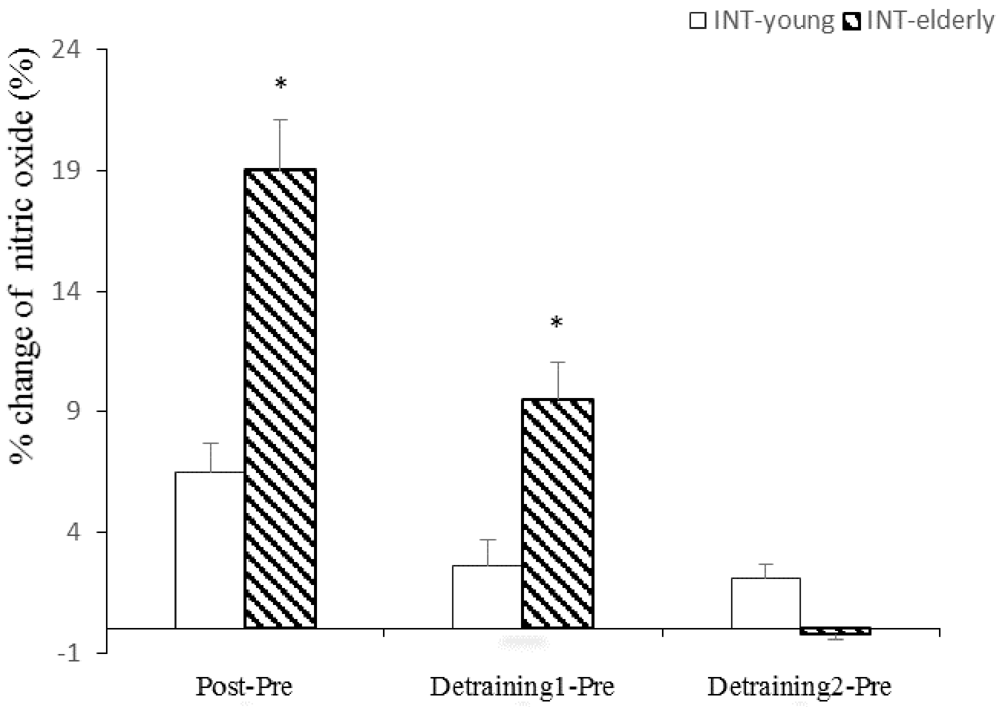



Figure 13 % change of nitric oxide in INT-young and INT-elderly

Data are mean ± SD.

SED-young = Sedentary young subjects; INT-young = young subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions); Detraining1 = a month of detraining; Detraining2 = 6 weeks of detraining

* P<0.05 vs. Pre

The % change nitric oxide in INT-young and INT-elderly groups were shown in Figure 13. The INT-elderly group showed significant higher at Post-Pre and Detraining1-Pre (P<0.05) as compared to the INT-young group. There is no significant difference between groups at Detraining2-Pre.

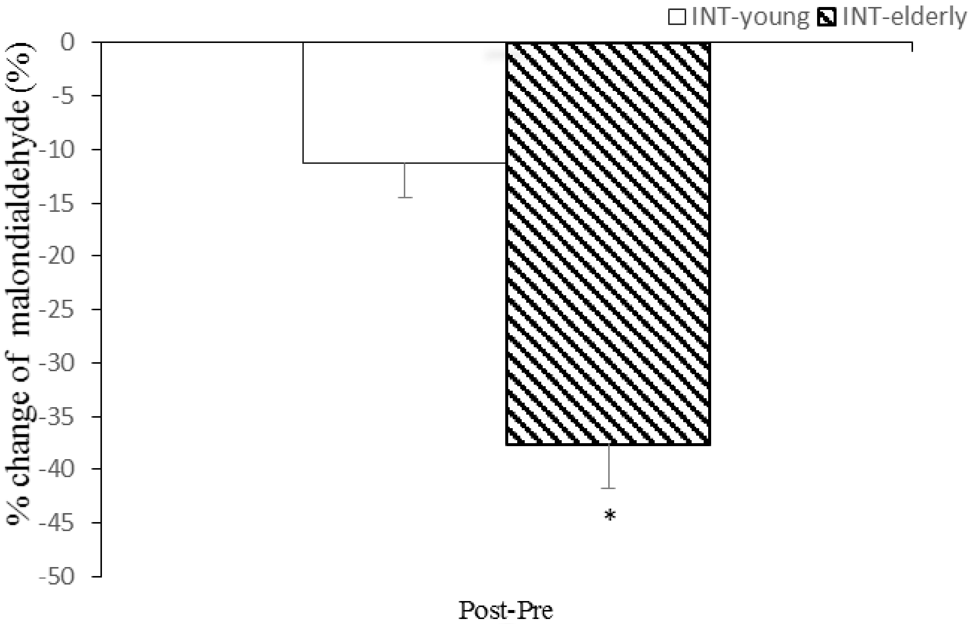



Figure 14 % change of malondialdehyde in INT-young and INT-elderly

Data are mean ± SD.

SED-young = Sedentary young subjects; INT-young = young subjects performed interval training; Pre = Pre-test; Post = Post-test (after 12 weeks of interventions); Detraining1 = a month of detraining; Detraining2 = 6 weeks of detraining

* P<0.05 vs. Pre

The % change malondialdehyde in INT-young and INT elderly were shown in Figure 14. The INT-elderly group showed significant difference at Post-Pre (P<0.05) as compared to the INT-young group.

## Discussion and conclusion

In this study, the experiment aims to investigate the potential of interval aerobic training program on age-associated endothelial function in the youngs and elderlies. Both SED-elderly and INT-elderly groups showed similar results in all parameters at baseline. And also, the similarity was observed at baseline in both SED-young and INT-young groups. However the elderly group (SED-elderly and INT-elderly) showed the significant difference in all parameters at baseline as compared to the young group (SED-young and INT-young). After 12 weeks of interventions, both the INT-elderly and the INT-young groups showed the decrement of body fat, systolic blood pressure, diastolic blood pressure, mean arterial pressure, baPWV, carotid IMT, and malondialdehyde, in the similar way as the increment of maximal $O_2$ consumption, Knee flexion muscular strength, knee flexion muscular strength, flow-mediated dilatation and nitric oxide. But only INT-elderly group had a significant decrease in body mass, and body mass index. Interestingly, both the INT-elderly and the SED-young group demonstrated the equality of body mass index, systolic blood pressure, diastolic blood pressure, mean arterial pressure, maximal $O_2$ consumption, baPWV and malondialdehyde at 12 weeks of the exercise program.

At the detraining phase, the INT-elderly showed the improvement of flow-mediated dilatation, baPWV, and nitric oxide at 12 weeks and a month of detraining and the value of these parameters return to the baseline at 6 weeks of detraining. Only the enhancement of baPWV value in the INT-elderly could maintain even after 6 weeks of detraining. In the INT-young group, flow-mediated dilatation, baPWV, and nitric oxide return to baseline after 4 weeks of detraining. In addition, the % change of flow-mediated dilation, nitric oxide, baPWV, and malondialdehyde were higher in the INT-elderly group.

**The effects of interval aerobic training on health-related physical fitness in the youngs and elderlies**

In the present study, the results showed that the SED group (SED-young, SED-elderly) showed no significant change in all parameters while the interval aerobic training group (INT-young and INT-elderly groups) could enhance biological and health-related physical fitness parameters.

Exercise training, non-pharmacological therapeutic strategy, is known to benefit healthy and non-healthy individuals in many aspects such as health-related physical fitness, diabetic control, vascular function, and cardiovascular risk factors (mitranun et al., 2014; Jakicic et al., 2003; Kadoglou et al., 2007; Wisloff et al., 2007). In this study, we focused on the effects of interval aerobic training which is the potential candidate of aerobic training type and show the superior results on health-related physical fitness as compared to the traditional aerobic exercise (Mitranun et al., 2014; Wisloff et al., 2007). In this study, we used the training protocol as four-six intervals of high-intensity work (80-85%$VO_2$peak)/low-intensity active recovery (50-60%$VO_2$peak) ratio of 1 min:4 min which is similar as our previous report (Mitranun et al; 2014)ใ While other previous treadmill interval exercise for health studies used four intervals of 4 min at 80-90% VO2peak following 3 min at 50-60% VO2peak (Rognmo et al., 2004) and four intervals of 4 min at 90-95% peak heart rate following 3 min at 50-70% peak heart rate (Wisloff et al., 2007). The difference points of our exercise protocol from other health and disease studies are the number of intervals performed, the duration of the recovery between bouts and a progression in training intensity. However, our interval aerobic training program in this study is still sufficient for improving aerobic capacity in both young and elderly subjects.

Advanced aging impairs the biological and health-related parameters which several studies supported the benefit of exercise to optimizing age-related change in body composition ( ACSM, 2018. The present study supported the potential of exercise in elderly subjects. Moreover, the interval aerobic training program could restore the impairment of body mass index and maximal $O_2$ consumption similar as sedentary young subjects. In aspect of maximal oxygen consumption, there is a number of studies supported the increment of PGC1 alpha, master regulator of mitochondrial biogenesis, through exercising (Brown et al., 2010). PGC1 alpha, intensity-dependent (Egan et al., 2010), had a high correlation with VO2peak (r=0.71)

(Wisloff et al., 2007). Thus, this might explain the underlying mechanism of the interval aerobic exercise contributing the great benefit to aerobic capacity in elderly subjects (Mitranun et al., 2014)

**The effects of interval aerobic training on endothelial dependent vasodilatation in the youngs and elderlies**

The present study, the results showed that the sedentary group (SED-young and SED-elderly groups) showed no significant change in all parameters. In both interval aerobic training groups (the INT-elderly and the INT-young groups) the decrement of baPWV, carotid IMT, and malondialdehyde were observed, in the similar way as the increment of flow-mediated dilatation and nitric oxide.

Endothelial dysfunction is an early event of the atherosclerotic process which associated with increased pulse wave velocity (PWV), an indicator of arterial stiffness (van Popele et al., 2001) and low magnitude of flow-mediated dilation (FMD), one of the noninvasive standard for assessing in vivo of endothelial-dependent vasodilation (Betik et al., 2004). Aging is one of the major factors for the development of endothelial dysfunction (Lakatta and Levy, 2003). Old age individuals have vascular stiffness, loss of vascular elasticity and having vascular endothelial dysfunction (Santos-Parker et al., 2014). The increment of PWV and the decrement of FMD can be observed in advancing age even in adults without any significant cardiovascular disease (Eskurza et al., 2004; Lakatta and Levy, 2003). The mechanism of aging in vascular changes can be explained by oxidative stress of increased superoxides resulted in collagen deposition, fragmentation of elastin, protein oxidation and formation of advanced glycation end products (Santos-Parker et al., 2014). Our study also found the significant impairment of the old group as compared to the young group at baseline. However, after 12 weeks of interval aerobic training, both exercise group ( young and elderly) showed the improvement of baPWV, FMD, malondialdehyde, and nitric oxide. Conversely, the previous study in both male and female elderlies involving 4x4 min of interval aerobic training at an intensity 80-90% of HRmax alternated with 60-70 % HRmax, 3 times per week for 12 weeks did not potent enough to change central PWV (Deiseroth et al, 2019) . Thus, the gender might be the one of essential factors (Seals et al., 2019).

Carotid intima-media thickness (Carotid IMT) is a surrogate marker for atherosclerosis which the increment of CIMT is associated with increased risk for cardiovascular events. A recent systemic review show the strongly and linearly relation of age and carotid IMT in both individuals with and without cardiovascular disease (van et al., 2018). Consistently, our study report the higher result of carotid IMT in the elderly group. However, the decrement of carotid IMT was found after 12 weeks of intervention in both young and elderly group. The proposed mechanism may involve the decrement of blood pressure in our both groups which the high blood pressure is a potent factor of carotid IMT (Qu and Qu, 2015). It seems reasonable to suggest that interval aerobic training increased blood flow and shear stress to a greater extent and, in turn, improved the NO bioavailability as well as endothelium-dependent vasodilation (Ribeiro et al., 2010). These functional measurement data are consistent with the decrement of plasma MDA, an index of lipid peroxidation and oxidative stress, which the increase of nitric oxide considered to decrease level of reactive oxygen species (Chakraphan et al., 2005). Thus, the summation effects result in the enhancement of vascular function.

Arterial stiffness of middle-aged and older men who involve regular aerobic training is lower when compared to sedentary status matched for similar aged and gender, but close to young men (Vaitkevicius et a., 1993). But only 12 weeks of our interventions, the elderly group performing interval aerobic training could reverse baPWV and malondialdehyde values similar as the sedentary young age. To understand these mechanisms and the difference between old and young age, we followed up the change of vascular parameters after cessation of training. The results showed that FMD, baPWV and nitric oxide values restores to baseline after a month of detraining in the young group whereas the old group could sustain after a month of detraining and return to baseline at 6 weeks of detraining. Surprisingly, baPWV in the old group can maintain even 6 weeks of detraining.

The increment of plasma nitrite in the old subjects following an ergometer exercise test with a stepwise increase in force is lower as compared to the young subjects. However, our study was designed to investigate in chronic effects and the change of vascular parameters might be dissimilar. In this study, the factors that leads to sustain vascular parameters in the old group might not be “actual values” by reason of the lower actual values in the elderly group. On the other hand, it might be the % change of these parameters due to the exercise intervention which calculated from baseline. The % change of these parameters seem to be higher in the older groups. Based on these rationales, higher % enhancement of nitric oxide and malondialdehyde bring about the greater improvement of FMD and baPWV in the old group.

### Conclusion

We conclude that interval aerobic training is an effective program for improving health-related physical fitness and age-associated endothelial function in the youngs and elderlies. The potential of the training is to reverse in some parameters of endothelial dysfunction in older age to same level as young age individuals and can maintain endothelial function after detraining. This study will be beneficial to recommend interval aerobic exercise program as a treatment for the improvement the endothelial function in the process of aging.

### Limitation of this study

1. The numbers of subjects in each intervention group may be considered small.
2. In this study, we could not control daily life behaviors of our subjects which may affect the results of biological data, blood chemistry and blood flow data.

## References

American College of Sports Medicine. 2018. ACSM's guidelines for exercise testing and prescription. Lippincott Williams & Wilkins

Brown, G. C., Murphy, M. P., Jornayvaz, F. R., & Shulman, G. I. 2010. Regulation of mitochondrial biogenesis. Essays in biochemistry, *47*, 69-84.

Bertovic, D.A., Waddell, T.K., Gatzka, C.D., Cameron, J.D., Dart, A.M. and Kingwell, B.A. 1999. Muscular strength training is associated with low arterial compliance and high pulse pressure. Hypertension. 33, 1385-1391.

Betik, A.C., Luckham, V.B. and Hughson, R.L. 2004. Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. American Journal of Physiology-Heart and Circulatory Physiology. 286, H442-H448.

Bilfinger, T.V. and Stefano, G.B. 2000. Human aortocoronary grafts and nitric oxide release: relationship to pulsatile pressure. The Annals of Thoracic Surgery. 69, 480-485.

Brunner, H., Cockcroft, J.R., Deanfield, J., Donald, A., Ferrannini, E., Halcox, J., Kiowski, W., Luscher, T.F., Mancia, G., Natali, A., Oliver, J.J., Pessina, A.C., Rizzoni, D., Rossi, G.P., Salvetti, A., Spieker, L.E., Taddei, S. and Webb, D.J. 2005. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. Journal of hypertension. 23, 233-246.

Chakraphan, D., Sridulyakul, P., Thipakorn, B., Bunnag, S., Huxley, V. H., & Patumraj, S. 2005. Attenuation of endothelial dysfunction by exercise training in STZ-induced diabetic rats. Clinical hemorheology and microcirculation, 32(3), 217-226.

Clifford, P.S. and Hellsten, Y. 2004. Vasodilatory mechanisms in contracting skeletal muscle. Journal of Applied Physiology. 97, 393-403.

Corretti, M.C., Anderson, T.J., Benjamin, E.J., Celermajer, D., Charbonneau, F., Creager, M.A. and Vogel, R. 2002. Guidelines for the ultrasound assessment of endothelial dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology. 39, 257-265.

Cortez-Cooper, M.Y., DeVan, A.E., Anton, M.M., Farrar, R.P., Beckwith, K.A., Todd, J.S. and Tanaka, H. 2005. Effects of high intensity resistance training on arterial stiffness and wave reflection in women. American Journal of Hypertension. 18, 930-934.

Cosio-Lima, L. M., Thompson, P. D., Reynolds, K. L., Headley, S. A., Winter, C. R., Manos, T., ... and Germain, M. 2006. The acute effect of aerobic exercise on brachial artery endothelial function in renal transplant recipients.Preventive cardiology, 9, 211-214.

Deanfield, J., Donald, A., Ferri, C., Giannattasio, C., Halcox, J., Halligan, S., Lerman, A., Mancia, G., Oliver, J.J., Pessina, A.C., Rizzoni, D., Rossi, G.P., Salvetti, A., Schiffrin, E.L., Taddei, S. and Webb, D.J. 2005. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial factors of the European Society of Hypertension. Journal of Hypertension. 23, 7-17.

Deiseroth, A., Streese, L., Köchli, S., Wüst, R. S., Infanger, D., Schmidt-Trucksäss, A., & Hanssen, H. 2019. Exercise and arterial stiffness in the elderly: A combined cross-sectional and randomized controlled trial (EXAMIN AGE). Frontiers in physiology, 10, 1119.

D'Amico, J., Betlach, M., Senkarik, R., Smith, R. and Voight, M. 2007. Return to golf following left total hip arthroplasty in a golfer who is right handed. North American Journal of Sports Physical Therapy. 2, 251-261.

Dhindsa, M., Sommerlad, S.M., DeVan, A.E., Barnes, J.N., Sugawara, J., Ley, O. and Tanaka, H. 2008. Interrelationships among noninvasive measures of post-ischemic macro-and microvascular reactivity. Journal of Applied Physiology. 105, 427-432.

Donato, A. J., Eskurza, I., Silver, A. E., Levy, A. S., Pierce, G. L., Gates, P. E., & Seals, D. R. (2007). Direct Evidence of Endothelial Oxidative stress with aging in humans relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappa-B. Circulation Research. 100(11), 1659-1666.

Egan, B., Carson, B. P., Garcia-Roves, P. M., Chibalin, A. V., Sarsfield, F. M., Barron, N., ... & O'Gorman, D. J. 2010. Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor γ coactivator-1α mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. The Journal of physiology, 588(10), 1779-1790.

Endermann, D.H. and Schiffrin, E.L. 2004. Endothelial dysfunction. Journal of American Society of Nephrology. 15, 1983-1992.

Eskurza, I., Monahan, K. D., Robinson, J. A., & Seals, D. R. 2004. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. The Journal of physiology, 556, 315-324.

Finnoff, J.T., Smith, J., Low, P.A., Dahm, D.L. and Harrington, S.P. 2003. Acute hemodynamic effects of abdominal exercise with and without breath holding. Archives of Physical Medicine and Rehabilitation. 84, 1017-1022.

Fleenor, B. S., Sindler, A. L., Marvi, N. K., Howell, K. L., Zigler, M. L., Yoshizawa, M., & Seals, D. R. (2013). Curcumin ameliorates arterial dysfunction and oxidative stress with aging. Experimental Gerontology. 48, 269-276.

Giles, T.D., Sander, G.E., Nossaman, B.D. and Kadowitz, P.J. 2012. Impaired vasodilation in the pathogenesis of hypertension: focus on nitric oxide, endothelial-derived hyperpolarizing factors, and prostaglandins. The Journal of Clinical Hypertension. 14, 198-205.

Gonzales, J.U., Thompson, B.C., Thistlethwaite, J.R. and Scheuermann, B.W. 2010. Association between exercise hemodynamics and changes in local vascular function following acute exercise. Applied Physiology, Nutrition, and Metabolism. 36, 137-144.

Harris, R. A., Padilla, J., Hanlon, K. P., Rink, L. D., & Wallace, J. P. 2008. The flow-mediated dilation response to acute exercise in overweight active and inactive men. Obesity, 16, 578-584.

Harvey, P.J., Morris, B.L., Kubo, T., Picton, P.E., Su, W.S., Notarius, C.F. and Floras, J.S. 2005. Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women. Journal of Hypertension. 23, 285-292.

Jurva, J.W., Phillips, S.A., Syed, A.Q., Syed, A.Y., Pitt, S., Weaver, A. and Gutterman, D.D. 2006. The effect of exertional hypertension evoked by weight lifting on vascular endothelial function. Journal of the American College of Cardiology. 48, 588-589.

Lakatta, E. G., & Levy, D. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises part I: aging arteries: a "set up" for vascular disease. Circulation. 107, 139-146.

Lambiase, M.J., Dorn, J., Thurston, R.C. and Roemmich, J.N. 2014. Flow-mediated dilation and exercise blood pressure in healthy adolescents. Journal of Science and Medicine in Sport. 17, 425-429.

Lauer, T., Heiss, C., Balzer, J., Kehmeier, E., Mangold, S., Leyendecker, T., ... & Rassaf, T. 2008. Age-dependent endothelial dysfunction is associated with failure to increase plasma nitrite in response to exercise. Basic research in cardiology, 103(3), 291-297.

London, G.M. and Guerin, A.P. 1999. Influence of arterial pulse and reflected waves on blood pressure and cardiac function. American Heart Journal. 138, S220-S224.

Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., ... and Benetos A. 2001. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension, *37*, 1236-1241.

Maiorana, A., O'Driscoll, G., Dembo, L., Cheetham, C., Goodman, C., Taylor, R. and Green, D. 2000. Effect of aerobic and resistance exercise training on vascular function in heart failure. American Journal of Physiology-Heart and Circulatory Physiology. 279, H1999-H2005.

Millgård, J. and Lind, L. (1998). Acute hypertension impairs endothelium-dependent vasodilatation. Clinical Science. 94, 601-608.

Mitchell, G. F., Hwang, S. J., Vasan, R. S., Larson, M. G., Pencina, M. J., Hamburg, N. M., ... and Benjamin, E. J. 2010. Arterial stiffness and cardiovascular events the Framingham Heart Study. Circulation, 121, 505-511.

Mitranun, W., Deerochanawong, C., Tanaka, H. and Suksom, D. 2014. Continuous vs interval training on glycemic control and macro-and microvascular reactivity in type 2 diabetic patients. Scandinavian Journal of Medicine & Science in Sports. 24, e69-e76.

Miyachi, M., Donato, A.J., Yamamoto, K., Takahashi, K., Gates, P.E., Moreau, K.L. and Tanaka, H. 2003. Greater age-related reductions in central arterial compliance in resistance-trained men. Hypertension. 41, 130-135.

Moro, T., Thomas, E. and Bosco, G. 2011. Blood pressure and heart rate response to two resistance training technique of different intensity. The European Journal of Sports Studies. 1, 46-56

Phillips, S.A., Das, E., Wang, J., Pritchard, K. and Gutterman, D.D. 2011. Resistance and aerobic exercise protects against acute endothelial impairment induced by a single exposure to hypertension during exertion. Journal of Applied Physiology. 110, 1013-1020.

Pratley, R., Nicklas, B., Rubin, M., Miller, J., Smith, A., Smith, M., Hurley, B. and Goldberg, A. 1994. Strength training increases resting metabolic rate and norepinephrine levels in healthy 50-to 65-yr-old men. Journal of Applied Physiology. 76, 133-137.

Pyke, K.E., Hartnett, J.A. and Tschakovsky, M.E. 2008. Are the dynamic response characteristics of brachial artery flow-mediated dilation sensitive to the magnitude of increase in shear stimulus? Journal of Applied Physiology. 105, 282-292.

Qu, B., & Qu, T. 2015. Causes of changes in carotid intima-media thickness: a literature review. Cardiovascular ultrasound, 13(1), 46.

Radak, Z., Zhao, Z., Koltai, E., Ohno, H. and Atalay, M. 2013. Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling. Antioxidants & redox signaling. 18, 1208-1246.

Rakobowchuk, M., McGowan, C.L., De Groot, P.C., Bruinsma, D., Hartman, J.W., Phillips, S.M. and MacDonald, M.J. 2005. Effect of whole body resistance training on arterial compliance in young men. Experimental Physiology. 90, 645-651.

Santos-Parker, J. R., LaRocca, T. J., & Seals, D. R. (2014). Aerobic exercise and other healthy lifestyle factors that influence vascular aging. Advances in Physiology Education. 38, 296-307.

Seals, D. R., Nagy, E. E., & Moreau, K. L. (2019). Aerobic exercise training and vascular function with ageing in healthy men and women. *The Journal of physiology*, *597*(19), 4901-4914.

Stoner, L., Young, J. M., Fryer, S. and Sabatier, M.J. 2012. The importance of velocity acceleration to flow-mediated dilation. International Journal of Vascular Medicine. 2012, 1-11.

Terra, D.F., Mota, M.R., Rabelo, H.T., Bezerra, L.M.A., Lima, R.M., Ribeiro, A.G., Vinhal, P.H., Ritti Dias, R.M. and Silva, F.M.D. 2008. Reduction of arterial pressure and double product at rest after resistance exercise training in elderly hypertensive women. Arquivosbrasileiros de Cardiologia. 91, 299-305.

Tjønna, A.E., Lee, S.J., Rognmo, Ø., Stølen, T.O., Bye, A., Haram, P.M., Wisløff, U. 2008. Aerobic Interval training versus continuous moderate exercise as a treatment for the metabolic syndrome. A pilot study. Circulation. 118, 346-354.

Umpierre, D. and Stein, R. 2007. Hemodynamic and vascular effects of resistance training: implications for cardiovascular disease. Arquivosbrasileiros de Cardiologia. 89, 256-262.

van den Munckhof, I. C., Jones, H., Hopman, M. T., de Graaf, J., Nyakayiru, J., van Dijk, B., ... & Thijssen, D. H. 2018. Relation between age and carotid artery intima‐medial thickness: a systematic review. Clinical cardiology, 41(5), 698-704.

Vaitkevicius, P. V., Fleg, J. L., Engel, J. H., O'Connor, F. C., Wright, J. G., Lakatta, L. E., ... & Lakatta, E. G. 1993. Effects of age and aerobic capacity on arterial stiffness in healthy adults. Circulation, 88(4), 1456-1462.

van Popele, N. M., Grobbee, D. E., Bots, M. L., Asmar, R., Topouchian, J., Reneman, R. S., ... & Witteman, J. C. 2001. Association between arterial stiffness and atherosclerosis The Rotterdam Study. *Stroke*, *32*, 454-460.

Varady, K.A., Bhutani, S., Church, E.C. and Phillips, S.A. 2010. Adipokine responses to acute resistance exercise in trained and untrained men. Medicine and Science in Sports and Exercise. 42, 456-462.

Verma, S., Buchanan, M.R. and Anderson, T.J. 2003. Endothelial function testing as a biomarker of vascular disease. Circulation. 108, 2054-2059.

Virdis, A., Ghiadoni, L., Gianarelli, C. and Taddie, S. 2010. Endothelial dysfunction and vascular disease in later life. Maturitas. 67, 20-24.

Wray, D.W., Uberoi, A., Lawrenson, L. and Richardson, R.S. 2005. Heterogeneous limb vascular responsiveness to shear stimuli during dynamic exercise in humans. Journal of Applied Physiology. 99, 81-86.

Walker, B. F. 2000. The prevalence of low back pain: a systematic review of the literature from 1966 to 1998. Journal of Spinal Disorders & Techniques. 13(3), 205-217.

Wisløff, U., Støylen, A., Loennechen, J.P., Bruvold, M., Rognmo, Ø., Haram, P.M., Tjønna, A.E., Helgerud, J., Slørdahl, S.A., Lee, S.J., Videm, V., Bye, A., Smith, G.L., Najjar, M., Ellingsen, Ø. and Skjærpe, T. 2007. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients a randomized study. Circulation. 115, 3086-3094.

Xia, W. H., Li, J., Su, C., Yang, Z., Chen, L., Wu, F., and Tao, J. 2012. Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men. Aging Cell, 11, 111-119.

Yang, Z., Xia, W. H., Su, C., Wu, F., Zhang, Y. Y., Xu, S. Y., and Tao, J. 2013. Regular exercise-induced increased number and activity of circulating endothelial progenitor cells attenuates age-related decline in arterial elasticity in healthy men. International Journal of Cardiology, 165, 247-254.

Zieman, S. J., Melenovsky, V., and Kass, D. A. 2005. Mechanisms, pathophysiology, and therapy of arterial stiffness. Arteriosclerosis Thrombosis and Vascular Biology, 25, 932-943.

APPENDICES

# APPENDIX A
# การเผยแพร่ผลงานวิจัย

1. Mitranun, W., Suksom, D. High- intensity interval training can maintain the improvement of vascular function after a month of detraining in elderly. The 8th International Conference on Sports and Exercise Science "Toward Sports and Exercise Science in the Modern World", 20th - 22nd June, 2018, the Ambassador Hotel Bangkok, Thailand. <u>(The winner poster presentation awards).</u>

ภาพ A.1 แสดงบรรยากาศการนำเสนองาน หลักฐานการนำเสนองานและการรับรางวัลชนะเลิศ


The 8th International Conference
on Sports and Exercise Science
ICSSE
2018
GRAND


The 8th International Conference
on Sports and Exercise Science
Toward Sports and Exercise Science in the Modern World
The 1st
Poster Presentation Award
$120
Chulalongkorn University
สสส
United BMEC
GRAND
WARRIX
LWN
BEST ELIXIR
BJHMedical
PHILIPS
Udom

The 8th International Conference on Sp d Exercise Science
Toward Sports and Exercise Scienc e Modern world
Exercise Physiology (EP-P-02)
HIGH-INTENSITY INTERVAL TRA CAN MAINTAIN THE IMPROVEMENT OF VASCULAR FUNCTION AFTER A MONTH OF DETRAINING IN ELDERLY
Witid Mitranun1, Daroonwan Suksom2
1Department of Sport Science, Faculty of Physical Education, Srinakharinwirot University, Nakhon Nayok, Thailand
2Faculty of Sports Science, Chulalongkorn University, Bangkok, Thailand
Introduction
Purpose
Methods
Results
Conclusions
Acknowledgement

The 8th International Conference on Sports and Exercise Science
Toward Sports and Exercise Science in the Modern world

# Certificate of Achievement

Proundly Presented to

## WITID MITRANUN

Is hereby named an award winner

In recognition of having met the highest standard of excellence in

Poster presentation

*"High- intensity training can maintain the improvement of vascular function after a month of detraining in elderly"*

The 8th International Conference on Sports and Exercise Science
"Toward Sports and Exercise Science in the Modern World"
June 20 - 22, 2018
at the Ambassador Hotel Bangkok,Thailand

Assist.Prof.Wanchai Boonrod, Ph.D.
Conference Chairman

แสดงบทคัดย่อของงานประชุมระดับนานาชาติ The 8th International Conference on Sports and Exercise Science "Toward Sports and Exercise Science in the Modern World" ซึ่งได้รับรางวัลชนะเลิศ

# HIGH-INTENSITY INTERVAL TRAINING CAN MAINTAIN THE IMPROVEMENT OF VASCULAR FUNCTION AFTER A MONTH OF DETRAINING IN ELDERLY

**Witid Mitranun[1], Daroonwan Suksom[2]**
[1]Department of Sport Science, Faculty of Physical Education, Srinakharinwirot University, Nakhon Nayok, Thailand
[2]Faculty of Sports Science, Chulalongkorn University, Bangkok, Thailand

---

ABSTRACT

High-intensity interval training (HIIT) has a potential to improve vascular function while the enchancement and maintainance effects in elderlies are unknown.

**Purpose:** This study aimed to investigate the effects of HIIT on vascular fuction in elderly subjects.

**Methods:** Male and female elderly aged 64.3±3.6 were studied. All participants were divided into control (CON) groups (n=15) and HIIT group (n=15). The HIIT group was assigned to perform walking on treadmill for 30-40 min/day, 3 times/week for 12 weeks. The biological data, health related-physical fitness, flow-mediated dilation (FMD) data, pulse wave velocity (PWV) data, and blood pressure data were collected at baseline, after 12-week HIIT period, and 4 weeks of detraining.

**Results:** Both the CON and HIIT group showed similar results at the baseline of all parameters ($P > 0.05$). After 12 weeks, body fatness, heart rate at rest, and PWV decreased ($P < 0.05$) and leg muscle strength, maximal aerobic capacity, FMD increased significantly in the HIIT group ($P < 0.05$). At 4 weeks in detraining period, the changes of these parameters, excepting body fatness, were similar at after 12 weeks of intervention ($P > 0.05$).

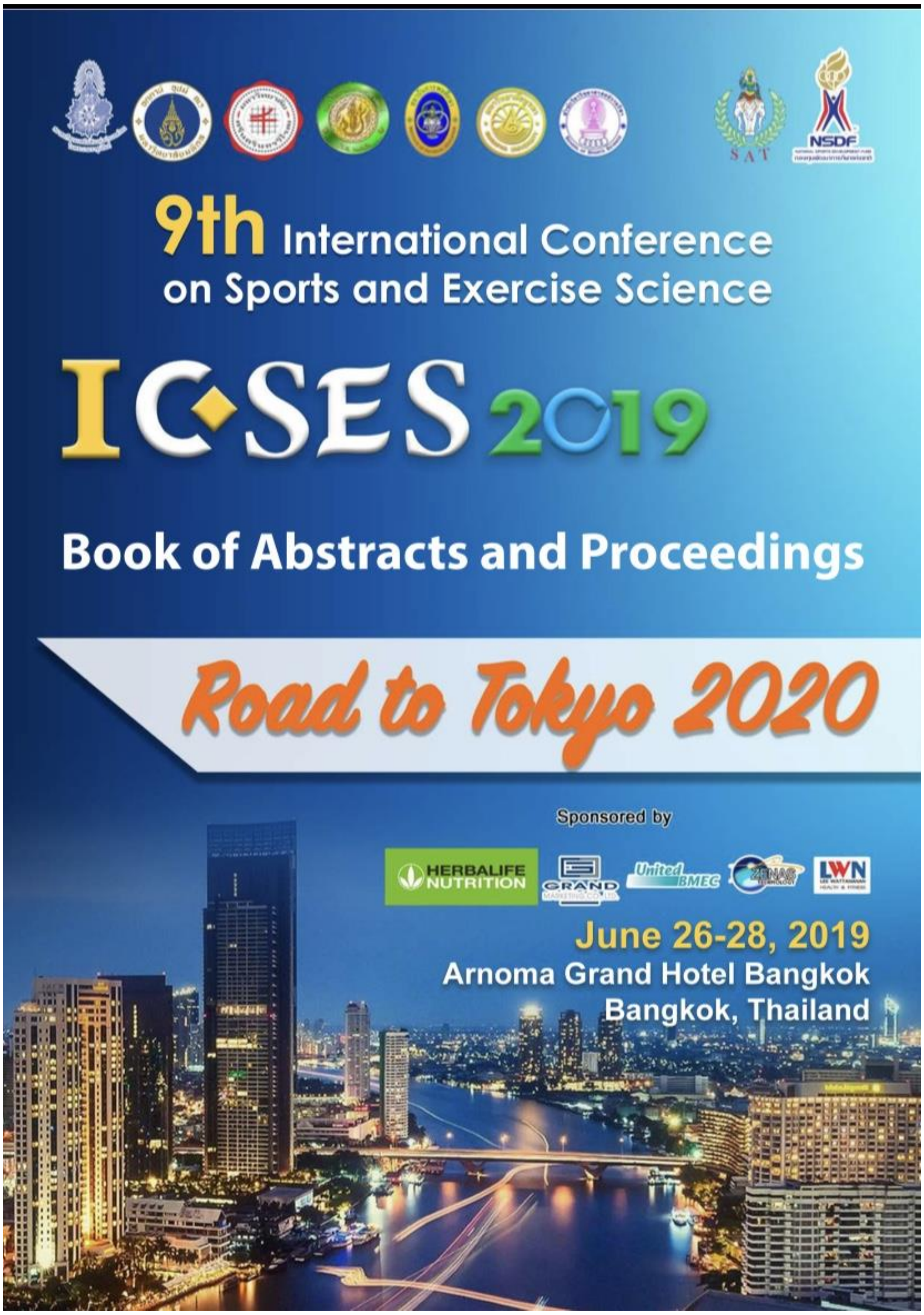
**Conclusion:** We concluded that high-intensity interval training has the potential to improve aerobic fitness; moreover, the training program can maintain the improvement of vascular function after a month of detraining.

**Key Word:** Vascular function, Aging, Aerobic exercise, Detraining, Pulse wave velocity

**Corresponding Author:** Dr. Witid Mitranun, Department of Sport Science, Faculty of Physical Education, Srinakharinwirot University, Nakhon Nayok, Thailand**,** E-mail. mitranunwitid@hotmail.com

2. นำเสนองานวิจัยในงานประชุมวิชาการระดับนานาชาติ The 8th International Conference on Sports and Exercise Science “Road to Tokyo 2020” ในหมวดการนำเสนอ “Young Scientist Forum” เรื่อง “Advantages and Disadvantages of Exercises on Vascular Function”

ภาพ A.2 แสดงหลักฐานการนำเสนองานวิจัย



# Advantages and Disadvantages of Exercises on Vascular Function

***Witid Mitranun, PhD***

**Department of Sports Science, Faculty of Physical Education, Srinakharinwirot University, Thailand**

There are many type of exercises to enhance vascular function (VF), especially aerobic exercise. Our previous study reported the beneficial effects of twelve weeks of aerobic training on macro- and microvascular reactivity in diabetes patients which both continuous training and high-intensity interval training (HIIT) were effective in improving glycemic control, aerobic fitness, and endothelium-dependent vasodilation, but the HIIT appears to confer greater improvements than the continuous training program. Our recent study also showed the potential of the training which HIIT can maintain the improvement of VF after a month of detraining in elderly. In the aspect of resistance exercise, our several investigations on the acute effects demonstrated various results on VF which depending on different exercise postures and pattern of force generation. In different exercise postures, the impairment of VF was observed in long duration of Plank exercise while Crunch exercise show the positive effects in untrained participants. In pattern of force generation, an improvement in VF was acutely observed in the dumbbell with elastic tubing, the VF was unchanged in elastic tubing, and in contrast the dumbbell alone resulted in an impairment of VF.

***Keywords*:** Nitric oxide, Blood flow, Flow-mediated dilatation, Arterial stiffness, Oxidative stress

Assist. Prof. Dr. Witid Mitranun is a lecturer at Department of Sports Science, Faculty of Physical Education, Srinakharinwirot University, Thailand. He teaches several issues of exercise physiology and exercise in special populations in the Bachelor, Master, and Philosophy programs. Previously, he had the Bachelor of Science (Sports Science) (First degree honour, Gold medal) from Chulalongkorn University. And also, he had the Master and the Doctor of Philosophy of Science (Sports Science) from Chulalongkorn University. His PhD research investigated the effects of continuous and interval aerobic training on glycemic control and endothelial dependent vasodilatation in type 2 diabetes patients (published in The Scandinavian Journal of Medicine & Science in Sports). After his graduation, he continued to study in the field of exercise and vascular function. The main research of interests are the type of exercises such as, high intensity interval training, weight training, functional training, and combining elastic and weight resistance that acutely/chronically affect vascular functions (Flow-mediated dilatation, vascular stiffness, and blood chemistries).


Certificate of Scientific Young Scientist Presentation

This is to certify that

**Witid Mitranun**

Presented in

**9th International Conference on Sports and Exercise Science**

**"Road to Tokyo 2020"**

Organized by the College of Sports Science and Technology, Mahidol University
& the Badminton Association of Thailand Under the Royal Patronage of His Majesty the King.

June 26-28, 2019 at Arnoma Grand Hotel Bangkok, Bangkok, Thailand

Chanin Lamsam, MD
ICSES Congress Chair

3. **Mitranun, W.**, Suksom, D. (2018). Effects of Interval Aerobic Training on Age-Associated Endothelial Function in the Youngs and Elderlies. TRF-OHEC Annual Congress 2018 (TOAC 2018), 10th - 12th January, 2018, The Regent Cha Am Beach Resort, Phetchaburi. (จัดโดย สกว.)

ภาพ A.3 แสดงหลักฐานการนำเสนองานวิจัย


4. ในขณะนี้ผู้วิจัยกำลังจัดทำ Manuscript เพื่อส่งเผยแพร่ในวารสารระดับนานาชาติ

# APPENDIX B

## Nitric oxide

### Reconstitution of Reagents

1. Enzyme cofactor

-Reconstitute with 1.1 ml of Assay Buffer. Aliquot desired amount and store at –20°C. Keep on ice during use. Store at –20°C.

2. Enhancer

-Reconstitute with 1.1 ml distilled water. Store at +4°C.

3. Nitrate Reductase

-Reconstitute to 1.1 ml with Assay Buffer. This dissolves slowly, so gently vortex 2-3 times over 15 minutes. Keep on ice during use. Store at +4°C.

4. Nitrate and Nitrite Standards

-Reconstitute with 100 ul of Assay Buffer. Vortex and mix well to generate 100 mM standard. Store at +4°C when not in use (do not freeze!). The reconstituted standard is stable for 4 months when stored at +4°C.

5. Griess Reagents R1 and R2

-Ready to use. Store at +4°C.

### Measurement of Nitrate + Nitrite

1. Nitrate standard curve: Mix 5 ul of the 100 mM reconstituted standard with 495 ul of Assay Buffer to generate 1 mM standard working solution. Note: DAN Probe reacts with nitrite, not nitrate. For routine total nitrite/nitrate assay, you may prepare a nitrate standard curve only. However, if you desire to measure nitrite, nitrate concentration separately, you may prepare a nitrite standard curve in the absence of Nitrate Reductase in the standard and assay samples. Nitrate = Total – Nitrite.

2. Preparation of samples: Up to 85 ul of sample can be added per assay and should be done in duplicate. When using less than 85 ul of sample, adjust volume to 85 ul with Assay Buffer. If the approximate nitrate/nitrite concentration is completely unknown, we recommend

that several dilutions be made. Urine can have high nitrate content and a 10 fold dilution should be used. Serum proteins will have a slight (~10%) effect on apparent nitrite levels. For best results serum filtrate from a 10Kd cutoff filter (Cat.No. PK-CA577-10KC-100) should be used. Typical urine levels are 0.2-2 mM and 1-
20 uM respectively. Typical normal serum levels are ~20 uM and ~2 uM for nitrate and nitrite respectively with various disease states elevating these levels significantly. The absorbance of samples should be in the linear range of the standard curve (0-10 nmol/well). If they fall outside this range, they should be rediluted and rerun.

3. Assay procedure:

-Add 85 ul sample and 115 ul Assay Buffer as blank well.

-Add 0, 2, 4, 6, 8, 10 ul of standard to each well and adjust to 85 ul.

- Add 85 ul of sample or dilution to each unknown well.

-Add 5 ul of the Nitrate Reductase mixture to each well (standards and unknowns).

-Add 5 ul of the enzyme cofactor to each well (standards and unknowns).

-Cover the plate and incubate at room temperature for 1 hour to convert nitrate to nitrite.

-Add 5 ul of the enhancer to each well and incubate 10 minutes.

-Add 50 ul of Griess Reagent R1 to each well (standards and unknowns).

-Add 50 ul of Griess Reagent R2 to each well (standards and unknowns).

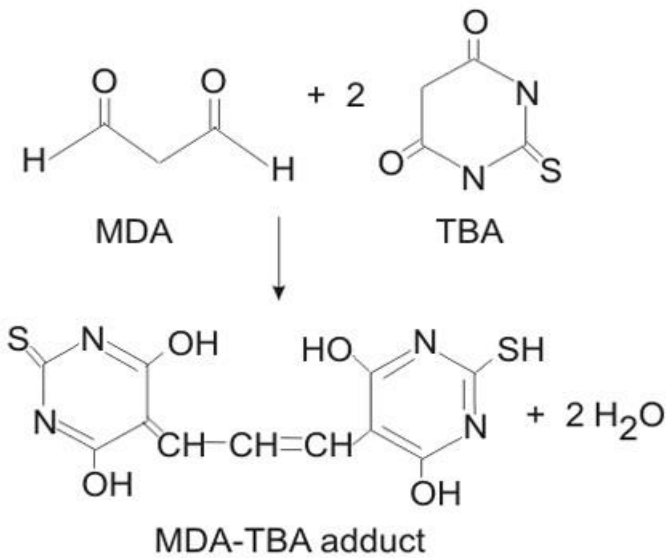
-Develop the color for 10 minutes at room temperature. The color is stable for about an hour.

-Read the absorbance at 540 nm using a plate reader.

# APPENDIX I

## Malondialdehyde

Malondiadehyde (MDA) is a ROS which is derived from the lipid oxidation of cell membrane polyunsaturated fatty acid and its formular is $CH_2$ $(CHO)_2$. This compound is highly reactive and toxic and is used as a biomarker to measure the level of oxidative stress. Thiobarbituric acid reactive substances method is used for analyzing level of MDA (Nanhini TA and Anuradha CV, 2003), by the concept that one molecule of MDA can react with 2 molecules of thiobarbituric acid (TBA). The chemical reaction and the method of measurement are described as follows:




**Reagents**

1. Phosphate buffer saline (PBS) pH 7.4

   -$Na_2HPO_4$ 2.27 g, $NaH_2PO_4$ 0.12 g and NaCl 8,18 g are dissolved in distilled water until total volume of solution is 1,000 ml. The solution is adjusted to pH 7.4.

2. 30% Trichloroacetic acid (TCA)

   -Dissolve thicholoacetic acid 30 g in distilled water until toltal volume of solution is 100 ml

3. Butylated hydroxyl toluene (BHT)

   -Dissolve Butylated hydroxyl toluene 88 mg in ethanol 10 ml

4. 1% thiobarbituric acid (TBA)

   -One g of 2-thiobarbituric acid in 100 ml distilled water

5. Malondialdehyde bis (diethylaceta) is used as external standard.

**Samples**

Use EDTA whole blood samples.

**Sample preparation**

Centrifuge whole blood for 10 minutes at 4˚C, 3500 rpm and then separate the plasma and erythrocytes. The erythrocytes are washed four times with 3 ml of 0.85% normal saline; centrifuging for 10 minutes at 4˚C, 3000 rpm after each wash.

**Measurement of MDA; the sequences of the test are:**

1. Draw 200 µl of each of washed erythrocytes, plasma, and diethylacetal (external standard) in three separate eppendorf tubes.
2. In each tube, add 800 µl of PBS, mix, and then add 30% TCA for erythrocyte lysis and protein precipitation
3. Add 25µl of BHT, mix, and then put in -20˚C refrigerator for 2 hours for the completeness of erythrocyte lysis and protein precipitation
4. Centrifuge at 12,000 rpm for 10 minutes.
5. Separate the supernatant into the new eppendorf tubes.
6. Add 250 µl of 1% TBA in each tube, mix, and boil in heat box (ACCUBLOCK™ Digital Dry Bath, Labnet International) at 103˚C for 15 minutes.
7. Stop the reaction by using cold water, draw 300µl of the solution and put in the 96-well-plate ELISA.
8. Measure light absorbance at 532 nm by using ELISA Reader (Enspire multilabel plate, Perkin-Elmer, USA)

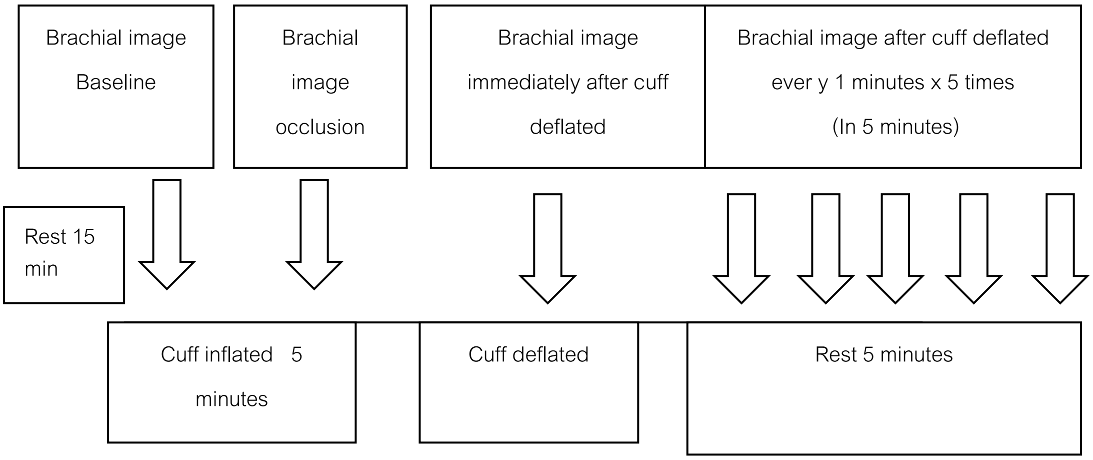
Construct the concentration graft of the external standard, and then calculate the lipid oxidation of the samples from the standard curve.

# APPENDIX H

## Vascular assessment

### Brachial artery characteristics assessment

Brachial artery characteristics were assessed with the ultrasound equipment (CX50, Philips, USA), using the occlusion technique on the right forearm. All subjects rested in the supine position for 20 min. The brachial artery was imaged above the antecubital fossa in the longitudinal plane. Baseline data was monitored for 1 min and then the cuff placed around the right forearm was inflated rapidly to 50 mmHg above systolic blood pressure for 5 min and deflated for 5 min of recovery (Corretti et al., 2002; Dhindsa et al., 2008). Mean blood velocity was collected by using the pulsed wave Doppler mode. Brachial analyzer program (Brachial Analyzer, Medical imaging applications, USA) was used for analyzing changes in vascular diameter. Shear rate was calculated by blood velocity/vascular diameter (Pyke et al., 2008). FMD was calculated using the formula FMD = (d2-d1) x 100/d1 when d1 is the averaged the brachial artery diameter at baseline, d2 is the averaged the maximal post occlusion brachial artery diameter (Naidu et al., 2011).




Protocol for ultrasound imaging

**Pulse velocity assessment**

Pulse wave velocity (PWV) method will be measured using a volume-plethysmographic device with four cuffs matched with oscillometric sensors, placed around the upper arms and ankles. Deflation phase, the pulse volume records of the bilateral brachial and tibial arteries are monitored. Oscillometric method is used to calculate the blood pressure of each lesion. Electrodes are placed on both wrists and a microphone is placed on the left edge of the sternum. Transit time ($\Delta T_{ba}$) is monitored by a time delay between the feet of the wave at the two sites. The distance between the two sites of PWV is calculated by using the height of the paticipants and $\Delta T_{ba}$.

$L_b$ = 0.2195 × height of the paicipants (cm) - 2.0734

$L_a$ = 0.8129 × height of the participants (cm) + 12.328.

PWV = $(L_a – L_b) / \Delta T_{ba}$


6