ผลของการออกกำลังกายระยะสั้นต่อความเข้มข้นไนไตรท์/ไนเตรตในซีรัม ในหญิงวัยหมดระดู

นางสาวสุกัญญา เอกสกุลกล้า

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EFFECT OF SHORT TERM EXERCISE ON SERUM NITRITE/NITRATE CONCENTRATION IN POSTMENOPAUSAL WOMEN

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Field of Study	Sposts Medicine
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สุกัญญา เอกสกุลกล้า : ผลของการออกกำลังกายระยะสั้นต่อความเข้มข้นไนไตรท์/ไนเตรตในซีรัมในหญิงวัยหมดระดู (EFFECT OF SHORT TERM EXERCISE ON SERUM NITRITE/NITRATE CONCENTRATION IN POSTMENOPAUSAL WOMEN) อ.ที่ปรึกษา : รศ.พญ.ธาดา สืบหลินวงศ์, อ.ที่ปรึกษาร่วม : ศ.นพ.นิมิต เตชไกรชนะ, 74 หน้า. ISBN 974-17-3535-9.

เป้าหมายของการวิจัยเชิงทดลองครั้งนี้ เพื่อศึกษาผลของการออกกำลังกายระดับปานกลางต่อความเข้มข้นของไนไตรท์/ไนเตรตในซีรัม ของหญิงวัยหมดระดู ที่มีสุขภาพดี 30 ราย อายุระหว่าง 45-60 ปี ซึ่งได้รับการสุ่มแบ่งเป็น 2 กลุ่ม กลุ่มละ 15 ราย ได้แก่กลุ่มออกกำลังกายด้วยการเดินลู่วิ่งและกลุ่มออกกำลังกายด้วยการปั่นจักรยาน ตามตารางการฝึกเดียวกัน ระยะเวลาศึกษารวม 23 วัน โดย ในวันที่ 1 เก็บตัวอย่างเลือด 8 มิลลิลิตร ทั้งก่อนและหลังการทดสอบวัดค่า VO_{2peak} เพื่อวิเคราะห์ระดับความเข้มข้นของไนไตรท์/ไนเตรตและไขมันในเลือด ระหว่างวันที่ 2-11 ซึ่งเป็นระยะควบคุม อาสาสมัครจะถูกขอร้องให้ปฏิบัติกิจวัตรประจำวัน และรับประทานอาหารตามปกติเป็นเวลา 10 วัน วันที่ 12 ทำการเก็บตัวอย่างเลือดอีกครั้ง ดังเช่นวันที่ 1 หลังจากนั้น ตั้งแต่วันที่ 13-22 เป็นช่วงออกกำลังกาย โดยอาสาสมัคร 15 ราย ในแต่ละกลุ่มจะออกกำลังกายตามวิธีที่ทำการสุ่มเลือกไว้ ด้วยความหนักระดับปานกลาง (50-60% VO_{2peak}) วันละ 30 นาที เป็นเวลา 10 วันจนครบกำหนด และทำการเก็บตัวอย่างเลือดครั้งที่ 3 ในวันที่ 23 เพื่อหลีกเลี่ยงผลแทรกซ้อนเฉียบพลันจากการทดสอบ VO_{2peak} ต่อการออกกำลังกายที่มีต่อระดับของไนไตรท์/ไนเตรต

ผลการศึกษาระดับความเข้มข้นของไนเตรตในซีรัมของอาสาสมัครทั้ง 30 ราย มีค่าเพิ่มขึ้นเล็กน้อย หลังการทดสอบ VO_{2peak} ในวันที่ 1 คือ 26.14 ไมโครโมล/ลิตร และคงค่าใกล้เคียงเดิมไปตลอดระยะที่ทำการศึกษา ส่วนค่าของไขมันในเลือดในกลุ่มเดินลู่วิ่ง พบว่าไตรกลีเซอไรด์ มีค่าลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.01$) ตรงข้ามกับค่าไขมันชนิดความหนาแน่นสูง (HDL-C) ที่กลับเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติที่ ($p<0.001$) ทั้งนี้เมื่อวิเคราะห์ผลการเปลี่ยนแปลงของไขมันในเลือดอาสาสมัคร 30 ราย ยังคงได้ผลในทำนองเดียวกัน ($p<0.005$) การเปลี่ยนแปลงของไขมันในเลือดที่เกิดขึ้นเป็นไปตามการตอบสนองที่ดีของร่างกายต่อการออกกำลังกาย อย่างไรก็ตาม ระดับของไนเตรตที่มีค่าเพิ่มขึ้นเล็กน้อยนั้น เป็นไปตามที่คาดไว้ นั่นคือการออกกำลังกายระดับปานกลางไม่มีผลต่อเปลี่ยนแปลงระดับความเข้มข้นของไนไตรท์/ไนเตรตในหญิงวัยหมดระดู

สาขาวิชา....เวชศาสตร์การกีฬา..... ลายมือชื่อนิสิต.....

ปีการศึกษา.....2546..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

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KEY WORD : MENOPAUSE/ MODERATE EXERCISE/ NITRIC OXIDE/ NITRITE/NITRATE

SUKANYA EKSAKULKLA : EFFECT OF SHORT TERM EXERCISE ON SERUM NITRITE/NITRATE CONCENTRATION IN POSTMENOPAUSAL WOMEN. THESIS ADVISOR : ASSOC. PROF. TADA SUEBLINVONG, M.D., THESIS CO-ADVISOR : PROF. NIMIT TAECHAKRAICHANA, M.D., 74 pp. ISBN 974-17-3535-9.

The aim of this experimental designed research was to study the effect of short term moderate exercise on serum nitrite/nitrate concentration in thirty healthy post menopause women. The volunteers whose age ranging from 45-60 years were randomly assigned into two groups of 15 subjects, namely, the treadmill group and the bicycle group performing similar schedule of exercise training. On day 1, of the 23 day of study, 8 ml, blood samples were obtained both prior to and after a VO_{2peak} test, for the analysis of serum nitrite/nitrate level and lipid profiles. During day 2-11, the control or run in period, all subjects were asked to live a regular sedentary lifestyle on their regular diet for 10 days. A second blood samples were taken on day 12 in the same manner as day 1. The exercise period, from day 13-22, both groups of 15 subjects each performed their assigned exercise mode at moderate intensity (50-60% VO_{2peak}) for 30 minutes per day for 10 consecutive days. The final third blood samples were obtained on day 23 to avoid contamination of short term exercise training over VO_{2peak} effect on serum nitrite/nitrate level.

Results on the concentration of serum nitrite in all thirty subjects exhibited a slight increase, 26.14 μmol/L, in post VO_{2peak} at day1 and remained at this level throughout the studied period. The lipid profiles of the treadmill group showed a significant decrease in serum triglycerides ($p<0.01$) while there was a significant increase in high density lipoprotein cholesterol (HDL-C) level with $p<0.001$. Similar lipid profiles changes were observed when analyzing all thirty subjects with $p<0.05$. The changes of lipid profiles were in accordance with the well accepted body response to exercise. However, the slight increase of nitrate was within expectation. Since, moderate intensity aerobic exercise did not change serum nitrite/nitrate in postmenopause women.

Field of study .....Sports Medicine.....

Academic .....2003.....

Student's signature .....

Advisor's signature .....

Co-advisor's signature .....

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LIST OF ABBREVIATIONS

BMI	=	body mass index
BW	=	body weight
TC	=	total cholesterol
dL	=	deciliter
HDL-C	=	High Density Lipoprotein Cholesterol
HR	=	heart rate
Kg	=	kilogram
L	=	liter
LDL-C	=	Low Density Lipoprotein Cholesterol
mg/dL	=	milligram/deciliter
NO	=	nitric oxide
NO_2^-	=	nitrite
NO_3^-	=	nitrate
Rpm	=	revolution per minute
μmol/l	=	micromole/liter
VO_{2peak}	=	peak oxygen uptake

CHAPTER I

INTRODUCTION

The nature of a normal cycle of human life begins with birth; a short period of fertility and reproduction, then comes aging, illness and ends finally in death. In woman, nature gives an important biomarker to mark the beginning and the end of the reproductive period which is known as the menstruation. The ending of reproductive life or menopause is defined by World Health Organization (WHO), 1981, “as the transition period in a woman's life when the ovaries stop producing eggs” (World Health Organ Tech Rep Ser 1981). Menopause also marks the beginning of aging process in woman. There are physiologic changes resulting from the depletion of hormone especially estrogen, called the menopausal syndrome. Where, some woman experience mild flushing symptoms to others with fairly severe emotional or physical abnormality. The pathophysiological or disease following menopause may include: osteoporosis, autonomic nervous system diseases, hypertension, cardiovascular diseases with abnormal system lipid metabolism (กองอนามัยครอบครัว 2539). In most cases, after the onset of menopause, women have increased levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), as well as decreased levels of high-density lipoprotein cholesterol (HDL-C) (Bush et al. 1987). Such plasma lipid profiles have been demonstrated to be associated with an increased risk of coronary artery disease, whereas high levels of high density lipoprotein cholesterol have been thought to protect against atherosclerosis and coronary artery disease. In addition to the presence of atherosclerotic lesions, cardiovascular disease is characterized by important abnormalities in vascular function. In particular, endothelial control of vascular tone, thrombosis, and platelet activity is impaired in patients with coronary artery disease (Hambrecht et al. 2000).

At present, it is accepted that the guidelines for the prevention of diseases following menopause are exercise and hormone replacement therapy (HRT). Physicians generally accept the idea that exercises promote vascular health, a concept that enjoys considerable support from epidemiological evidence (Vita and Keaney 2000). There is

an inverse relation between the level of physical activity and the incidence of cardiovascular diseases, and this relation persists after control for other risk factors for cardiovascular diseases (Oldridge et al. 1988). Exercise training also improves myocardial perfusion, coronary blood flow, resulting in increased shear stress on the surface of the endothelium (Schuler 1992). Endothelial cells respond to short-term increases in shear stress by producing vasodilator compounds such as prostacyclin and nitric oxide. Sustained increases in shear stress elicit an adaptive response in endothelial cells that is manifested, in part, by increased expression of the enzyme that catalyzes nitric oxide production (Vita and Keaney 2000). Since, there is evidence indicated that the endothelial function in animals that perform regular exercise is improved as a result of increased endothelial nitric oxide production and is better than that in animals that do not exercise (Sessa et al. 1994). Such adaptive responses of the endothelium also apply to the coronary circulation in humans (Hambrecht et al. 2000). Other conditions associated with menopause and aging are neurodegenerative diseases such as Alzheimer's disease, cancer, stroke, and a decline in the immunoresponse to pathogens, which have been shown to be directly related to the amount or incidence of nitric oxide available for biomolecular modification. Therefore, alterations in nitric oxide production could have a profound effect on both normal aging and disease conditions.

Nitric oxide (NO) has received much attention recently as one potential mediator of vascular tone, an endothelium-derived relaxing factor. A number of studies in both animals and humans have recognized that endothelially derived NO play as a role in blood flow regulation during acute, dynamic exercise (Dyke et al. 1995). In particular, it has been postulated that vasodilatation in active muscle promotes a pressure gradient and thus increased blood flow that stimulates NO production from upstream arteries (Green et al. 1996). NO mediated dilation of “feed” arteries can therefore permit increased microvascular flow without reduction in muscle perfusion pressure. With regular exercise it appears that there are adaptations in this system that may be partly responsible for the reduction in cardiovascular risk associated with the trained state (Delp et al. 1993). However, there are several studies on the effect of short term and acute exercise on the serum level of nitrites/nitrates which are the stable end product of nitric oxide (NO). In animal study, Johnson 2001 found that, short term exercise training

(7 days) enhances Ach-stimulated vasorelaxation in pulmonary arterial tissue of miniature porcine. Which adaptation is associated with increased expression of endothelial cell nitric oxide synthase (eNOS).

Acute vigorous exercise has been carried out in twenty-three healthy non-smoking males using single event treadmill exercise until exhausted (from pre-exercise to 5 min post-exercise). Using direct measure of NO by a NO-selective microelectrode, Kasuya et al. 2002 has shown that, acute vigorous exercise primes augmentation of NO release from the platelets of the about subjects. Although these two studies indicate that short term exercise play role on the augmentation of NO both in human tissue and in animal model. Most of the studies reviewed by Bolli 2001, demonstrate that long term exercise give better beneficial cardioprotective effect due to the increment of inducible nitric oxide synthase and increase circulatory NO level.

With the above mentioned controversies, this study is designed to examine whether short term exercise can affect the level of serum nitric oxide. The study will concentrate on the serum nitrite/nitrate (the stable end product of nitric oxide) change as well as changes on lipid profiles and general physical fitness in postmenopausal women after a short term exercise training.

Operational Definition

1. Aerobic exercise is defined as the physical activity using energy mainly from aerobic metabolism.
2. Moderate exercise is defined as the physical activity performed at intensity 50 – 65% VO_{2peak}, 30 minutes/day.
3. Short term exercise is defined as 10 consecutive days of exercise training.

CHAPTER II

LITERATURE REVIEW

Menopause, a natural phenomenon signified the end of reproductive period of women, occurs at an average age of 52 years, also defined as a woman's final menstrual period (Shangold 1996). This event results from the lack of endometrial stimulation by estrogen as the ovarian follicles become depleted. Hormonal alterations can produce symptoms such as hot flushes, night sweats, and vaginal dryness. And then, it relates about weight gain, bone loss, sleep disturbances, depression, and cardiovascular disease (CVD) (Shangold and Sherman 1998). There has been well documented that menopause and subsequent estrogen deprivation increase the risk of CVD in women. Before the menopause, women are at a decreased risk for CVD as compared to men, with men having between 3.5 and 4.5 times the risk over women. Within 10 years, following menopause, the risk of CVD in women increases to a level similar to that seen in men (Barrett-Connor 1994; Kafonek 1994).

Currently, hormone replacement therapy (estrogen with or without progestin) is the primary treatment for the symptoms and long term risks (CVD) associated with menopause. Physiologic effects of estrogen, such as arterial vasodilatation, decreased fibrinogen levels, increased high density lipoprotein cholesterol levels and decreased low density lipoprotein cholesterol levels, are likely to reduce cardiovascular risk (Cutson and Meuleman, 2000). But, adverse effects attributed to hormone replacement therapy include breast tenderness, breakthrough bleeding, cancer (breast or endometrial) and thromboembolic disorders. In addition, there are relative and absolute contraindications to the use of hormone replacement therapy (Ravnikar 1987; Grady et al. 1992). Also, alternative choice for menopausal women e.g. diet (high fiber, low fat), exercise, changed lifestyle, relaxation and stress reduction.

However some study found that age, gender and menopausal status are major genetic risk factors for coronary artery disease, while the cholesterol concentration, diet and lifestyle are largely environmental risk factors in an otherwise healthy population (Schaefer et al. 1994).

Aging is a physiological process that proceeds via structural and functional alterations in the vessel wall, causing an increase in the incidence of pathological conditions such as hypertension, coronary heart disease, cardiac insufficiency, and postural hypotension (Dohi et al. 1995; Gerhard et al. 1996). Endothelium dysfunction is also a common feature of aging which leads to some alterations in these cells, resulting in reduced relaxation and increased constriction (Luscher et al. 1993). Endothelium-mediated relaxation is reduced due to a decreased secretion and/or synthesis of endothelium-derived relaxing factor nitric oxide (EDRF-NO). In some tissues, the decrease in endothelium-mediated relaxation may promote endothelium-derived contracting factor (EDCF) (Busse et al. 1993; Luscher et al. 1991). According to some investigators, the decease in endothelium-mediated relaxation results from a decrease in the number of vasodilator receptors. Aging is linked with dysfunction of endothelium - the inner lining of the body's blood vessels, which plays a major role in maintaining a healthy circulation, and exercise is known to improve endothelial function. A lack of exercise (sedentary lifestyle) generally is considered a risk factor for atherosclerosis independent of its negative effects on body weight, blood pressure, and serum lipid values (Glasser et al. 1996). So important has physical activity and exercise come to be regarded in maintaining cardiovascular integrity that the American Heart Association has issued a position statement on its benefits (Fletcher et al. 1996). The statement affirms that physical inactivity is a recognized risk factor for coronary artery disease and has been related to increased cardiovascular mortality. Hornig et al. 1996, has pointed out that chronic immobilization or lack of adequate physical activity, whether by choice or as a result of disease, may be associated with reduced expression of NO synthase and thereby decreased synthesis of NO.

Nitric oxide (NO) is as a major signaling molecule in neurons and in the immune system, either acting within the cell in which it is produced or by penetrating cell membranes to affect adjacent cells. Nitric oxide is generated from arginine by the action of nitric oxide synthase (NOS) (Figure 2.1) (Moncada and Higgs 1993; บรรลือและคณะ 2540).

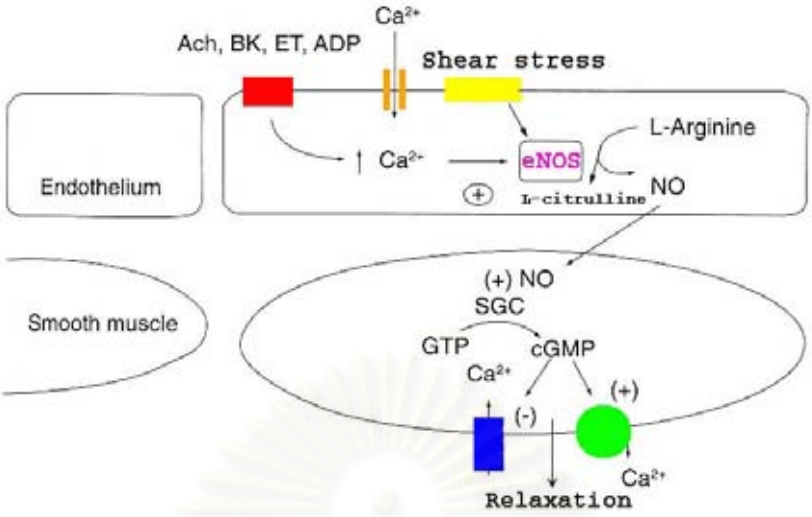


Figure 2.1 diagram of nitric oxide pathway

NO has a half-life of only a few seconds in vivo. However, since it is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes. NO has effects on neuronal transmission as well as on synaptic plasticity in the central nervous system. In the vasculature, NO reacts with iron in the active site of the enzyme guanylyl cyclase (GC), stimulating it to produce the intracellular mediator cyclic GMP (cGMP). This in turn enhances the release of neurotransmitters which play roles on smooth muscle relaxation and vasodilation (Figure 2.1), that is essential for the regulation of blood pressure. In the central nervous system nitric oxide is a neurotransmitter that underpins several functions, including the formation of memory. In the periphery, there is a widespread network of nerves, previously recognized as nonadrenergic and noncholinergic, that operate through a nitric oxide-dependent mechanism to mediate some forms of neurogenic vasodilatation and regulate various gastrointestinal, respiratory, and genitourinary tract functions. Nitric oxide also contributes to the control of platelet aggregation and the regulation of cardiac contractility (Moncada and Higgs 1993). In the extracellular milieu, NO reacts with oxygen and water to form nitrates and nitrites (Equation 1 and 2). NO toxicity is linked to its ability to combine with superoxide anions (O_2^-) to form peroxynitrite ($ONOO^-$) (Equation 3), an oxidizing free radical that can cause DNA fragmentation and lipid oxidation (Brown 1999; Lipton 1999; Murad 1998).

$2NO + O_2^- \longrightarrow N_2O_4 \xrightarrow{H_2O} NO_2^- + NO_3^- + 2H^+$ Equation 1

$2NO + O_2^- \longrightarrow N_2O_3 \xrightarrow{H_2O} 2NO_2^- + 2H^+$ Equation 2

$NO + O_2^- \longrightarrow ONO_2^- \xrightarrow{H^+} NO_3^-$ Equation 3

Lipid oxidation which occurred in LDL is known to associate with atherosclerosis, a possible coronary risk factor. Patients with coronary artery disease (CAD) and patients with hypercholesterolemia have been shown to have loss of NO-dependent vasodilation. NO-dependent vasodilation is impaired in the coronary and brachial circulation of patients with diabetes, hypertension, and cigarette smoking. Postmenopausal women also demonstrate impairment of vasomotor function. Thus, hypercholesterolemia, diabetes mellitus, hypertension, cigarette smoking, aging, sedentary lifestyle, and postmenopausal state, and established CAD are all associated with endothelial dysfunction (Cooke 1997; Teddei et al. 1996).

Since exercise can improve endothelium-dependent vasodilatation, Teddei et al. 2000 found that regular physical activity can at least in part prevent the age-induced endothelial dysfunction, probably the restoration of nitric oxide availability consequent to prevention of production of oxidative stress. Regular exercise benefits the heart and bones, helps regulate weight, and contributes to a sense of overall well-being and improvement in mood. Sedentary people are far more prone to coronary heart disease, obesity, high blood pressure, diabetes, and osteoporosis. Sedentary women may also suffer more from chronic back pain, stiffness, insomnia, and irregularity. They often have poor circulation, weak muscles, shortness of breath, and loss of bone mass. Depression can also be a problem. Women who regularly walk, jog, swim, bike, dance, or perform some other aerobic activity can more easily circumvent these problems and also achieve higher HDL cholesterol levels (Schiff 2002).

There are several studies both in human and experimental animals on short term exercise which demonstrate the beneficial outcomes. Lewis et al. 1999 studied in 9 hypercholesterolemic patients which cycle training 3x30 minutes/week at 65% VO_{2max} for 4 weeks. They found that basal release of endothelium-derived nitric oxide was increased.

Higashi et al. 1999 studied in 10 essential hypertension which brisk walking 30 minutes 5 to 7 times/week for 12 weeks. He found an increased release of nitric oxide, and that continued physical exercise alleviates impairment of reactive hyperemia in patients with essential hypertension.

Hambrecht et al. 2000 studied in 10 coronary artery disease which cycle ergometer 6x10 minutes/day for 4 weeks at 80% HR_{max}. They found that exercise training improves endothelium-dependent vasodilatation in patients with coronary artery disease.

Another study of patients whose physical activity was limited by congestive heart failure, flow-dependent dilation can be enhanced by physical training. After 4 weeks of hand-grip training, flow-dependent dilation was restored, most likely by increased endothelial release of NO (Clarkson et al. 1999).

In healthy subjects, Node et al. 1997 studied changes in the plasma concentration of NO in systemic blood in response to acute physical exercise in 16 healthy volunteers (60 ± 4 yr). Cycling exercise began at a workload of 25 Watts (W), which was increased every 1 minute in stepwise increments of 25 W. For all volunteers, exercise was discontinued when leg fatigue occurred. It was found that NO markers increased statistically. Since it is rather difficult to directly quantitative NO production in most circumstances, the measurement of serum nitrite/nitrate concentrations has been established as an alternative useful method to estimate nitric oxide production in both human and experimental animal. Maeda et al. 2001 studied in 8 healthy young subjects (20.3 ± 0.5 yr) exercised by cycling on a leg ergometer (70% VO_{2max} for 1 hour, 3-4 days/week) for 8 weeks. The result that chronic exercise caused an increase in production of NO suggested beneficial effects on the cardiovascular systems.

In animal, studies of the coronary vasculature have typically been performed in either pigs or dogs. Treadmill training for only 7 days in dogs enhanced flow-mediated and Acetylcholine (Ach)-induced dilation in epicardial coronary arteries (Wang et al. 1993), while training for 10 days increased nitrite release from both proximal coronary arteries and microvessels in response to Ach infusion (Sessa et al. 1994). Besides, short-term aerobic exercise program in humans (exercise consisted of 7 consecutive days of treadmill walking and cycle ergometry) also improves insulin sensitivity. It has been

confirmed that exercise could induce improvements in glucose and insulin metabolism (Brown 1997).

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND EQUIPMENT

The following pieces of equipment were used to assess physical performance and analyze blood samples.

Physical Performance Test :-

- Bicycle ergometer (Quinton instrument CO, Corival 400, USA)
- Treadmill ergometer (Quinton, Q 55 series 90, USA)
- Oxygen and carbon dioxide gas analyzer (Quinton Metabolic Cart, QMC, USA)
- Sphygmomanometer (Baumanometer®, Stand by and wall unit 33, USA)
- Stethoscope (3M™ Littmann™, Classic II S.E., USA)
- Heart rate monitor polar pacer (Polar Accurex Plus, Polar electro, Finland)
- A weighing scale (Yamato, DP-6100 GP, Japan)
- A scale for height

Blood analysis :-

- Microplate reader (Anthos 2010, Austria)
- Reagent kit for nitric oxide: nitrite/nitrate (R&D systems GmbH, USA)
  - Nitrate Reductase
  - Nitrate Reductase Storage Buffer
  - NADH
  - Nitrite Standard
  - Nitrate Standard
  - Reaction Buffer Concentrate
  - Griess Reagent I
  - Griess Reagent II

Miscellaneous :-

- Pipettes and pipette tips
- Deionized water
- Ice bath
- Vortex mixer
- Vivaspin 2 (10,000 Molecular Weight cutoff, Sartorius AG, Germany)

METHODS

A. Volunteers

The volunteers were 30 healthy postmenopausal women. The recruitment of volunteer was achieved via the public announcement and local advertisement at King Chulalongkorn Memorial Hospital, The Thai Red Cross Society.

1. Inclusion criteria

- Women who no history or evidence of Diabetes, Hypertension, Heart disease, Cancer
- 40-60 years of age
- Menopause at least 1 year
- No hormone replacement therapy used in the past 6 months
- Written informed consent provided

2. Exclusion criteria

- Surgical menopause
- Hypertension (resting > 160/90 mmHg) or on antihypertension medication
- Diabetes mellitus
- Heart disease
- Total cholesterol > 300 mg/dL or on lipid lowering medication
- Triglycerides > 400 mg/dL
- Body mass index (BMI) > 30 kg/m^2

- Regular conditioning exercise for twice a week at least 20 minutes duration time
- Unable to participate in the constitutive exercise program

The participants were recruited into the study after receiving both verbal and written informed consent. The study protocol has been approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University.

B. Study Designs

The participants were assigned into 2 groups by purposive sampling according to the modes of exercise training bicycle (non-weight bearing) or treadmill (weight bearing). Both groups underwent 3 parts of the study protocol as follows.

PART I (day 1-11): CONTROL/RUN IN PHASE

Day 1:

- Subjects arrived at Wellness Research Center, 1st Floor of the Physiology building, Department of Physiology, Faculty of Medicine, Chulalongkorn University. Weight was measured under light clothes no shoes.
- Blood pressure (BP) and heart rate assessment were performed after 10 minutes of rest. The mean of two measures separated by 30 seconds was recorded.
- Prior to a peak oxygen uptake (VO_{2peak}) test, 1st blood sample was collected after 8-12 hr fast.
- A total of 8 mL of blood was drawn from anticubital vein into a disposable syringe.
- The amount of 4 mL was added into heparinized tube and centrifuged at 4,000 rpm for 10 min. The separated plasma was then analyzed for lipid profiles. The rest of blood were kept in a non-anticoagulated tube and transferred to centrifuge at 3,000 rpm for 5 min. The serum was then separated and kept in –20°C. The serum was later used for Nitrite/Nitrate (NO_2^-/ NO_3^-) assays.

- Immediately post the VO_{2peak} test, the 2nd blood sample for NO_2^-/ NO_3^- and lipid profile was collected and processed as described above.

Day 2 – day 11: - **CONTROL DAYS/RUN IN PERIODS.**

- Subjects were on their regular daily activities

PART II (day 12-22): EXERCISE PHASE

Day 12: - The processes as in day 1 were repeated.

Day 13 – day 22: - **EXERCISE DAYS**

- Subjects arrived at Wellness Research Center, 1st Floor of the Physiology building, Department of Physiology, Faculty of Medicine, Chulalongkorn University.
- Exercise at 50-65% VO_{2peak} work rate individually, for 30 min/day either by bicycle or treadmill ergometer with heart rate monitor
- Warm up 10 min before exercise and cool down 10 min after exercise using stretching maneuvers, (Figure 3.4).

PART III (day 23): PROTOCOL FINAL TESTING

Day 23: The processes as in day 1 were repeated.

FLOW CHART OF THE PROTOCOL

PART I (day 1-11): control/run in phase

Day 1:

Subjects arrived at Wellness Research Center, 1st Floor of the Physiology building, Department of Physiology, Faculty of Medicine, Chulalongkorn University.

↓

sit resting prior to blood pressure and heart rate assessment (Figure 3.1)

↓

collect 8 mL fasting blood from anticubital vein (Figure 3.2) (1st blood collection)
- → 4 mL into clotted blood tube
- → 4 mL into heparinized tube

↓

peak oxygen uptake (VO_{2peak}) test (Figure 3.3)

↓

collect 8 mL blood from anticubital vein immediately after the VO_{2peak} test (2nd blood collection)

↓

- 4 mL into clotted blood tube
- 4 mL into heparinized tube

Day 2 – day 11: - Control days

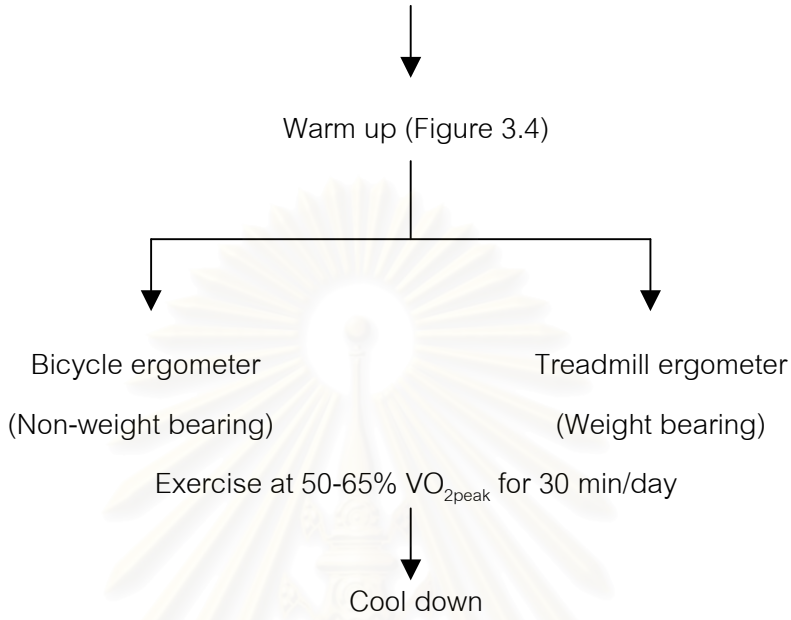
- Subjects were on their regular daily activities

PART II (day 12-22): exercise phase

Day 12: - The processes as in day 1 were repeated.

Day 13 – day 22: - Exercise days

Subjects arrived at Wellness Research Center, 1st Floor of the Physiology building, Department of Physiology, Faculty of Medicine, Chulalongkorn University



PART III (day 23): protocol final testing

Day 23: The processes as in day 1 were repeated.

Figure 3.1 Measuring the resting blood pressure and heart rate

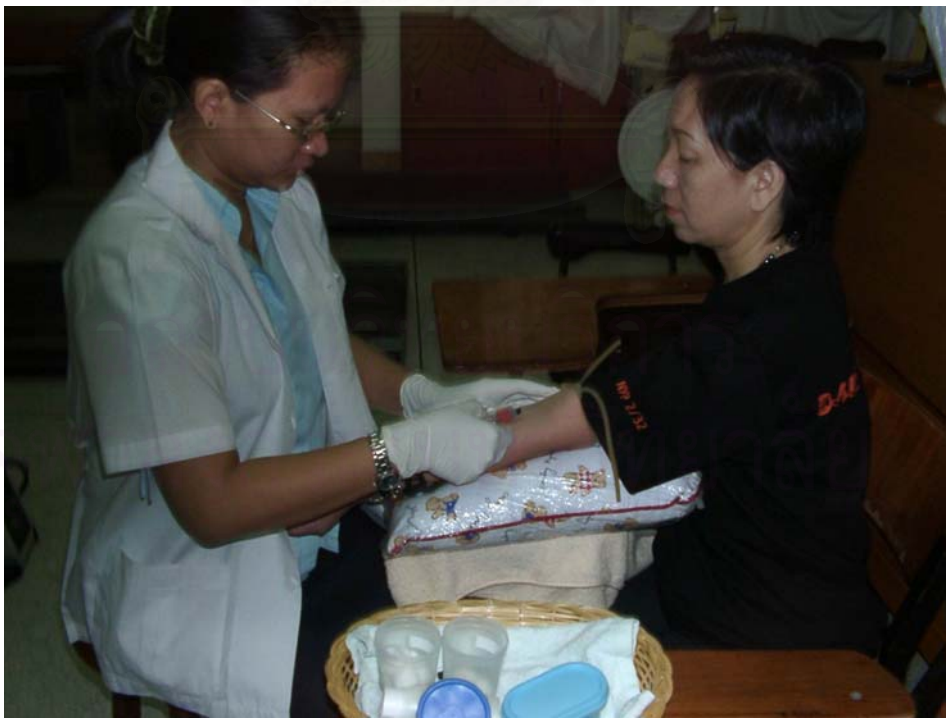


Figure 3.2 Collecting blood sample

Figure 3.3 VO_{2peak} assessment

C. VO_{2peak} assessment

The Ordinary ramp protocol on a cycle ergometer (Corival 400) was used to assess the VO_{2peak}. The subjects initially pedaled at 50-60 rpm with no resistance for 3 minute and then the load was increased to achieved an incremental work rate of 25 watt every minute. The test was terminated at volitional fatigue (Figure 3.3).

Figure 3.4 Warm up and cool down stretching maneuvers

D. Storage of serum samples

The clotted blood samples were centrifuged at 3,000 rpm, 4°C for 5 minutes. The serum was separated into 0.5 mL aliquots and stored at -20°C for the quantitative of serum nitrite/nitrate concentration.

Lipoprotein – Lipid Analysis

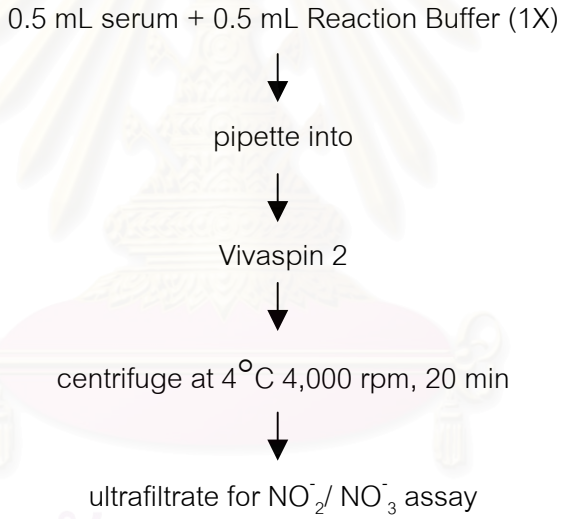
Lipid profiles, including total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were determined using automate analyzer (Integra 400 plus, Roche Diagnostics GmbH, Mannheim, Germany), which analyzed by the Special Laboratory at King Chulalongkorn Memorial Hospital. Total cholesterol level was measured by enzymatic, colorimetric method (CHOD/PAP) with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine. Triglyceride levels was

measured by enzymatic, colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazone. High-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels were determined by homogeneous enzymatic colorimetric assay.

Assays for Nitric Oxide Metabolites

1. **Sample preparation**

Preparation of sample was performed before all assays. All samples required at least a 2-fold dilution with Reaction Buffer (1X). After dilution, samples were ultrafiltered through a 10,000 molecular weight cutoff filter (Vivaspin 2) to eliminate proteins. The ultrafiltrate of serum must be freshly prepared prior to analysis of nitrite/nitrate as follows:



2. **Reagent preparation**

All reagents of the nitrite/nitrate test kit (except nitrate reductase) were brought to room temperature before use. Deionized water was used when reconstituting or diluting reagents to avoid nitrite/nitrate contamination.

The following 5 reagents were treated as shown in the diagrams below.

2.1. Reaction Buffer (1X):

10 mL Reaction Buffer Concentrate (10X) + 90 mLdeionized water

↓ mixed

100 mL Reaction Buffer (1X)

storage at 2-8°C

2.2. Stock NADH:

Reconstitution of NADH

NADH 1 vial + 0.5 ml deionized water

↓

3 min at room temperature,

slightly agitate

↓

stock NADH

↓

storage at -20°C

2.3. Working NADH:

immediately before use NADH (stock) 1 vol + deionized H_2O 2 vol

↓

working NADH, to be dispended 25 μl/ well;

kept on ice during the assay

2.4. Stock Nitrate reductase:

Reconstitution of Nitrate reductase

Nitrate reductase 1 vial + 1.0 ml Nitrate reductase storage buffer

↓

Vortex

↓

15 min at room temperature

↓

Vortex vigorously

↓

15 min at room temperature

↓

Aliquot

storage at -20°C

2.5. Working Nitrate reductase:

Immediately before use, nitrate reductase was diluted as described below.

The number of standard and sample wells to be used was determined (blank wells not included).

2.5.1. Nitrate reductase (μl) = (# wells + 2) x 10 μl.

2.5.2. Reaction Buffer (μl) = volume on 5.1 x 1.5

2.5.3. Add volumes from steps 5.1 and 5.2 to a tube, vortex.

2.5.4. Place on ice and use within 15 minutes of dilution, 25 μl /well.

3. Preparation of Nitrite Standards for Standard Curve Calibration

An amount of 180 μl of Reaction Buffer (1X) was pipetted into a 200 μmol/L well. Another volume of 100 μl of Reaction Buffer (1X) was pipetted into the remaining standard wells. The amount of 20 μl of the 2,000 μmol/L nitrite standard stock was used to produce a dilution series (Table 3.1). Each well was mixed thoroughly and the pipette tip was changed between each transfer. The 200 μmol/L standard served as the high concentration and the Reaction Buffer (1X) served as the zero standard (0 μmol/L).

Table 3.1 Working Nitrite Standard

	Micro well						
(µl)	1	2	3	4	5	6	7
1. Reaction buffer (1x)	180	100	100	100	100	100	100
2. Standard NO_2^- 2,000 µmol/L	20	-	-	-	-	-	-
3. Transfer from 1--->2 ; 2 --->3; 3 --->4; 4 --->5; 5 --->6; 6 --->7, 100 µl each	100	100	100	100	100	100	out
4. Final volume	100	100	100	100	100	100	200
5. Pipette out 100 µl	-	-	-	-	-	-	100
6. Aliquot duplicate well	50	50	50	50	50	50	50
	50	50	50	50	50	50	50
7. Final concentration µmol/L	200	100	50	25	12.5	6.25	3.12

4. Preparation of nitrate standards for standard curve calibration

An amount of 180 µl of Reaction Buffer (1X) was pipetted into a 200 µmol/L well. An amount of 100 µl of Reaction Buffer (1X) was added into the remaining standard wells. An amount of 20 µl of the 1,000 µmol/L nitrate standard stock was used to produce a dilution series (Table 3.2). Each well was thoroughly mixed and the pipette tip was changed between each transfer. The 100 µmol/L standard serves as the high concentration and the Reaction Buffer (1X) serves as the zero standard (0 µmol/L).

Table 3.2 Working Nitrate Standard

(µl)	Micro well					
	1	2	3	4	5	6
1. Reaction buffer (1x)	180	100	100	100	100	100
2. Standard NO_3^- 1,000 µmol/L	20	-	-	-	-	-
3. Transfer from 1--->2; 2 --->3; 3 --->4; 4 --->5; 5 --->6, 100 µl each	100	100	100	100	100	out
4. Final volume	100	100	100	100	100	200
5. Pipette out 100 µl	-	-	-	-	-	100
6. Aliquot duplicate well	50	50	50	50	50	50
	50	50	50	50	50	50
7. Final concentration µmol/L	100	50	25	12.5	6.25	3.12

5. Nitrite assay procedure

This assay procedure measures the concentration of endogenous nitrite present in the sample. All reagents were brought to room temperature before use. All samples and standards were assayed in duplicate.

5.1 All reagents, working standards, and samples were prepared as directed in the previous sections (1, 2 and 3).

5.2 Excess microplate strips were removed from the plate frame, and returned them to the storage bag and proceed as shown in Table 3.3.

5.3 A volume of 200 µL of Reaction Buffer (1X) was added to the Blank wells.

5.4 A volume of 50 µL of Reaction Buffer (1X) was added to the zero standard wells.

5.5 A volume of 50 µL of Nitrite Standard or sample was added to the remaining wells.

5.6 A volume of 50 µL of Reaction Buffer (1X) was added to all standard and sample wells.

5.7 A volume of 50 µL of Griess Reagent I was added to each well except the Blank wells.

5.8 A volume of 50 μL of Griess Reagent II was added to each well except the Blank wells. Well mixed by gently tapping the side of the plate.

5.9 All well were incubated for 10 minutes at room temperature.

5.10 The optical density (OD) of each well was determined using a microplate reader set at 540 nm.

Table 3.3 Nitrite assay procedure

μl	Blank well	Micro well								serum ultrafiltrate
		Zero	1	2	3	4	5	6	7	
1. Reaction buffer (1x)	200	-	-	-	-	-	-	-	-	-
2. Reaction buffer (1x)	-	50	-	-	-	-	-	-	-	-
3. Nitrite standard	-	-	50	50	50	50	50	50	50	-
	-	-	50	50	50	50	50	50	50	-
4. Sample ultrafiltrate	-	-	-	-	-	-	-	-	-	50
	-	-	-	-	-	-	-	-	-	50
5. Reaction buffer (1x)	-	50	50	50	50	50	50	50	50	50
6.Griess reagent I	-	50	50	50	50	50	50	50	50	50
7.Griess reagent II	-	50	50	50	50	50	50	50	50	50
8. Final volume	200	200	200	200	200	200	200	200	200	200
9. Tapping	Tapping gently									
10. Incubate	10′ Room temperature									
11. Standard conc. μmol/L	-	0	200	100	50	25	12.5	6.25	3.12	?
12. OD 540 nm	0									

6. Nitrate reduction assay procedure

This assay procedure measures total nitrite by converting nitrate to nitrite. To determine the nitrate concentration in the sample, the endogenous nitrite concentration measured from the Nitrite Assay Procedure must be subtracted from the converted nitrite concentration measured in this assay procedure.

The reconstituted NADH and Nitrate Reductase were kept on ice throughout the duration of the assay. All other reagents were brought to room temperature before use. All samples and standards were assayed in duplicate.

6.1 All reagents, working standards, and samples were prepared as directed in the previous sections (1, 2 and 4).

6.2 Excess microplate strips were removed from the plate frame, and returned them to the storage bag and proceed as shown in Table 3.4.

6.3 A volume of 200 μL of Reaction Buffer (1X) was added to the Blank wells.

6.4 A volume of 50 μL of Reaction Buffer (1X) was added to the zero standard wells.

6.5 A volume of 50 μL of Nitrite Standard or sample was added to the remaining wells.

6.6 A volume of 25 μL of NADH was added into all standard and sample wells.

6.7 A volume of 25 μL of Nitrate Reductase was added into all standard and sample wells. Each well was mixed and covered with the adhesive strip.

6.8 Incubate for 30 minutes at 37°C.

6.9 A volume of 50 μL of Griess Reagent I was added to all wells except Blank wells.

6.10 A volume of 50 μL of Griess Reagent II was added to all wells except Blank wells. Well mixed by gently tapping the side of the plate.

6.11 All wells were incubated for 10 minutes at room temperature.

6.12 The optical density (OD) of each well was determined using a microplate reader set at 540 nm.

Table 3.4 Nitrate reduction assay procedure

µl	Blank well	Micro well							serum ultrafiltrate
		Zero	1	2	3	4	5	6	
1. Reaction buffer (1x)	200	-	-	-	-	-	-	-	-
2. Reaction buffer (1x)	-	50	-	-	-	-	-	-	-
3. Nitrate standard	-	-	50	50	50	50	50	50	-
	-	-	50	50	50	50	50	50	-
4. Sample ultrafiltrate	-	-	-	-	-	-	-	-	50
	-	-	-	-	-	-	-	-	50
5. NADH working	-	25	25	25	25	25	25	25	25
6. Nitrate reductase	-	25	25	25	25	25	25	25	25
7. Mixed, covered	Covered with adhesive strip mixed								
8. Incubate	30′ 37°C								
9.Griess reagent I	-	50	50	50	50	50	50	50	50
10.Griess reagent II	-	50	50	50	50	50	50	50	50
11. Tapping	Mixed by tapping gently								
12. Incubate	10′ Room temperature								
13. Final volume	200	200	200	200	200	200	200	200	200
14. Standard conc. µmol/L	0	0	100	50	25	12.5	6.25	3.12	?
15. OD 540 nm	0								

7. Calculation of the results

The duplicate readings were averaged for each standard and sample and subtracted the average zero standard optical density.

Determination of the concentration of nitrate in the sample:

7.1 The endogenous nitrite concentration (X µmol/L) was measured using the Nitrite Assay Procedure.

7.2 The total nitrite concentration (Y µmol/L) was measured after the conversion of nitrate to nitrite using the Nitrate Reduction Assay Procedure.

7.3 Determine the nitrate concentration in the sample by subtracting endogenous nitrite concentration from the total nitrite concentration, as indicated by the following equation.

Nitrate concentration = (Y - X) μmol/L

E. Statistical Analysis

The results ware reported as mean and standard deviation (SD). All data were analyzed using the Statistical Package for the Social Science (SPSS) version 11.5. The distribution of data was tested for normal distribution. If the data was normally distributed, the paired t-test was used to test for significant difference between two conditions within the same group i.e. either pre and post VO_{2peak} test, or the tests applied at either before or after exercise training. Unpaired t-test was used to test for significant difference among different groups i.e. the treadmill and the bicycle groups. If data were not normally distributed (non-parametric), then Wilcoxon Signed Ranks test was used instead of paired t-test and the Mann-Whitney U test was used in place of Unpaired t-test. Difference was considered significant at a p-value ≤ 0.05.

CHAPTER IV

RESULTS

The design of this study is experimental research with randomized sampling. This study protocol was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University.

Thirty healthy post-menopausal women were enrolled in this 23 days program. They took the VO_{2peak} test on day 1, day 12, and day 23, represented baseline or control phase, exercise phase and protocol final testing. During day 2 to day 11 which was the control phase, subjects were asked to retrain their regular daily living and eating style. However, during day 13 to day 22, the same group of volunteers was equally randomized into two groups of exercise procedures either of treadmill or bicycle training for 30 minutes daily for 10 consecutive days (exercise phase).

4.1 The physical characteristics of volunteers

The mean $\pm$ SD of anthropometric measures and other basal characteristics of all volunteers were shown in Table 4.1. Brief results were as follows: height 154.58 $\pm$ 5.20 cm, body weight 55.00 $\pm$ 9.65 kg, body mass index (BMI) 22.97 $\pm$ 3.59 kg/m^2, resting heart rate 73.7 $\pm$ 6.55 beats/min, resting systolic blood pressure 113 $\pm$ 9.52 mmHg, resting diastolic blood pressure 73 $\pm$ 6.50 mmHg, and peak oxygen uptake (VO_{2peak}) 19.11 $\pm$ 3.92 mL/kg/min.

Table 4.1 The physical characteristics data *

	MEAN	SD
Age (yr)	53.53	3.59
Height (cm)	154.58	5.20
Body weight (kg)	55.00	9.65
BMI (kg/m^2)	22.97	3.59
Resting heart rate (beats/min)	73.70	6.55
Resting systolic blood pressure (mmHg)	113	9.52
Resting diastolic blood pressure (mmHg)	73	6.50
VO_{2peak} (mL/kg/min)	19.11	3.92

* Total subject number = 30

4.2 The standard curve for nitrite/nitrate assay

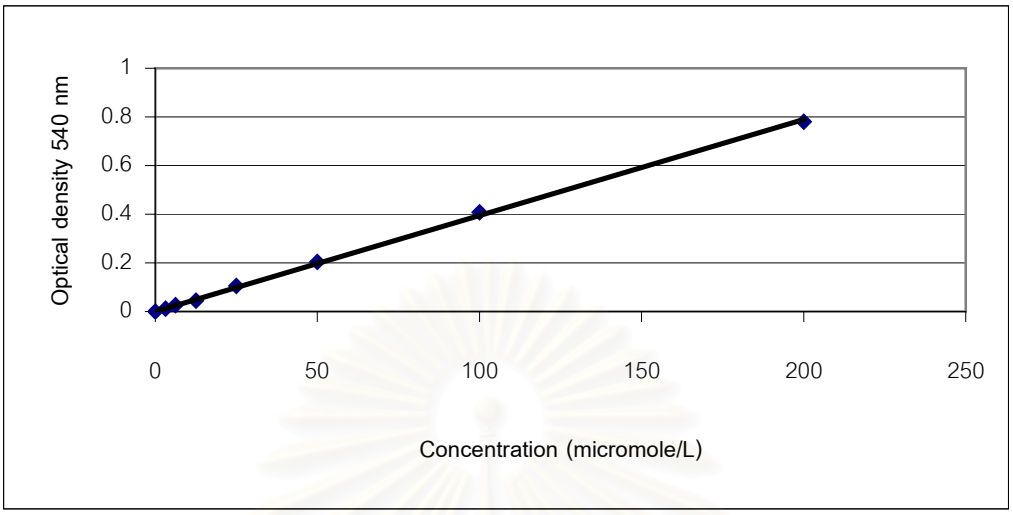
4.2.1 Nitrite standard curve

Using the 2,000 μmol/L of nitrite standard supplied with the assay kit, a serial dilution was prepared according to the manufacturers protocol. The optical density at 540 nm of the final concentration of nitrite standard at 0, 3.12, 6.25, 12.5, 25, 50, 100, and 200 μmol/L was presented in Table 4.2 A. The data were plotted in Figure 4.1 using the average of two assays.

Table 4.2 A The nitrite concentration and the OD at 540 nm

Nitrite concentration (μmol/L)	OD 540 nm
zero	0.0000
3.12	0.0115
6.25	0.0265
12.50	0.0445
25.00	0.1055
50.00	0.2035
100.00	0.4080
200.00	0.7805

Figure 4.1 The nitrite standard curve



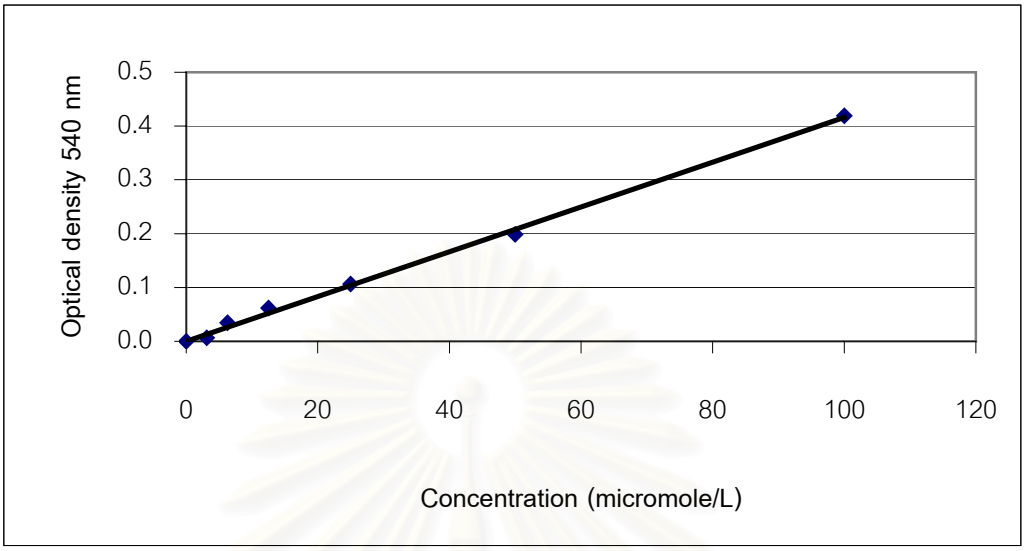
4.2.2 Nitrate standard curve

Using the 1,000 μmol/L of nitrate standard supplied with the assay kit, a serial dilution was prepared according to the manufacturers protocol. The optical density at 540 nm of the final concentration of nitrate standard at 0, 3.12, 6.25, 12.5, 25, 50, and 100 μmol/L was shown in Table 4.2 B. The data were plotted in Figure 4.2 using the average of two assays.

Table 4.2 B The nitrate concentration and the OD at 540 nm

Nitrate Concentration (μmol/L)	OD 540 nm
zero	0.00000
3.12	0.00625
6.25	0.03475
12.50	0.06175
25.00	0.10650
50.00	0.19875
100.00	0.41900

Figure 4.2 The nitrate standard curve



4.3. Assay of serum nitrite/nitrate

On day 1, day 12, and day 23 each subject was asked to perform VO_{2peak} test. The blood samples were collected before VO_{2peak} test (pre VO_{2peak} test) and after VO_{2peak} test (post VO_{2peak} test) for further assay of nitrite / nitrate and lipid profiles.

4.3.1 Effect of exercise training on serum nitrite/nitrate concentration

From Figure 4.3.1 A and Figure 4.3.1 B, the pre VO_{2peak} concentration of serum nitrite/nitrate taken at day 1, 12, and 23 showed no significant differences. In the same manner, comparison of the post VO_{2peak} concentration of serum nitrite/nitrate resulted in no significant differences as well, (Figure 4.3.1 A & B; Table 4.3.1).

To search for the effect of acute exercise (pre-post VO_{2peak}) on the concentration of serum nitrite/nitrate, the pre VO_{2peak} concentration of serum nitrite/nitrate of day 1, 12, and 23 was compared to the post VO_{2peak} concentrations of the same period. Although the results did not exhibit any significant differences, (Figure 4.3.1 A & B; Table 4.3.1). But when observed the concentration of serum nitrate at day 1, the post VO_{2peak} serum nitrate concentration was 26.14 μmol/L and stayed at this level through out the study period. While the pre VO_{2peak} serum nitrate was 22.67 μmol/L at day 1. Whether this

slightly increase of serum nitrate could be a response to acute exercise or not, it needed further investigation.

Table 4.3.1 Serum nitrite/nitrate concentration at day 1, day 12, and day 23 of treadmill group, bicycle group and the summative of both.

Exercise Mode	Serum concentration (μmol/L)	Baseline or control phase		Exercise phase		Protocol final testing	
		Day 1		Day 12		Day 23	
		Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}
Treadmill (n=15)	Nitrite	1.46 ± 2.19	1.29 ± 3.58	1.94 ± 3.44	1.03 ± 1.39	0.79 ± 1.02	0.83 ± 1.14
	Nitrate	22.62 ± 16.99	29.12 ± 18.65	23.17 ± 18.25	23.46 ± 18.54	27.69 ± 18.76	26.85 ± 18.84
Bicycle (n=15)	Nitrite	1.25 ±1.78	1.26 ± 2.17	0.84 ± 1.23	0.73 ± 1.11	1.31 ± 2.11	0.75 ± 1.80
	Nitrate	22.72 ± 21.04	23.16 ± 19.29	29.13 ± 30.86	29.94 ± 30.13	26.78 ± 21.29	27.26 ± 21.30
Total (n=30)	Nitrite	1.36 ± 1.97	1.27 ± 2.91	1.39 ± 2.60	0.88 ± 1.24	1.05 ± 1.65	0.79 ± 1.48
	Nitrate	22.67 ± 18.79	26.14 ± 18.89	26.15 ± 25.10	26.70 ± 24.80	27.24 ± 19.72	27.05 ± 19.76

Figure 4.3.1 A Serum nitrite concentration of 30 subjects at day 1, day 12, and day 23, a comparison of the pre VO_{2peak} to post VO_{2peak} test.

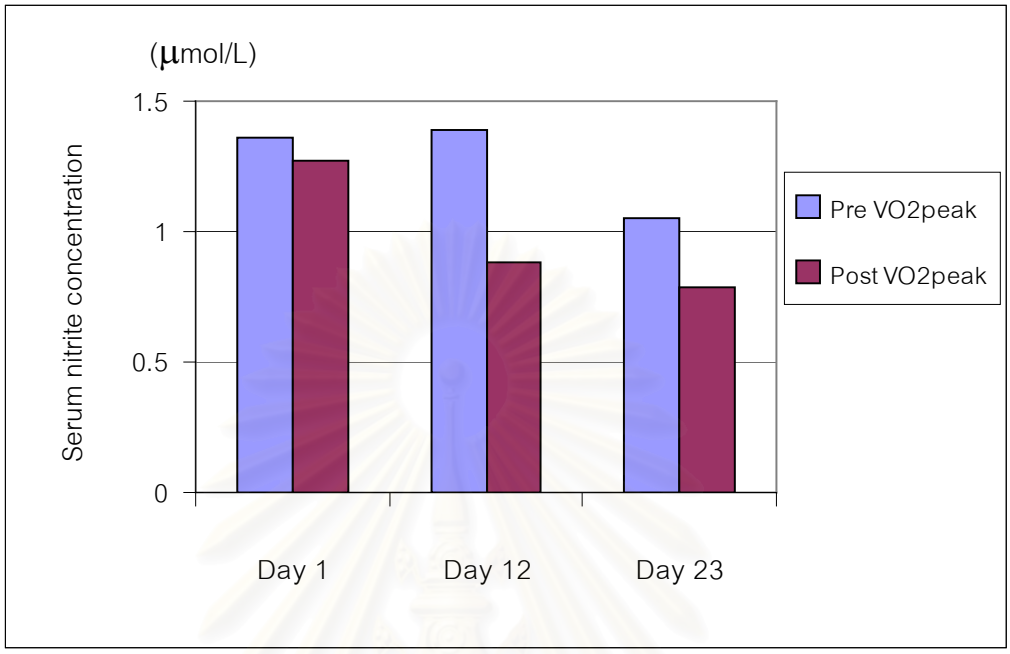
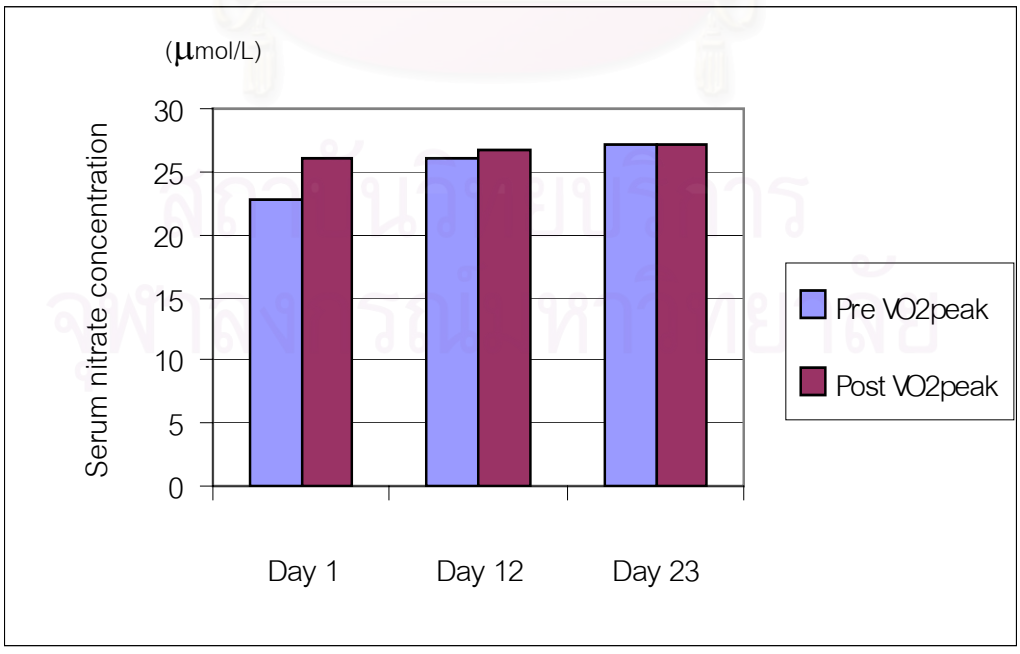


Figure 4.3.1 B Serum nitrate concentration of 30 subjects at day 1, day 12, and day 23, a comparison of the pre VO_{2peak} to post VO_{2peak} test.



4.3.2 Effect of exercise mode on the concentration of serum nitrite/nitrate

The modes of exercise either by treadmill or bicycle did not affect the concentration of serum nitrite/nitrate, the data was shown in Figure 4.3.1 A & B; Table 4.3.1. Further analysis by subtracting the individual post VO_{2peak} concentration of serum nitrite/nitrate from the pre VO_{2peak} concentrations, the results were presented in Table 4.3.2. The negative mean differences signified no increment in the concentration of serum nitrite/nitrate mediated by acute exercise via the VO_{2peak} test.

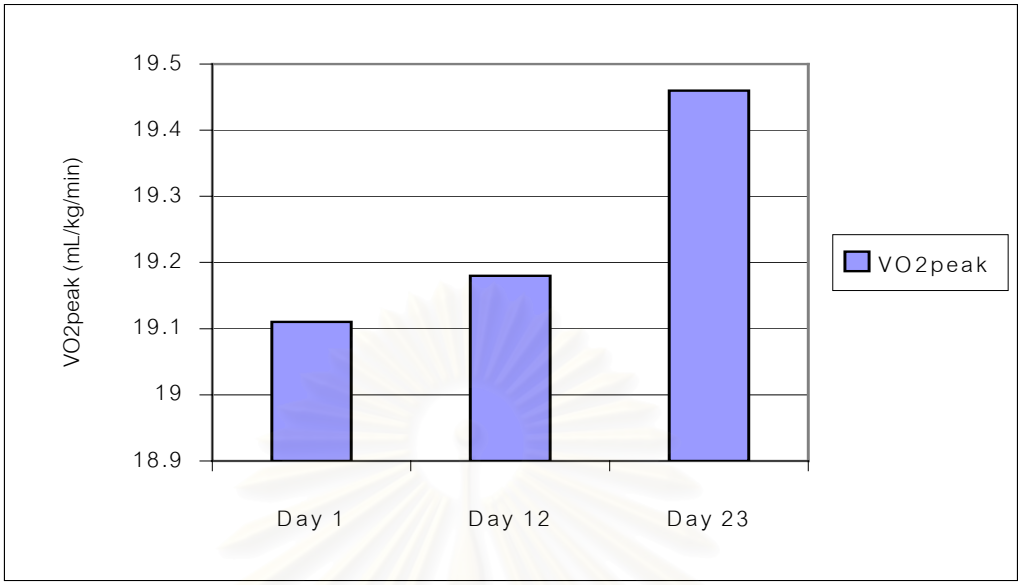
Table 4.3.2 The mean difference of serum nitrite/nitrate at day 1, day 12, and day 23 in treadmill and bicycle groups

Exercise Mode	Serum concentration (μmol/L)	Mean difference of pre and post VO_{2peak}		
		Day 1	Day 12	Day 23
Treadmill (n=15)	Nitrite	-0.17 ± 1.98	-0.91 ± 2.7	0.03 ± 0.76
	Nitrate	6.50 ± 14.49	0.29 ± 1.51	-0.84 ± 9.52
Bicycle (n=15)	Nitrite	0.01 ± 1.66	-0.11 ± 1.21	-0.56 ± 1.82
	Nitrate	0.43 ± 5.35	0.82 ± 5.02	0.48 ± 2.95

4.4 Comparative study of peak oxygen uptake (VO_{2peak}) in subjects during the control phase and the exercise phase.

The VO_{2peak} tests on day 1, 12, and 23 were performed in every subjects in order to demonstrate the effect of exercise training on oxygen uptake, the result was shown in Figure 4.4. It was noticed that the mean of the peak oxygen uptake (VO_{2peak}) increased from 19.1 mL/kg/min at day 1 up to 19.5 mL/kg/min at the end of exercise training period (day 23). Although the VO_{2peak} at day 23 increased without statistic significance, but it implied an improvement of the physical fitness of all volunteers. Besides, all volunteers felt their own wellness and wished to continue further exercise practices.

Figure 4.4 VO_{2peak} at day 1, day 12, and day 23 of all subjects



4.5 Effect of exercise training on lipid profiles

The lipid profiles test package comprised of the tests for total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C). The data for lipid profiles of the group on treadmill and the bicycle group were presented in Table 4.5 together with data of the summative of both groups. The result of the treadmill group in Figure 4.5 A exhibited highly statistic significant decrease of serum triglycerides level ($p<0.01$) when comparing the serum TG level of day 23 to day 12. Whilst, the HDL-C of this treadmill group increased significantly with $p< 0.05$. (Figure 4.5 A). There was no statistical significantly change observed in the lipid profiles of the bicycle group. The analysis of the summative data on lipid profiles on both groups of thirty volunteers was shown in Figure 4.5 B. The statistically significant changes ($p<0.05$) of both TG and HDL-C on day 23 as compared to day 12, still remained.

The effect of acute exercise on the concentration of lipid profiles, when compared the pre VO_{2peak} concentration of lipid profiles at day 1, 12, and 23 to the post VO_{2peak} concentrations of the same period, the results showed significant differences of all parameters ($p<0.01$), (Figure 4.5 C-F, Table 4.5).

Table 4.5 Blood lipid profiles in subjects at day 1, day 12, and day 23

Exercise Mode	Lipid profiles (mg/dL)	Baseline or control phase			Exercise phase			Protocol final testing		
		Day 1			Day 12			Day 23		
		Pre VO_{2peak}	Post VO_{2peak}	Percent change	Pre VO_{2peak}	Post VO_{2peak}	Percent change	Pre VO_{2peak}	Post VO_{2peak}	Percent change
Treadmill (n=15)	TC	226.13 ± 34.19	238.87 ± 35.13	*5.63*	227.4 ± 38.52	241.73 ± 43.24	*6.30*	231.73 ± 36.52	242.33 ± 41.03	*4.57*
	TG	105.53 ± 60.95	115.07 ± 71.87	*9.04*	113.07 ± 64.86	121.93 ± 70.35	*7.84*	87.07 ± 45.22	93.67 ± 48.39	*7.58*
	HDL-C	68.6 ± 14.79	73.6 ± 17.14	*7.29*	68 ± 13.65	73.13 ± 13.79	*7.54*	73.07 ± 13.4	78 ± 14.09	*6.75*
	LDL-C	146.47 ± 37.52	150.47 ± 38.48	*2.73*	146.87 ± 36.63	158.07 ± 45.24	*7.63*	149 ± 36.51	155.6 ± 38.97	*4.43*
Bicycle (n=15)	TC	218.87 ± 28	231.87 ± 25.77	*5.94*	220.2 ± 20.88	230.67 ± 25.41	*4.75*	214.07 ± 24.44	227.6 ± 24.03	*6.32*
	TG	90.87 ± 23.79	98.87 ± 28.49	*8.80*	87.47 ± 25.54	99 ± 31.85	*13.18*	82.2 ± 26.02	90.87 ± 28.25	*10.55*
	HDL-C	69.33 ± 13.03	74.33 ± 16.24	*7.21*	69.47 ± 14.5	73.53 ± 15.25	*5.84*	71.13 ± 13.97	75.53 ± 14.58	*6.19*
	LDL-C	139.4 ± 31.68	148.8 ± 31.83	*6.74*	137.87 ± 26.41	147.2 ± 32.07	*6.77*	133.93 ± 28.49	140.27 ± 27.87	*4.73*
Total (n=30)	TC	222.5 ± 30.93	235.37 ± 30.48	*5.78*	223.8 ± 30.66	236.2 ± 35.3	*5.54*	222.9 ± 31.83	234.97 ± 33.88	*5.41*
	TG	98.2 ± 46.07	106.97 ± 54.34	*8.93*	100.27 ± 50.15	110.47 ± 54.91	*10.17*	84.63 ± 36.33	92.27 ± 38.96	*9.03*
	HDL-C	68.97 ± 13.7	73.97 ± 16.4	*7.25*	68.73 ± 13.85	73.33 ± 14.29	*6.69*	72.07 ± 13.53	76.77 ± 14.14	*6.52*
	LDL-C	142.93 ± 34.31	149.63 ± 34.71	*4.69*	142.37 ± 31.71	152.63 ± 38.93	*7.21*	141.47 ± 33.08	147.93 ± 34.19	*4.57*

Figure 4.5 A Lipid profiles of pre VO_{2peak} test on day 1, day 12, and day 23 of the *treadmill group*

* Significant difference of triglycerides level between day 12 and day 23 ($p < 0.01$)

** Significant difference of HDL-C level between day 12 and day 23 ($p < 0.001$)

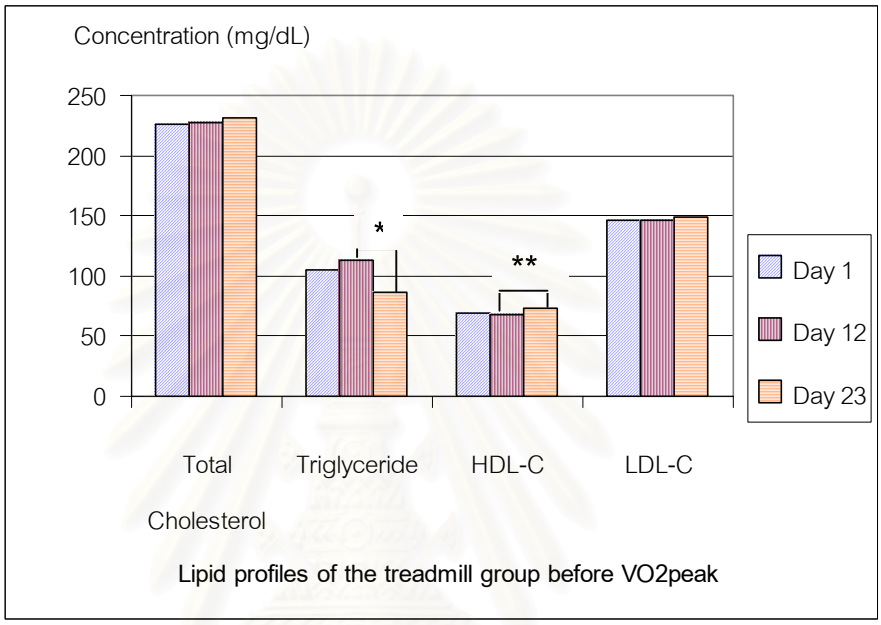


Figure 4.5 B Lipid profiles pre VO_{2peak} test of *30 subjects* at day 1, day 12, and day 23

* Significant difference of TG and HDL-C between day 12 and day 23 ($p < 0.01$).

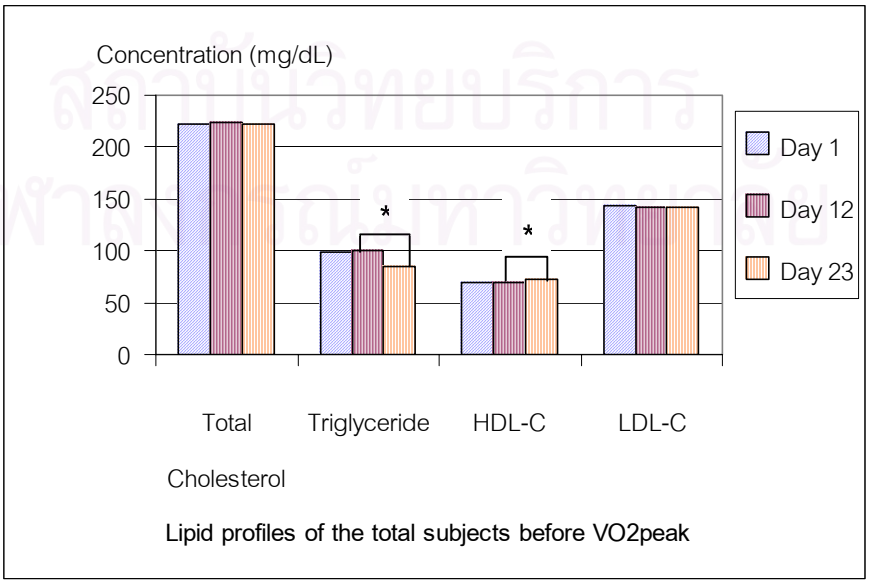


Figure 4.5 C Total cholesterol pre and post VO_{2peak} test of *30 subjects* at day 1, day 12, and day 23

* Significant difference of total cholesterol between pre and post VO_{2peak} ($p < 0.01$).

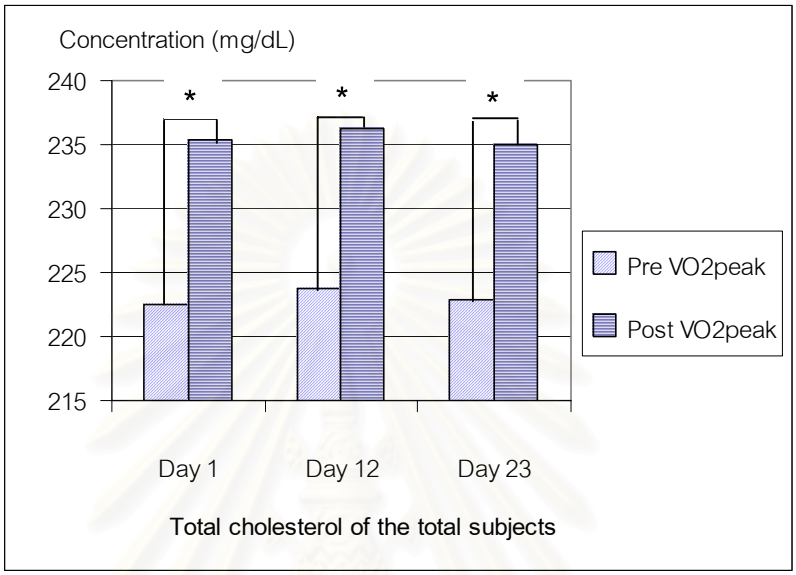


Figure 4.5 D Triglycerides pre and post VO_{2peak} test of *30 subjects* at day 1, day 12, and day 23

* Significant difference of triglycerides between pre and post VO_{2peak} ($p < 0.01$).

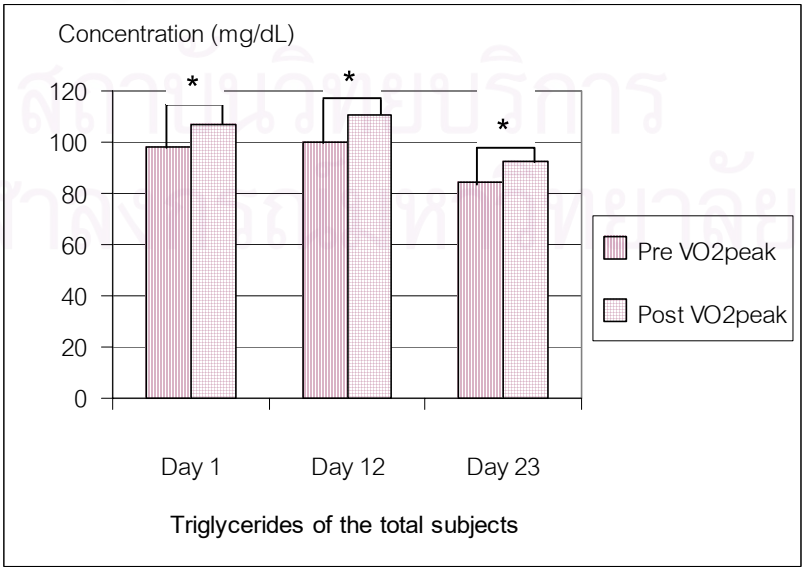


Figure 4.5 E HDL-C pre and post VO_{2peak} test of *30 subjects* at day 1, day 12, and day 23

* Significant difference of HDL-C between pre and post VO_{2peak} ($p < 0.01$).

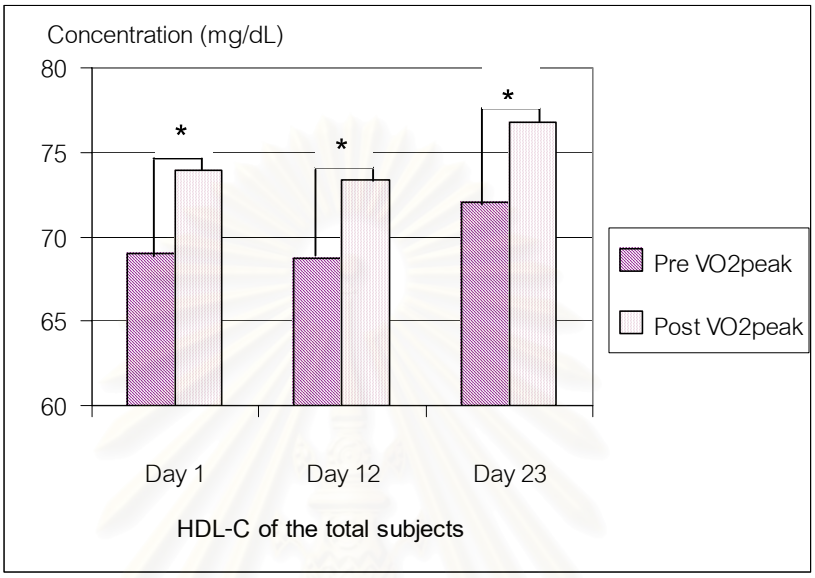
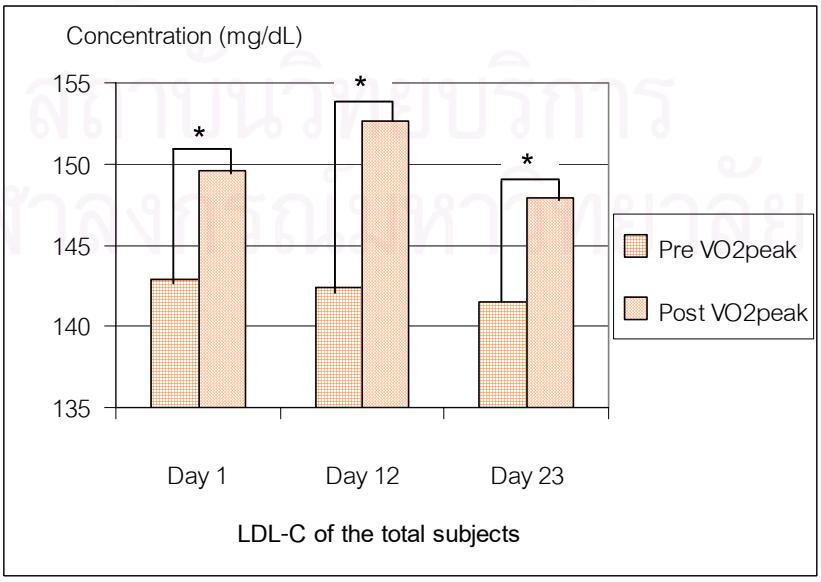


Figure 4.5 F LDL-C pre and post VO_{2peak} test of *30 subjects* at day 1, day 12, and day 23

* Significant difference of LDL-C between pre and post VO_{2peak} ($p < 0.01$).



CHAPTER V

DISCUSSION AND CONCLUSION

The metabolic product of NO (represented by nitrite/nitrate concentration) in systemic venous blood in thirty healthy postmenopausal women in the present study was compared between the control phase (n=30) and exercise phase (n=30). The exercise phase was divided into two groups as treadmill group (n=15) and bicycle group (n=15).

In this study, there was no significant change of serum nitrite/nitrate concentration due to the exercise. There were no significant different of the serum concentration of nitrite/nitrate when comparing pre and post VO_{2peak} test on day 1, day 12, and day 23. But, at day 1 post VO_{2peak} serum nitrate concentration was 26.14 μmol/L and stayed at this level throughout the study period. There was no significant different in mode of exercise, represent treadmill and bicycle ergometer. Concerning physical fitness of subjects, short term exercise training did not changed peak oxygen uptake.

The present study, there was no significant change of physical fitness after exercise training. But, after short term exercise could decrease triglycerides and increase HDL-C significantly. There were significant different of lipid profiles (all parameter) when comparing pre and post VO_{2peak} test on day 1, day 12, and day 23. In mode of exercise, after short term exercise could decrease triglycerides and increase HDL-C significantly in treadmill group only.

The coefficient of variation (CV) of lipid profiles, including TC, TG, HDL-C and LDL-C for the intra-assay 1.8%, 0.5%, 1.7%, and 1.5%, respectively. The inter-assay were 0.9%, 1.4%, 2.7%, and 1.9%, respectively.

The volunteers in the present study were aged between 46-60 years with the serum nitrite as 1.37±1.97 μmol/L and serum nitrate as 22.67±18.79 μmol/L which were similar to the observation by Toprakci et al. 2000. They found that NO release declines with age in healthy people, with the most pronounce decrease between 46 and 60 years of age as shown in Table 5.1.

Table 5.1 Nitrite and nitrate levels in different age groups (values are expressed as median, minimum, and maximum values) (Toprakci et al. 2000)

Age groups (years)	n	Nitrite (μmol/L)	Nitrate (μmol/L)
I (6-15)	13	1.6 (0.8-5.5)	47.2 (12.8-95.2)
II (16-30)	13	1.6 (0.8-5.0)	35.2 (0.8-76)
III (31-45)	15	1.9 (0.8-6.8)	22.4 (2.4-38.4)
IV (46-60)	14	1.8 (1.0-4.9)	12.4 (0.8-42)
VI (>61)	14	1.15 (0.8-3.9)	16.4 (0.8-38.4)

Other investigation also observed decreased NO production not only in pathological states but also during physiological aging (Clelland et al. 1997 and Genhard et al. 1996). The observed decreased NO levels in aging individuals were proclaimed result of free radicals in aging process. Higher plasma concentrations of nitric oxide among the athletes, suggesting a higher basal production of NO that may cause lower blood pressure were reported by Poveda et al. 1997, therefore physical activity or daily exercise that has positive health benefit in terms of normalizing blood pressure might be due to the increase NO production. In order to improved the endothelial function in postmenopasual individuals, the present study was aimed to endeavor the effect of short term exercise training on the level of nitrite/nitrate concentration.

In this study, at day 1 and day 12 pre VO_{2peak} serum nitrate concentration was 22.67 and 26.15 μmol/L, respectively. It was possibly, volunteers may be adjust activity to prepare participate exercise training program (placebo effect). Thus, we do not known and could not control, but we attempted to ask volunteers do not change activity.

Nevertheless in animal studies, Treadmill training for only 7 days in dogs enhanced flow-mediated and Ach-induced dilation in epicardial coronary arteries (Wang et al. 1993), while training for 10 days increased nitrite released from both proximal coronary arteries and microvessels in response to Ach infusion (Sessa et al. 1994). The animal study, facilitated researcher to control every modality in experimental i.e. intensity,

duration of exercise, diet. Then, in long term training, Maeda et al. 2000 found that, 8-weeks exercise training increased nitrite/nitrate concentration in serum. It possibly in this study that duration of training was not long enough to cause the rising nitrite/nitrate concentration in serum or might be the adaptive function of endothelium.

In this study, the mean differences of serum concentration of nitrate (between pre and post VO_{2peak} test) at day1 showed the larger effect of exercise than day 12 and day 23 (from 3.48 μmol/L to 0.55 μmol/L to–0.19 μmol/L, respectively). It possibly, these results were chronic effect on top of acute. All volunteers in this study were sedentary individuals and they have never been exposed to the treadmill or bicycle ergometer. This would lead to the microtrauma because of muscle strain from overexertion. Furthermore, the emotional stress presumably from fear of blood sampling may cause the rise of serum nitrate concentration.

Physical exercise increase coronary blood flow, resulting in increased shear stress on the surface of the endothelium (Schuler 1992). Endothelial cells responded to short-term increase in shear stress by producing vasodilator compounds such as prostacyclin and nitric oxide. Sustained increases in shear stress elicited an adaptive response in endothelial cells that was manifested, in part, by increased expression of the enzyme that catalyzes nitric oxide production (Vita and Keaney 2000). During physical and mental stress, increases in coronary blood flow because of sympathetically mediated increases in cardiac output also augment shear stress across the endothelium, producing coronary and systemic arterial dilation. Vasodilation during stress may also be mediated by epinephrine and norepinephrine activation of adrenoceptors on the endothelium, with enhanced synthesis and release of nitric oxide.

However, Node et al. 1997 found that, the plasma concentration of NO was responded to acute physical exercise in 16 healthy volunteers with average at 60 $\pm$ 4 years (9 men and 7 women). The cycling exercise began with a workload of 25 W, which was increased every 1 minute in stepwise increments of 25 W. For all volunteers, exercise was discontinued when leg fatigue occurred. It demonstrated the NO markers increase statistically. It was possibly, different of physical fitness when compared between race, which majority of foreigners (Japanese) was active more than Thai. In addition, Poveda et al.1997, studied in 10 healthy subjects which were tested by a

modified Bruce protocol (treadmill), they did not find any significant increase in plasma levels of nitrites or nitrates after acute exercise. However, the limitation of this study was not control diet of all subjects, merely advice subjects avoid diet which composed of nitrite/nitrate only e.g. food with preservative, sausage, ham. But, resting nitrite/nitrate concentration in serum between this study and Node et al. 1997 were similar (24.02 vs 24.6 μmol/L).

About physical fitness, short term exercise training did not increased peak oxygen uptake, which was the same as well as studies in short term aerobic exercise program for 7 day (Brown et al. 1997).

The present study, triglycerides was significantly decreased and HDL-C was significantly increased after short term exercise training. In general, exercise-trained and physically active individuals generally exhibit lower plasma concentrations of triglycerides and higher levels of HDL-C than their untrained, sedentary counterparts. Some of the potential mechanism by which exercise modifies plasma and lipoprotein profile are related to increases in lipoprotein lipase (LPL) and lecithin cholesterol acid transferase (LCAT) activity (Tall 1990) HDL contains LCAT, and the enzyme catalyzes a reaction that gathers free cholesterol and returns it to the liver. LPL decreases HDL_2 breakdown and increases the use of triglycerides (HDL_2 is a major class of HDL). In addition, exercise lowers triglycerides by increasing insulin receptor activity and reduces abdominal body fat (Despres et al. 1988). Abdominal body fat, commonly seen postmenopausally, was associated with decreased liver LPL activity, impairing the breakdown of triglycerides (Barret-Connor et al. 1997; Tall 1990). In addition, when compared the concentration of lipid profiles between treadmill and group at day 12 and 23, the results showed triglycerides was significantly decreased and HDL-C was significantly increased after short term exercise training in treadmill group only.

The result in Table 4.5 showed that, acute exercise-induced increase lipid profiles (all parameter) significantly when compared between pre and post VO_{2peak} test in each day (day 1, 12, and 23). The promptly rising of lipid profiles was possibly, the catecholamine response to exercise resulting in increase lipolysis of adipose tissue triacylglycerols and, presumably, intramuscular triacylglycerols (Horowitz and Kliein 2000). In addition, increases in adipose tissue and muscle blood flow decrease fatty acid

reesterification and facilitate the deliverly of released fatty acids to skeletal muscle. Compared with untrained persons exercising at the same absolute intensity, persons who have undergone endurance training have greater fat oxidation during exercise without increased lipolysis (Horowitz and Kliein 2000). Therefore, acute exercise induced the increase of lipolysis more than needed for fatty acid oxidation in sedentary individuals as seen in this study. Furthermore, in this study, blood sample was collected For examine plasma volume change in 5 subjects. The results showed that, there was a Change in approximately 7-8%, which gave no significance as compared to the whole pasema volume.

Conclusion

1. Short term moderate exercise training could not change the concentration of serum nitrite/nitrate in postmenopausal women both treadmill and bicycle group.
2. Acute exercise increased the concentration of serum nitrite/nitrate in postmenopausal women both treadmill and bicycle group on the first day.
3. Short term moderate exercise training could decrease in triglycerides and increased high density lipoprotein cholesterol levels in postmenopausal women in treadmill group.
4. Further study should include longer duration of exercise training, and should be diet control, which might provide the distinct results.

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APPENDICES

APPENDIX A

Table A The Physical Characteristics of Volunteers

Number	Age	Height (c.m.)	Weight (k.g.)	BMI (kg/m^2)	VO_{2peak} (mL/kg/min)
1	54	145	41	19.50	27.07
2	54	154	47	19.82	22.55
3	51	151	54	23.68	14.26
4	52	153	70	29.90	15.29
5	56	154	63	26.56	12.86
6	58	147.50	51	23.44	16.27
7	53	155	68	28.30	20.74
8	54	163	57	21.45	18.60
9	50	155	52	21.64	18.82
10	53	153	41	17.51	25.06
11	54	157	60	24.34	20.33
12	56	150	42	18.67	17.62
13	58	158	42	16.82	17.87
14	53	158	49	19.63	23.80
15	50	158	58	23.23	23.46
16	49	153	43	18.37	22.20
17	50	149	56	25.22	17.73
18	51	144	43	20.74	22.63
19	57	150	55	24.44	11.95
20	49	155	65	27.06	21.67
21	55	160	53	20.70	22.15
22	60	160	77	30.08	15.23
23	58	156	57	23.42	17.54
24	59	158.5	69	27.47	13.97

Table A The Physical Characteristics of Volunteers (continue)

Number	Age	Height (c.m.)	Weight (k.g.)	BMI (kg/m^2)	VO_{2peak} (mL/kg/min)
25	57	165	70	25.71	15.86
26	52	147.5	54	24.82	22.93
27	47	163	49	18.44	20.50
28	50	155	56	23.31	16.36
29	58	158	56	22.43	22.91
30	48	152	52	22.51	15.00

APPENDIX B

Table B I Nitrite Concentration in Serum

Number	Control phase		Exercise phase		Protocol final testing	
	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}
1	0.07	0.00	0.07	0.00	0.33	0.00
2	6.86	7.11	0.00	0.00	6.23	6.85
3	8.11	14.07	13.06	3.23	0.00	0.00
4	1.94	1.61	0.00	2.72	0.00	0.00
5	0.07	0.00	0.07	0.20	0.20	0.27
6	0.71	0.68	0.68	1.09	0.16	1.22
7	0.79	0.25	0.62	0.62	1.02	1.25
8	0.00	0.16	1.71	0.16	1.02	1.55
9	1.48	0.62	1.02	0.24	0.86	2.14
10	2.62	0.00	1.02	0.16	1.71	0.00
11	1.02	0.79	0.16	0.00	1.25	0.00
12	1.71	2.40	3.78	0.48	1.48	0.00
13	1.90	0.95	0.06	0.00	0.00	0.00
14	0.00	0.25	2.77	4.25	2.02	3.25
15	0.00	0.00	3.07	3.45	0.57	0.77
16	3.36	0.32	0.00	0.00	0.00	0.00
17	1.43	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.48	0.00	0.25
19	0.62	0.00	0.00	0.00	0.92	0.00
20	0.32	1.23	3.04	0.62	1.53	0.57
21	3.94	1.83	5.46	3.04	3.64	2.74
22	0.42	0.40	0.67	0.26	0.15	0.40
23	0.00	0.13	0.02	0.15	0.67	0.00
24	0.02	0.00	0.00	0.13	0.29	0.00

Table B I Nitrite Concentration in Serum (continue)

Number	Control phase		Exercise phase		Protocol final testing	
	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}
25	0.79	0.00	0.67	0.00	0.02	0.00
26	0.06	5.39	2.20	2.01	6.43	0.00
27	0.38	0.00	0.32	0.00	0.27	0.00
28	0.00	0.00	0.68	1.76	0.12	0.00
29	0.51	0.00	0.51	1.08	0.71	2.40
30	1.55	0.00	0.00	0.25	0.00	0.00

Table B II Nitrate Concentration in Serum

Number	Control phase		Exercise phase		Protocol final testing	
	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}
1	35.38	42.94	19.78	29.69	17.56	24.04
2	63.84	51.60	87.89	88.46	32.67	31.02
3	47.61	44.84	43.27	45.44	65.42	71.78
4	68.73	69.72	83.82	73.70	40.89	40.86
5	14.98	33.12	64.51	64.94	17.10	18.48
6	6.49	10.70	13.45	20.12	8.61	7.03
7	12.83	30.91	54.70	55.28	35.14	45.25
8	10.75	61.67	12.49	15.31	16.09	23.11
9	15.63	11.02	25.50	20.94	47.27	48.93
10	15.12	19.43	75.33	83.51	10.87	15.36
11	12.95	25.03	12.18	13.27	41.18	17.57
12	22.14	28.41	9.37	12.46	82.08	84.25
13	5.80	5.14	60.39	57.91	21.43	19.29
14	71.07	70.85	6.99	5.29	12.81	7.26
15	9.37	12.55	4.58	4.48	12.42	16.43
16	15.72	15.40	10.30	10.57	42.70	39.40
17	9.27	11.63	8.58	6.99	8.71	9.11
18	21.94	24.45	8.98	6.20	31.72	33.13
19	5.93	6.30	18.79	18.92	17.36	14.36
20	6.23	9.49	5.91	6.69	8.68	9.89
21	25.19	25.54	30.74	31.39	63.58	51.12
22	19.56	12.78	15.63	14.57	23.64	24.54
23	7.93	8.16	7.86	8.77	51.94	48.23
24	8.20	7.07	9.38	10.30	13.58	11.80

Table B II Nitrate Concentration in Serum (continue)

Number	Control phase		Exercise phase		Protocol final testing	
	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}	Pre VO_{2peak}	Post VO_{2peak}
25	9.27	12.95	6.98	6.84	8.09	7.76
26	45.04	36.90	4.81	6.88	5.27	6.43
27	19.87	18.15	29.43	28.22	21.23	37.75
28	14.39	8.07	13.95	12.49	13.22	9.83
29	28.18	35.73	27.13	26.92	34.19	27.93
30	30.66	33.62	11.75	14.50	11.70	9.70

APPENDIX C

Table C I The Level of Lipid Profiles Pre and Post VO_{2peak} Test at Baseline or Control Phase

Number	Pre VO_{2peak}				Post VO_{2peak}			
	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL
1	263	131	66	184	266	144	70	185
2	196	71	70	123	213	81	74	146
3	199	40	86	111	207	50	89	113
4	242	86	68	157	243	71	68	166
5	246	97	66	157	262	103	69	162
6	259	83	60	176	281	101	67	200
7	240	179	44	165	256	193	51	173
8	218	200	45	161	245	225	50	178
9	232	93	56	170	235	98	58	172
10	191	87	61	113	210	100	60	122
11	294	238	58	216	298	284	60	221
12	208	66	79	131	225	80	83	141
13	202	127	106	90	224	141	124	98
14	214	83	88	137	221	71	92	139
15	173	106	57	113	188	112	67	127
16	286	55	76	218	292	58	78	202
17	183	58	61	113	183	57	62	117
18	247	70	68	168	257	73	71	177
19	249	73	56	184	258	79	59	187
20	253	140	68	177	279	153	74	191
21	243	62	89	155	260	65	86	165

Table C I The Level of Lipid Profiles in Pre and Post VO_{2peak} Test at Baseline or Control Phase (continue)

Number	Pre VO_{2peak}				Post VO_{2peak}			
	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL
22	198	89	53	137	215	101	56	149
23	215	81	72	144	232	91	80	156
24	210	138	69	127	228	159	75	136
25	187	83	67	102	196	86	70	107
26	209	68	85	109	222	67	89	112
27	177	65	72	90	192	71	79	99
28	220	146	74	117	235	157	78	124
29	215	83	64	132	221	86	67	133
30	206	48	85	111	217	52	113	91

Table C II The Level of Lipid Profiles Pre and Post VO_{2peak} Test at Exercise Phase

Number	Pre VO_{2peak}				Post VO_{2peak}			
	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL
1	269	92	63	178	280	114	70	205
2	199	90	66	115	218	100	71	124
3	203	41	80	124	225	45	93	132
4	234	98	65	155	248	97	68	162
5	273	120	63	188	286	135	66	206
6	206	110	59	144	223	141	64	155
7	222	172	44	163	233	181	50	170
8	228	262	47	163	259	294	57	186
9	233	59	60	174	239	64	61	193
10	190	87	89	105	175	80	86	95
11	290	218	74	207	309	227	79	214
12	231	61	70	163	253	75	76	175
13	200	155	100	96	232	171	110	109
14	199	86	75	122	207	92	82	131
15	219	76	68	119	201	94	77	128
16	320	69	83	224	343	85	86	268
17	195	54	68	122	201	53	70	125
18	236	58	79	155	249	64	83	163
19	213	68	39	158	228	77	41	169
20	240	162	59	157	260	163	65	171
21	226	58	85	141	253	65	94	155

Table C II The Level of Lipid Profiles in Pre and Post VO_{2peak} Test at Exercise Phase (continue)

Number	Pre VO_{2peak}				Post VO_{2peak}			
	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL
22	214	121	62	145	221	132	65	151
23	206	112	60	119	223	147	63	129
24	241	94	74	153	256	103	78	162
25	202	84	66	114	209	81	69	118
26	224	68	84	120	226	77	86	121
27	195	67	78	102	200	69	79	105
28	188	113	54	110	198	123	57	114
29	202	93	61	120	216	106	66	127
30	216	60	87	115	215	59	88	116

Table C III The Level of Lipid Profiles Pre and Post VO_{2peak} Test at Protocol Final Testing

Number	Pre VO_{2peak}				Post VO_{2peak}			
	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL
1	227	92	64	145	248	102	70	159
2	198	105	76	105	207	123	80	109
3	227	48	85	124	242	50	95	132
4	216	76	63	152	236	73	66	156
5	208	135	62	139	220	143	66	153
6	267	76	74	187	275	85	80	194
7	232	157	46	171	242	166	50	177
8	236	182	50	169	253	199	55	185
9	224	68	55	165	234	79	57	167
10	178	71	77	104	197	87	83	116
11	259	107	79	178	270	114	87	186
12	225	47	69	163	249	52	84	174
13	205	121	108	97	231	138	111	111
14	208	71	79	119	208	87	85	126
15	180	85	53	110	192	93	57	116
16	334	66	86	239	357	73	93	254
17	201	43	71	128	204	39	73	129
18	223	55	71	144	221	59	72	142
19	227	72	54	166	238	76	57	170
20	279	134	75	195	295	140	78	196
21	248	70	89	159	253	67	93	161

Table C III The Level of Lipid Profiles Pre and Post VO_{2peak} Test at Protocol Final Testing (continue)

Number	Pre VO_{2peak}				Post VO_{2peak}			
	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL	TC mg/dL	TG mg/dL	HDL-C mg/dL	LDL-C mg/dL
22	213	74	66	150	219	79	68	158
23	194	78	68	117	211	96	72	126
24	235	149	77	128	238	153	79	130
25	182	65	69	101	191	69	71	106
26	230	73	88	125	246	78	94	128
27	201	46	79	108	209	53	84	113
28	219	81	69	123	239	91	77	130
29	202	39	68	123	211	48	72	123
30	209	53	92	110	213	56	94	111

APPENDIX D

The Case Report Form (CRF)

Subject initial……..

Subject number……..

Study name

Effect of short term exercise on serum nitrite/nitrate concentration in postmenopausal women

Principle Investigator name

Miss Sukanya Eksakulkla

Screening visit

Date……

Subject initial……

Subject number……..

Inclusion Criteria

	Yes	No
1. สตรีวัยหมดระดูอย่างน้อย 1 ปี	☐	☐
2. อายุ 40-60 ปี	☐	☐
3. มีสุขภาพดีสามารถออกกำลังกายตามโปรแกรมที่กำหนดเอาไว้ได้	☐	☐
4. ไม่เคยได้รับฮอร์โมนทดแทนหรือเคยได้รับก่อนเข้าร่วมการศึกษา อย่างน้อย 6 เดือน	☐	☐
5. ยินยอมเข้าร่วมศึกษาวิจัยด้วยความเต็มใจ	☐	☐

Exclusion Criteria

	Yes	No
1. เคยได้รับการตัดรังไข่ทั้ง 2 ข้าง	☐	☐
2. มีความดันเลือดสูง (ความดันเลือดขณะพักมากกว่า 160/90 มม.ปรอท) หรือได้รับยาลดความดัน	☐	☐
3. เป็นโรคเบาหวาน	☐	☐
4. ผู้ป่วยโรคหัวใจ	☐	☐
5. ระดับโคเลสเตอรอลมากกว่า 300 มิลลิกรัม/เดซิลิตรหรือ ได้รับยาลดไขมันในเลือด	☐	☐
6. ระดับไตรกลีเซอไรด์มากกว่า 400 มิลลิกรัม/เดซิลิตร	☐	☐
7. Body mass index (BMI) มากกว่า 30 กิโลกรัม/เมตร^2	☐	☐
8. ออกกำลังกายเป็นประจำอย่างน้อย 2 ครั้ง/สัปดาห์ นาน 20 นาที/ครั้ง เป็นเวลาติดต่อกัน 1 สัปดาห์ขึ้นไปก่อนเข้าร่วมการศึกษา	☐	☐
9. ไม่สามารถออกกำลังกายต่อเนื่องตามที่กำหนดไว้ได้	☐	☐

Investigator's signature……

Baseline visit

Group……

Date……..

Subject initial…….

Subject number……..

ส่วนสูง.....cm.

น้ำหนัก.....….kg.

Resting heart rate……..beat/min

BP……..mmHg

ผลการตรวจทางห้องปฏิบัติการ

Lipid profile		**หน่วย**
Total Cholesterol	……..	mg/dL
Triglyceride	……..	mg/dL
HDL-C	……..	mg/dL
LDL-C	……..	mg/dL

Nitric oxide		
Nitrite	……..	μmol/L
Nitrate	……..	μmol/L

VO_2peak	……..	mL/kg/min
50% VO_{2peak}	……..	mL/kg/min
65% VO_{2peak}	……..	mL/kg/min

Investigator's signature……

Data during exercise

Group……

Subject initial……..

Subject number……..

	วันที่ 1	วันที่ 2	วันที่ 3	วันที่ 4	วันที่ 5
น้ำหนัก (kg)					
resting HR (beat/min)					
BP (mmHg)					
intensity (% VO_{2peak})					

	วันที่ 6	วันที่ 7	วันที่ 8	วันที่ 9	วันที่ 10
น้ำหนัก (kg)					
resting HR (beat/min)					
BP (mmHg)					
intensity (% VO_{2peak})					

Investigator's signature……

Last visit

Group……

Date……..

Subject initial……

Subject number……..

ผลการตรวจทางห้องปฏิบัติการ

Lipid profile		**หน่วย**
Total Cholesterol	……..	mg/dL
Triglyceride	……..	mg/dL
HDL-C	……..	mg/dL
LDL-C	……..	mg/dL

Nitric oxide		
Nitrite	……..	μmol/L
Nitrate	……..	μmol/L

VO_2peak	……..	mL/kg/min
50% VO_{2peak}	……..	mL/kg/min
65% VO_{2peak}	……..	mL/kg/min

Investigator's signature……

Study summary

Group……

Date……..

Subject initial…….

Subject number……..

การนัดหมายครั้งนี้เป็นครั้งสุดท้ายเพราะ

☐ การเก็บข้อมูลครบถ้วนสมบูรณ์

☐ ไม่สามารถเก็บข้อมูลได้ครบถ้วนสมบูรณ์ เนื่องจาก

- ☐ อาสาสมัครมีความประสงค์จะออกจากการศึกษา
- ☐ อาสาสมัครไม่มาตามนัด และไม่สามารถติดต่อได้
- ☐ อาสาสมัครไม่สามารถมาตามนัดได้

เนื่องจาก

……

……

……

Investigator's signature……

ประวัติความเจ็บป่วยระหว่างการศึกษา

Group……

Date……..

Subject initial…….

Subject number……..

Diagnosis	อาการ	เริ่มวันที่	ระยะเวลา (วัน)	การรักษา

Investigator's signature……

APPENDIX E

เอกสารชี้แจงข้อมูล/คำแนะนำแก่ผู้เข้าร่วมโครงการ
(Patient Information Sheet)

ชื่อโครงการ ผลของการออกกำลังกายระยะสั้นต่อความเข้มข้นไนไตรท์/ไนเตรตในซีรัม ในหญิงวัยหมดระดู

Effect of short term exercise on serum nitrite/nitrate concentration in postmenopausal women

ชื่อผู้วิจัย นางสาวสุกัญญา เอกสกุลกล้า ผู้วิจัย

รองศาสตราจารย์แพทย์หญิงธาดา สืบหลินวงศ์ อาจารย์ที่ปรึกษาโครงการวิจัย

ศาสตราจารย์นายแพทย์นิมิต เตชไกรชนะ อาจารย์ที่ปรึกษาโครงการวิจัยร่วม

แพทย์หรือผู้ดูแลที่ติดต่อได้

1. รองศาสตราจารย์แพทย์หญิงธาดา สืบหลินวงศ์ ภาควิชาชีวเคมี คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทรศัพท์ (02) 256-4482 (ที่ทำงาน)
2. ศาสตราจารย์นายแพทย์นิมิต เตชไกรชนะ ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทรศัพท์ (02) 256-4163 ต่อ 218 (ที่ทำงาน)
3. นางสาวสุกัญญา เอกสกุลกล้า สาขาเวชศาสตร์การกีฬา ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทรศัพท์ (02) 256-4267

สถานที่วิจัย ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
Wellness Center ตึก อปร. ชั้น 6 โรงพยาบาลจุฬาลงกรณ์

ความเป็นมาของโครงการ

โรคหัวใจและหลอดเลือด (cardiovascular disease) เป็นปัญหาหนึ่งที่พบมากขึ้นในหญิงวัยหมดระดูโดยอาจจะเกี่ยวข้องกับความผิดปกติในการทำงานของเอนโดธีเลียม ซึ่งเป็นผลจากฮอร์โมนเอสโตรเจนที่ลดลงร่วมกับความเสื่อมจากการที่มีอายุมากขึ้น ในภาวะปกติเอนโดธีเลียมมีบทบาทสำคัญต่อการปรับความตึงและโครงสร้างของหลอดเลือด ตลอดจนสร้าง relaxing factor

คือ ไนตริกออกไซด์ ซึ่งทำหน้าที่ปกป้องหลอดเลือดไม่ให้เกิดการเปลี่ยนแปลงที่นำไปสู่ภาวะหลอดเลือดแดงแข็ง (atherosclerosis) และการเกิดลิ่มเลือดที่ทำให้เส้นเลือดอุดตัน (thrombosis) ได้ โดยมีการศึกษายืนยันว่าการออกกำลังกายเป็นประจำสามารถชะลอการเสื่อมหรือการเปลี่ยนแปลงตามอายุของเอนโดธีเลียมได้ นั่นคือลดปัจจัยเสี่ยงของการเกิดโรคหัวใจและหลอดเลือดได้ จึงเป็นที่น่าสนใจว่าการออกกำลังกายในช่วงระยะเวลาสั้น ๆ จะมีผลสามารถเพิ่มความเข้มข้นไนไตรท์/ไนเตรตในซีรัม (ไนไตรท์/ไนเตรตในซีรัมเป็นตัวบ่งชี้ถึงการสังเคราะห์ไนตริกออกไซด์ทางอ้อม) ในหญิงไทยวัยหมดระดูได้หรือไม่

วัตถุประสงค์ เพื่อศึกษาผลของการออกกำลังกายที่ความหนักปานกลางเป็นเวลา 10 วันติดต่อกันต่อการเปลี่ยนแปลงความเข้มข้นไนไตรท์/ไนเตรตในซีรัมในหญิงวัยหมดระดู

รายละเอียดที่จะปฏิบัติต่อผู้เข้าร่วมโครงการ

1. ท่านจะได้รับการสัมภาษณ์โดยผู้วิจัยเพื่อถามข้อมูลทั่วไป ประวัติความเจ็บป่วยในอดีต ความเจ็บป่วยปัจจุบัน ประวัติการใช้ฮอร์โมนทดแทน และประวัติการรับประทานยาที่ใช้เป็นประจำ
2. ท่านจะได้รับการทดสอบและประเมินร่างกายที่โรงพยาบาลจุฬาลงกรณ์รวม 3 ครั้ง ประกอบด้วยการวัดอัตราการเต้นของหัวใจ ความดันเลือด ทดสอบความสามารถในการใช้ออกซิเจนสูงสุด และเก็บตัวอย่างเลือดเพื่อนำไปวิเคราะห์ความเข้มข้นไนไตรท์/ไนเตรตและไขมันในเลือด โดยที่ท่านจะได้รับการเจาะเลือดก่อนและหลังการทดสอบรวมทั้งหมด 6 ครั้ง (ครั้งละ 8 มิลลิลิตร ประมาณ 1 ช้อนโต๊ะ) ปริมาณเลือดนี้ไม่ทำให้ท่านเกิดภาวะโลหิตจางแต่อย่างไร
3. ท่านจะได้เข้าร่วมโปรแกรมออกกำลังกายรวม 10 วัน ขั้นตอน คือ ท่านจะได้รับการทดสอบและประเมินร่างกายครั้งที่ 1 และหยุดพัก 10 วัน และต่อมาได้รับการทดสอบและประเมินร่างกายครั้งที่ 2 และเข้าโปรแกรมการออกกำลังกายโดยการปั่นจักรยานหรือเดินบนลู่วิ่งที่ความหนักระดับปานกลาง 30 นาทีต่อวัน เป็นจำนวน 10 วันติดต่อกันและหลังสิ้นสุดโปรแกรมในวันถัดไปท่านได้รับการทดสอบและประเมินร่างกายครั้งที่ 3 เป็นอันสิ้นสุดโครงการ

ประโยชน์และผลข้างเคียงที่จะเกิดแก่ผู้เข้าร่วมโครงการ

1. ท่านจะได้รับการทดสอบร่างกายและตรวจทางห้องปฏิบัติการเพื่อประเมินสมรรถภาพทางกาย
2. ท่านจะได้รับการตรวจและเฝ้าระวังอย่างดีในระหว่างที่ทำการทดสอบด้วยการออกกำลังกาย
3. ท่านอาจจะได้รับความเจ็บปวดจากการเจาะเลือดบ้างเล็กน้อย แต่อย่างไรก็ตามพยาบาลที่เจาะเลือดจะดูแลและให้คำแนะนำท่านเป็นอย่างดี

4. ท่านอาจจะมีอาการปวดเมื่อยบ้างหลังออกกำลังกาย โดยผู้วิจัยจะแนะนำหรืออธิบายการอบอุ่นร่างกายให้กระจ่างชัดและอยู่ภายใต้การดูแลจากผู้ทำการวิจัยอย่างใกล้ชิด

การเก็บข้อมูลเป็นความลับ

ผู้วิจัยขอยืนยันว่า ข้อมูลเกี่ยวกับตัวท่านจะถูกเก็บไว้เป็นความลับ และจะใช้สำหรับงานวิจัยนี้เท่านั้น และชื่อของท่านจะไม่ปรากฏในแบบฟอร์มการเก็บข้อมูลและในฐานข้อมูลทั่วไป ผู้วิจัยจะสร้างใช้ฐานข้อมูลลับที่มีชื่อของท่านไว้ต่างหาก โดยมีผู้วิจัยเพียงท่านเดียวเท่านั้นที่ทราบรายละเอียดของข้อมูลนี้

ผู้วิจัยขอกราบขอบพระคุณท่านที่ให้ความร่วมมือมาเข้าโครงการวิจัย และขอให้ท่านตระหนักว่า ไม่ว่าท่านจะเข้าร่วมโครงการนี้หรือไม่ก็ตามจะไม่มีผลต่อการรักษาพยาบาลที่จะได้รับจากโรงพยาบาลนี้และเมื่อท่านเข้าร่วมโครงการนี้แล้วท่านสามารถออกจากโครงการได้ทุกเวลาเมื่อท่านต้องการโดยแจ้งให้ผู้วิจัยทราบก่อน

ใบยินยอมเข้าร่วมการวิจัย (Consent form)

การวิจัยเรื่อง ผลของการออกกำลังกายระยะสั้นต่อความเข้มข้นไนไตรท์/ไนเตรตในซีรัม ในหญิงวัยหมดระดู

วันให้คำยินยอม วันที่ ..... เดือน.....พ.ศ.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่เกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมในโครงการวิจัยนี้เมื่อใดก็ได้ และเข้าร่วมโครงการวิจัยนี้ โดยสมัครใจ และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็น ด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม
(.....)

ลงนาม.....พยาน
(.....)

ลงนาม.....ผู้ทำวิจัย
(.....)

BIOGRAPHY

Miss Sukanya Eksakulkla was born on March 17, 1978 in Bangkok, Thailand. She graduated the Bachelor degree in Physical Therapy from the Faculty of Allied Health Scince, Chulalongkorn University in 2000.