ผลของการออกกำลังกายที่ระดับความหนักปานกลางเทียบกับชี่กงต่อไลโปโปรตีนฟอสโฟไลเปส เอทู และการทำหน้าที่ของเยื่อบุผนังหลอดเลือดแดงในกลุ่มอาการเมตาบอลิก

นางบวรลักษณ์ ทองทวี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาวิทยาศาสตร์การแพทย์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

THE COMPARATIVE EFFECTS OF MODERATE INTENSITY EXERCISE AND QIGONG ON LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2 AND ENDOTHELIAL FUNCTION IN METABOLIC SYNDROME

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Medical Science
Faculty of Medicine
Chulalongkorn University
Academic Year 2015

Thesis Title	THE COMPARATIVE EFFECTS OF MODERATE INTENSITY EXERCISE AND QIGONG ON LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2 AND ENDOTHELIAL FUNCTION IN METABOLIC SYNDROME
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บวรลักษณ์ ทองทวี : ผลของการออกกำลังกายที่ระดับความหนักปานกลางเทียบกับชี่กงต่อไลโปโปรตีนฟอสโฟไลเปส เอทูและการทำหน้าที่ของเยื่อบุผนังหลอดเลือดแดงในกลุ่มอาการเมตาบอลิก (THE COMPARATIVE EFFECTS OF MODERATE INTENSITY EXERCISE AND QIGONG ON LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2 AND ENDOTHELIAL FUNCTION IN METABOLIC SYNDROME) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. นพ. สมพล สงวนรังศิริกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. นพ. สมเกียรติ แสงวัฒนาโรจน์, 93 หน้า.

จุดมุ่งหมายของการศึกษา เพื่อเปรียบเทียบประสิทธิผลของการออกกำลังกายที่ระดับความหนักปานกลางเปรียบเทียบ การฝึกชี่กง ต่อระดับไลโปโปรตีนฟอสโฟไลเปสเอทู และการทำงานของเยื่อบุผนังหลอดเลือดแดง ซึ่งวัดจากการไหลของเลือดบริเวณแขน ในคนที่มีกลุ่มอาการเมตาบอลิก การศึกษานี้ใช้การวิจัยเชิงทดลองแบบสุ่มในกลุ่มตัวอย่างเพศหญิงที่มีกลุ่มอาการเมตาบอลิก อายุระหว่าง 40 ถึง 65 ปีที่พักอาศัยในเขตกรุงเทพมหานครและปริมณฑล จำนวน 83 ราย กลุ่มตัวอย่างได้รับการสุ่มเป็น 3 กลุ่ม โดย 1) กลุ่มออกกำลังกายที่ระดับความหนักปานกลาง จำนวน 28 ราย 2) กลุ่มฝึกชี่กง จำนวน 28 ราย และ 3) กลุ่มควบคุมที่ใช้ชีวิตวิถีเดิมร่วมกับการให้สุขศึกษา จำนวน 27 ราย กลุ่มออกกำลังกายทั้ง 2 กลุ่ม ได้รับโปรแกรมการฝึก เป็นเวลา 60 นาทีต่อวัน 4 วันต่อสัปดาห์ นาน 12 สัปดาห์ การเก็บรวบรวมข้อมูล 4 ระยะ ได้แก่ ก่อนดำเนินโปรแกรม สัปดาห์ที่ 4 สัปดาห์ที่ 8 และสัปดาห์ที่ 12 หลังดำเนินโปรแกรมตามลำดับ โดยการวัดระดับไลโปโปรตีนฟอสโฟไลเปสเอทู และการไหลของเลือดบริเวณแขน วิเคราะห์ข้อมูลโดยใช้สถิติเชิงพรรณนาการทดสอบความแตกต่างระหว่างกลุ่มด้วยการทดสอบความแปรปรวนแบบวัดซ้ำ ผลการศึกษาพบว่า ระดับไลโปโปรตีนฟอสโฟไลเปสเอทูลดลงอย่างมีนัยสำคัญในการฝึกทั้งสองรูปแบบ เมื่อเทียบกับกลุ่มควบคุมที่ 12 สัปดาห์ โดยระดับไลโปโปรตีนฟอสโฟไลเปสเอทู เท่ากับ 157.53±26.67, 167.25±20.37 และ 191.39±50.23 ไมโครกรัมต่อลิตร ในกลุ่มออกกำลังกายที่ระดับความหนักปานกลาง กลุ่มฝึกชี่กง และกลุ่มควบคุมตามลำดับ นอกจากนี้พบการไหลของเลือดที่แขนเพิ่มขึ้นอย่างมีนัยสำคัญ ในสัปดาห์ที่ 8 และสัปดาห์ที่ 12 พบว่าการไหลของเลือดบริเวณแขนเพิ่มขึ้นในการฝึกทั้งสองรูปแบบ เมื่อเปรียบเทียบกับกลุ่มควบคุม โดยพบว่าการไหลของเลือดบริเวณแขนในสัปดาห์ที่ 8 เป็น 786.57±155.58, 951.16±144.19 และ 630.97±193.24, มิลลิลิตรต่อ 100 มิลลิลิตรของเนื้อเยื่อ ในกลุ่มออกกำลังกายที่ระดับความหนักปานกลาง กลุ่มฝึกชี่กง และกลุ่มควบคุมตามลำดับ ส่วนในสัปดาห์ที่ 12 พบการไหลของเลือดบริเวณแขนเป็น 846.47±164.14, 1092.07±172.79 และ 638.61±203.81 มิลลิลิตรต่อ 100 มิลลิลิตรของเนื้อเยื่อ ในกลุ่มออกกำลังกายที่ระดับความหนักปานกลาง กลุ่มฝึกชี่กง และกลุ่มควบคุมตามลำดับ จากผลการศึกษาจะเห็นได้ว่าภายหลังการฝึกทั้งสองรูปแบบ นาน 12 สัปดาห์สามารถลดระดับไลโปโปรตีนฟอสโฟไลเปสเอทู และเพิ่มการทำงานของเยื่อบุผนังหลอดเลือดแดง ดังนั้นชี่กงจึงเป็นกิจกรรมทางกายอีกรูปแบบหนึ่งที่เหมาะสมสำหรับผู้ที่มีกลุ่มอาการเมตาบอลิก เนื่องจากทำได้ง่ายและมีประโยชน์เทียบเท่ากับรูปแบบการออกกำลังกายที่แนะนำโดยทั่วไป

สาขาวิชา วิทยาศาสตร์การแพทย์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5275364430 : MAJOR MEDICAL SCIENCE
KEYWORDS: METABOLIC SYNDROME / QIGONG / MODERATE INTENSITY EXERCISE / LIPOPROTEIN ASSOCIATED PHOSPHOLIPASE A2 / ENDOTHELIAL FUNCTION

BORWARNLUCK THONGTHAWEE: THE COMPARATIVE EFFECTS OF MODERATE INTENSITY EXERCISE AND QIGONG ON LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2 AND ENDOTHELIAL FUNCTION IN METABOLIC SYNDROME. ADVISOR: ASSOC. PROF. SOMPOL SAGUANRUNGSIRIKUL, M.D., CO-ADVISOR: ASST. PROF. SOMKIAT SANGWATANAROJ, M.D., 93 pp.

This randomized controlled trial examined the effects of moderate intensity exercise and Qigong on level of lipoprotein associated phospholipase A_2 (Lp-PLA_2) and endothelial function which measured by forearm blood flow in people with metabolic syndrome. Eighty three female participants with metabolic syndrome, age between 40-65 years lived in Bangkok and perimeter were randomly assigned to three groups including 1) moderate intensity exercise (MIE) group (n=28), 2) Qigong group (n=28), and 3) control group with usual activity and health education (n=27). The training group performed training 60 minutes per day, 4 days per week last for 12 week. Data were collected before, 4 week, 8 week, and 12 week after program initiation by measuring the level of Lp-PLA_2 and forearm blood flow. Repeated measured ANOVA were used to test the program efficacy. The results revealed that the two exercise groups had statistically significant reduced the level of Lp-PLA_2 compared with control group at 12^{th} week (157.53±26.67, 167.25±20.37, and 191.39±50.23 μg/L in MIE, Qigong, and control group respectively). Moreover, the data showed the statistically significant increased forearm blood flow at 8^{th} week compared to the control group as 786.57±155.58, 951.16±144.19, and 630.97±193.24 ml/100 ml tissue in MIE, Qigong, and control group respectively. Also in the 12^{th} week, there was significantly increased the forearm blood flow as 846.47±164.14, 1092.07±172.79, and 638.61±203.81 ml/100 ml tissue in MIE, Qigong, and control group respectively. The findings indicated that after performing the two training program for 12 week, it can reduce the level of Lp-PLA_2 and increased endothelial function. Therefore, Qigong is a beneficial program for people with metabolic syndrome since it easy to perform and also benefit to general physical activity that advised for people with metabolic syndrome.

Field of Study: Medical Science Student's Signature .....
Academic Year: 2015 Advisor's Signature .....
Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor Assoc. Prof. Sompol Sanguanrungsirikul for the continuous support of my Ph.D. study and research, for his excellent guidance, patience, and motivation. I would like to thank my co-advisor Asst. Prof. Somkiat Sangwatanaroj for his excellent guidance about knowledge and skill of Qigong.

I also would like to thank my thesis committee Prof. Vilai Chentanez, Prof. Narisa Futrakul, and Asst. Prof. Adisai Buakhamsri for scarify their time, insightful comments and suggestion.

My sincere thanks also to Assoc. Prof. Wilai Anomasiri , Asst. Prof. Thananya Thongtan , and department of Biochemmistry for their help on the instrument and laboratory skill.

My special thanks go to co-workers in sports medicine program, department of physiology for their helps and support.

I would like to thank my volunteers for their participation in this study and express heartfelt to the Research Grant, 90th anniversary of the Chulalongkorn University fund (Ratchadaphiseksomphot endowment fund) and Thammasat University for the financial support.

Finally, my deep appreciation is extended to my parents, my husband, my son for their loves and supporting throughout my life.

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LIST OF ABBREVIATION

ABI	Ankle Brachial Index
ACSM	American College of Sports Mediciine
AUC	Area under the curve
CAVI	Cardio Ankle Vascular Index
CRP	C-reactive protein
FBF	Forearm blood flow
FRAP	Ferric reducing antioxidant power
Lp-PLA_2	Lipoprotein associated phospholipase A_2
LysoPC	Lisophosphatidylcholine
MDA	Malondialdehyde
NCEP ATP III	The National Cholesterol Education Program's Adult Treatment Panel III
NEFA	Non esterified fatty acid
NO	Nitric oxide
OxNEFFA	Oxidized nonesterified free fatty acid
PAI-1	Plasminogen activator inhibitor-1
TCM	Traditional Chinese medicine
VO_{2peak}	Peak oxygen consumption

CHAPTER I
INTRODUCTION

Background and Rationale

A group of interrelated common clinical disorders, including obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia is also known as metabolic syndrome (Mets) (Eckel, Grundy, & Zimmet, 2005) The prevalence of Mets is approximately 20-25% of the world's adult population (36% in the United States, 17.8-34% in Europe, and 12.8-41.1% in Asia) (Alberti, Zimmet, & Shaw, 2006). Metabolic syndrome is associated with increases risk for vascular morbidity as well as mortality as shown in several studies. Hyperlipidemia is a known risk factor for atherosclerosis amongst the various risk factors that effects the pathogenesis of atherosclerosis due to Mets (Ford, 2005). A crucial step in the development of a local inflammatory response, endothelial dysfunction, as well as atherosclerosis in Mets is due to the accumulation of low-density lipoprotein (LDL) in sub-endothelial space and its subsequent oxidative modification (Itabe, Obama, & Kato, 2011).

Endothelial cell can be described as a thin layer of simple squamous cells that lines the interior surface of blood vessels controlling vascular homeostasis. The well endothelial cell is optimally placed, therefore able to respond to both physical as well as chemical signals by production of factors which regulate cellular adhesion, vascular tone, thromboresistance, vessel wall inflammation, and smooth muscle cell proliferation. The importance of endothelial cell is the vascular tone. This is achieved by production and released several vasoactive substances. This causes the relaxation and constriction of vessel such as nitric oxide. Nitric oxide plays an important role in maintaining the vascular wall in a gentle state by inhibition of inflammation, thrombosis, and cellular proliferation in normal vascular physiology (Onat, Brillon,

Colombo, & Schmidt, 2011). However, prolonged repeated exposure to risk factors can in the end expend the protective effect of endothelial cell and causes endothelial dysfunction, atherosclerosis and vascular disease. The important factors of endothelial cell dysfunction is the abnormality of metabolism such as dyslipidemia, obesity, and hyperglycemia (Cozma et al., 2009; Onat et al., 2011).

Oxidative stress is the crucial mechanism of endothelial dysfunction in Mets. Due to the accumulation of LDL in sub-endothelial space, LDL can be induced to oxidize LDL (oxLDL). oxLDL then contributes to inducing oxidative stress and cause endothelial dysfunction (Ishigaki, Oka, & Katagiri, 2009; Zambon, Pauletto, & Crepaldi, 2005). Oxidative modification of LDL is associated with the formation of a number of reactive substances such as lipid peroxides, aldehydes, lysophospholipids, and oxysterols during the production of oxLDL. Moreover, lipid oxidation induced inflammatory process to endothelial dysfunction and atherosclerotic (Mertens & Holvoet, 2001).

Lipoprotein associated phospholipase A_2 (Lp-PLA_2) is the enzyme that hydrolyzes oxidative polyunsaturated fatty acids to producing lysophosphatidylcholine (LysoPC) and oxidized nonesterified fatty acids (OxNEFFA). OxNEFFA were also potent monocyte activity and LysoPC up-regulates inflammatory mediators which includes cytokines, adhesion molecules and the chemotactic mediator MCP-1. Macrophage is the most significant active source for Lp-PLA_2 in the vascular wall even though Lp-PLA_2 accumulates in the vascular wall along with the retention of LDL particle. Lp-PLA_2 may become a mediator of secondary LDL modifications and oxidative stress in line with oxidative response to inflammation theory of atherosclerosis. Beyond an indicator function, Lp-PLA_2 may serve as an active link between oxidative stress and inflammation with important implications for atherosclerosis cardiovascular disease (Colley, Wolfert, & Cobble, 2011). Recently

data supports that Lp-PLA_2 is a cardiovascular risk biomarker and the increasing of Lp-PLA_2 level in plasma nearly double the risk for primary and secondary cardiovascular events (Ballantyne et al., 2004; Corson, Jones, & Davidson, 2008; Packard et al., 2000).

Reduced aerobic fitness as well as endothelial function in individuals with the metabolic syndrome and had been proposed to be independent and strong predictors of mortality relative to other established risk factors (Ghisi, Durieux, Pinho, & Benetti, 2010). Nevertheless, previous studies shown that, moderate intensity exercise which conventional type such as walking, bicycling benefit on reduced risk factor of atherogenesis such as lipid profiled (LDL-C, HDL-C, Triglyceride), inflammatory cytokine (TNF-α, C-reactive protein), anti-inflammatory cytokine (adiponectin) and reactive oxygen specie (ROS) (DeSouza et al., 2000; Fuchsjager-Mayrl et al., 2002; Higashi & Yoshizumi, 2004). The demonstrated of moderate intensity exercise (30 minutes every day significant improved Mets risk and better than vigorous intensity (Johnson et al., 2007). Moreover, moderate intensity exercise influenced which percentage of body weight loss more than height intensity exercise (Church, 2011).Similarly with the study effects of moderate intensity exercise in diabetes patients found that decreased blood sugar and post-prandial glucose after training with moderate intensity exercise (van Dijk et al., 2013). In addition, aerobic exercise with moderate intensity was benefits on Mets risk and suggest to implicated for training in Mets group (Kaur, 2014).

Furthermore in eastern countries, Qigong is one modality of traditional Chinese medicine (TCM) and were selected for alternative physical activity. It puts a greater emphasis on; 1) internal process, such as meditation, visualization, and breathing 2) external process on outward movement (Jahnke, Larkey, Rogers, Etnier, & Lin, 2010). For TCM describes the effects of Qi through to the meridian during Qigong practices were clamed as the major mechanism for improved abnormality

and all risk factor. But for clinical application, the previous studies the use of Qigong were demonstrated the benefits of Qigong practice in many population group such as hypertension (Lee, Pittler, Guo, & Ernst, 2007), cardiovascular disease (Hartley et al., 2015), diabetes (Hartley et al., 2015), cancer (Oh, Butow, Mullan, & Clarke, 2008), pain (Vincent, Hill, Kruk, Cha, & Bauer, 2010), anxiety & depression (Wang et al., 2013). In conclusion, Qigong is a light intensity and mental training, thereafter decreasing the risk factor of Mets (Y. F. Chao, Chen, Lan, & Lai, 2002).

For metabolic patients, most of their have obese problem and limit for conventional style exercise. In the other hand, Qigong was had simply, less limitation for metabolic patient. Although, the effects of moderate intensity exercise (physical adaptation) and Qigong, low intensity training (mental and physical adaption) on Lp-PLA_2level and endothelial function in metabolic syndrome is unknown. In this research, were designed to determine and compare the effects of moderate intensity exercise and eastern style Qigong on the Lp-PLA_2 and endothelial function in metabolic syndrome.

Research questions

1. Are there any changes on Lp-PLA_2 and endothelial function in metabolic syndrome after 12 week of Qigong and moderate intensity exercise?
2. Is there a difference on Lp-PLA_2 and endothelial function in metabolic syndrome between Qigong and moderate intensity exercise after 12 week of training?

Objectives

1. To determine effects of Qigong and moderate intensity exercise in metabolic syndrome on endothelial function and Lp-PLA_2 level after 12 week of training.

2. To compare effects of Qigong and moderate intensity exercise in metabolic syndrome on endothelial function and Lp-PLA_2 level after 12 week of training.

Hypothesis

After 12 weeks of the training program of metabolic syndrome volunteers, there was a decrease serum Lp-PLA_2 level and increase of endothelial function when compared with the controlled group.

Conceptual Framework

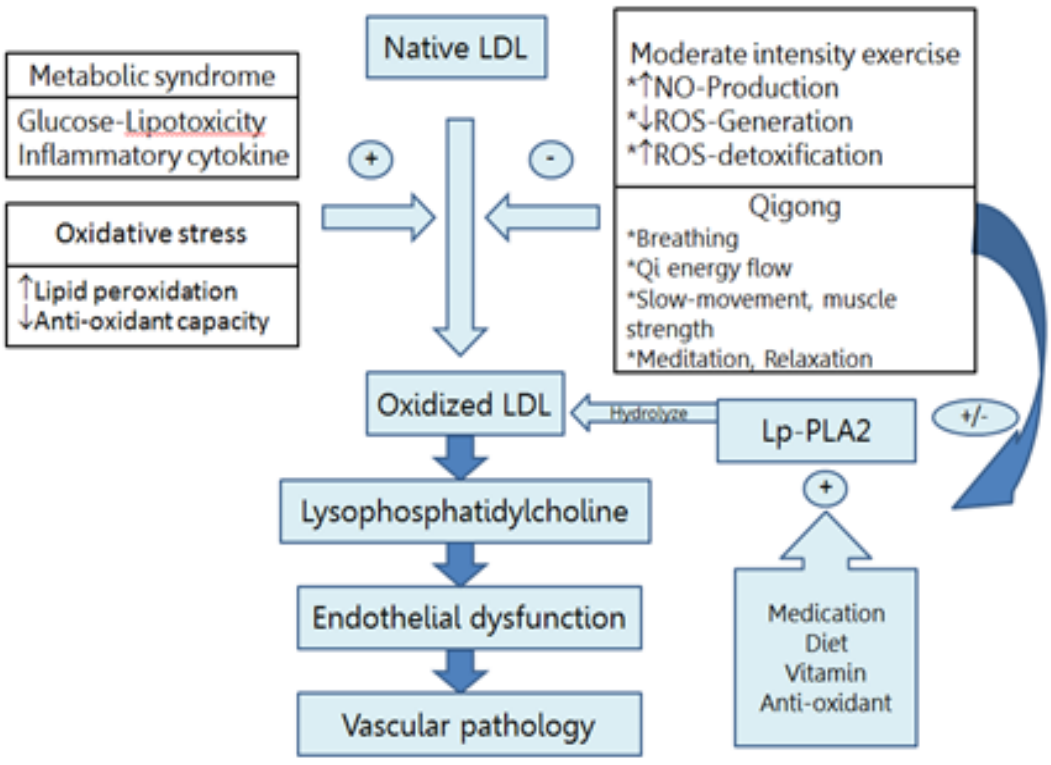


Figure 1 Conceptual Framework

Scope of research

This is an experimental research of Thai metabolic syndrome who engaged in 12 week (1 hour a day, 4 day/week, 12 week) of Qigong and moderate intensity exercise.

The study approval was obtained from the University Ethics Committee, Institutional Review Board, Faculty of Medicine, Chulalongkorn University (COA No.321/2013, IRB.471/55) written informed consent was obtained from each participant prior to participation. On attendance, participants were given the details of the research procedure and risk involved, and remained of their right to withdraw at any stage.

Assumptions

1. All volunteers participated as subjects in this study are voluntary.
2. The study population was metabolic syndrome, age between 40 and 65 years and defined according to The National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATPIII) criteria for metabolic syndrome and all of the participants living in Bangkok and perimeter.
3. The equipment was calibrated for standard accuracy and reliability.

Limitations

1. The metabolic syndrome people participated as subjects who were recruited following the inclusion criteria.
2. The result of study may not be refer to the general population in all age or the other type of exercise.

Key Words

Metabolic syndrome, Qigong, Moderate intensity exercise, Lipoprotein associated phospholipase A_2, Endothelial function

Operational definition

1. Metabolic syndrome defined according to NCEP ATPIII criteria for metabolic syndrome (≥3 criteria) , the criteria consist of

   1.1 Triglycerides ≥150 mg/dL

1.2 HDL cholesterol ≤ 40 mg/dL in men, < 50 mg/dL in women

1.3 Fasting plasma glucose ≥ 100 mg/dL

1.4 Waist circumference > 102 cm (40 in) in men, > 88 cm (35 in) in women

1.5 Blood pressure ≥ 130/85 mmHg

2. Endothelial function is the mechanism of endothelial cell to control vasodilation of the arteries, express by using the area under the curve (AUC) of the reactive hyperemia after venous occlusion plethysmography forearm blood flow technique.
3. Lipoprotein associated phospholipase A_2 is the enzyme that produced by cells of the monocyte/macrophage series under the control of inflammatory mediators and lead to endothelial dysfunction and vascular pathology.
4. Qigong is the Tradition Chinese Medicine that effect on mental and physical training, Gung-Im-Ju-Jai-Gong style by Qigong master Yang were performed to practice for 12 week (1 hour a day, 4 days/week). It consists of

   4.1 500 repetition arm swing (forward-backward)

   4.2 18 postures of body movements

   4.3 3 sets of finger movements at standing position
5. Moderate intensity exercise is recommend intensity as the best benefit in Mets, the brisk walking exercise on treadmill at 70-75% of peak oxygen consumption (VO_{2peak}) for 12 week (1 hour a day, 4 day/week) were performed to practice. It consists of warming up 10 minutes, walking on treadmill 40 minute with intensity of 70-75% of VO_{2peak} and cooling down 10 minute. The heart rate, blood pressure, and rating of perceived exertion (RPE) were monitored during training period.

Expected benefits and applications

1. More knowledge will be gained regarding the effect of Qigong and moderate exercise intensity on endothelial function and Lipoprotein associated-Phospholipase A_2 in metabolic syndrome.
2. To realize the effectiveness of exercise in metabolic syndrome.
3. Providing recommendation for type of exercise that reduce evidence of endothelial dysfunction on metabolic syndrome.
4. Providing recommendation Qigong for promotion in generally physical activity strategies.
5. Providing the preliminary data for the further research.

CHAPTER II
BACKGROUND AND LITERATURE REVIEW

Metabolic syndrome

Obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia together are a group of interrelated common clinical disorders which can be called metabolic syndrome (Mets) (Eckel et al., 2005). The group itself comprises of related component that expansion the hazard from claiming stroke and atherosclerotic cardiovascular disease (Moller & Kaufman, 2005). As statistic show, 20-25% of the world's adult population is suffering from metabolic syndrome. The prevalence of Mets differs due to geographic location, nationality, population characteristics as well as Mets criteria with 36% being in the United States, 17.8-34% in Europe, and 12.8-41.1% in Asia (Zimmet, Magliano, Matsuzawa, Alberti, & Shaw, 2005). Focusing specifically at the Thai population, Thai adult age ≥ 20 years is 23.2% (26.8% in women and 19.5% in men). It has been found to be higher in urban areas in comparison with rural areas (23.1% in urban and 17.9% in rural) as per results of the fourth national health examination survey (Pongchaiyakul, Nguyen, Wanothayaroj, Karusan, & Klungboonkrong, 2007).

Components of Metabolic Syndrome

The National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) (Grundy, Brewer, Cleeman, Smith, & Lenfant, 2004) indicated the 6 components of the metabolic syndrome which relates to cardiovascular disease as follow: 1) Abdominal obesity, 2) Atherogenic dyslipidemia, 3) Elevated blood pressure, 4) Insulin resistance, 5) A proinflammatory state, and 6) A prothrombotic state. And identified to 5 criteria (see Table 2.1)

Table 1 NCEP ATP III Clinical Identification of the Metabolic Syndrome (≥3 of 5 criteria) (Grundy, Brewer, et al., 2004)

Risk Factor	Defining Level
Dyslipidemia	
Triglycerides	≥ 150 mg/dL
HDL cholesterol	Men ≤ 40 mg/dL
	Women ≤ 50 mg/dL
Insulin resistance	
Fasting glucose	≥ 100 mg/dL
Abdominal obesity	
Waist circumference	Men >102 cm (40 in)
	Women > 88 cm (35 in)
Blood pressure	≥ 130/ 85 mmHg

Pathogenesis of Metabolic Syndrome (Grundy, Brewer, et al., 2004)

The possible etiological categories: obesity, disorders of adipose tissue, insulin resistance, and a constellation of independent factors act upon vascular pathology according to the component of metabolic syndrome.

Obesity and abnormal body fat distribution

Abdominal obesity largely correlates with metabolic risk factors. Excess adipose tissue releases many products that evidently exacerbate these risk factors. This comprises of nonesterified fatty acids (NEFA), cytokines, plasminogen activator inhibitor-1 (PAI-1), as well as adiponectin. A high plasma NEFA level overloading the muscle and liver with lipid may result in insulin resistance. A high C-reactive protein (CRP) levels along with obesity may indicate cytokine excess and a pro-inflammatory state. An increase in PAI-1 contributes to a prothrombolic state and on the contrary,

low adiponectin levels along with obesity relates to worsening of metabolic risk factor (Figure 2)

Insulin resistance

When the muscle is overloaded with lipid from high plasma NEFA levels, some excess NEFA in all likelihood is diverted to the liver, promoting fatty liver as well as atherogenic dyslipidemia in the state of insulin resistance. In addition, hyperinsulinemia is likely to enhance output of very low-density lipoprotein triglycerides. Insulin resistance in muscle affects glucose intolerance. This therefore can worsen the condition by increased hepatic gluconeogenesis and raised blood pressure due to a variety of mechanisms.

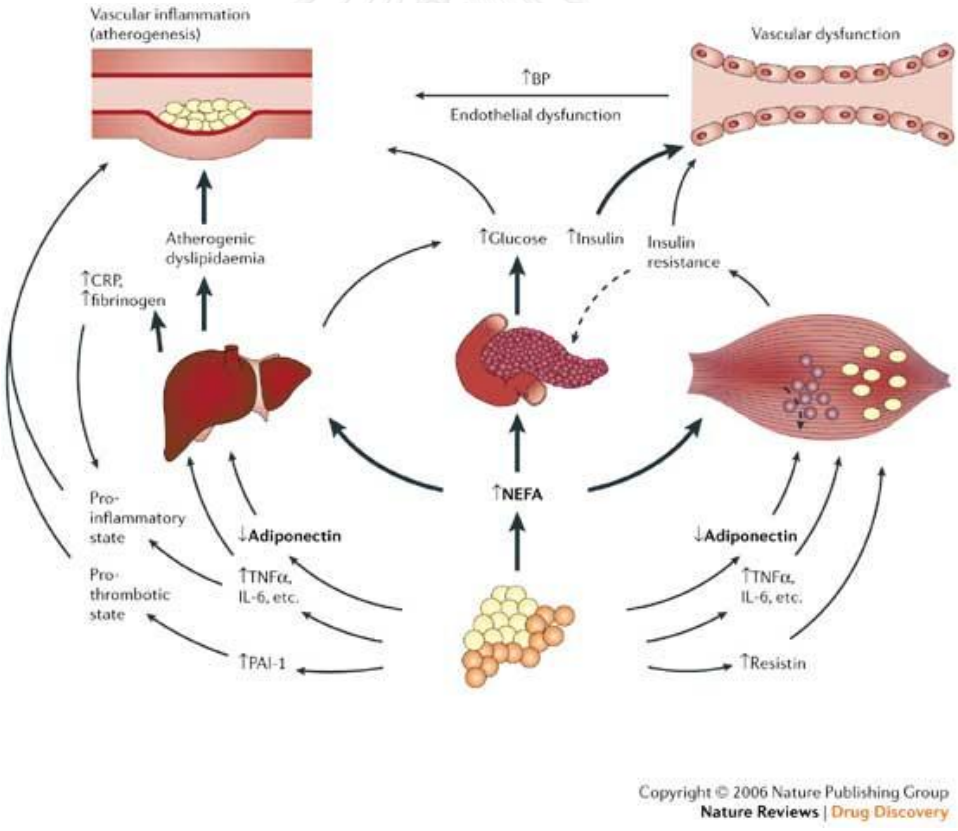


Figure 2 Pathogenesis of Metabolic syndrome (Grundy, 2006)

Endothelial function

Endothelial cell is a known mono-cellular structure which lines the entire vascular structure of the vascular tree. Hence, it plays an important role in a multitude of fundamental physiological pathways as follows: hemostasis, transportation of metabolites between the blood and the tissues, angiogenesis, repair of vascular lesions, as well as control muscle tone (Cozma et al., 2009). Furthermore, endothelial dysfunction can cause arterial stiffness and remodeling which as a result might be an important factor that causes the onset and lead to progression of cardiovascular disease. Vascular homeostasis is regulated by normal endothelium consisting of six major functions: 1) modulation of vascular permeability; 2) modulation of vasomotor tone; 3) modulation of coagulation homeostasis; 4) regulation of inflammation and immunity; 5) regulation of inflammation and immunity; regulation of cell growth; and 6) oxidation of LDL-cholesterol. Endothelial dysfunction is caused by the abnormalities of the metabolism in metabolic syndrome. This comprise of hyperglycemia, insulin resistance, and excessive fatty acid. An increase in the possibilities of vascular endothelial damage can result in the increase of cardiovascular risk (Deanfield, Halcox, & Rabelink, 2007).

Endothelial Dysfunction

Endothelial dysfunction defined as the decrease of vasodilator particularly nitric oxide (NO), and the enhancement of vasoconstrictor. Beside with endothelial structural and functional consequences, arterial remodeling and vascular stiffness represent the connection between cardiovascular risk and the beginning of atherosclerosis (Cozma et al., 2009). The cause of declined NO level can be due to: 1) the reduced endothelial nitric oxide synthase (eNOS) production, 2) the inhibition of the co-factors for eNOS synthesis, and 3) the degradation or inactivation of NO by reactive oxygen species (ROS) (Cai & Harrison, 2000).

Metabolic syndrome and endothelial dysfunction (Cozma et al., 2009)

The visceral adipose tissue is also thought to be the major source of these free fatty acids. The lipid overflow-ectopic fat model displays how fat are accumulated in the visceral organs rather than subcutaneously. If extra energy was stored in insulin-sensitive subcutaneous adipose tissue as evidence shows, it is believed that the individual maybe protected from the possibility of developing metabolic syndrome. Nevertheless, triglyceride surplus will accumulate at unwanted areas such as the liver and heart due to insufficient amount of subcutaneous adipose tissue or when this tissue becomes more insulin resistant. Because of the increase of the fat in the liver, the hepatic glucose production would thereafter increase resulting in hyperglycemia

Role of insulin resistance

The increase in insulin levels resulting from insulin resistance activates the sympathetic-adrenergic system as well as the renin-angiotensin system. Insulin resistance is associated with an increase in the production of endothelin 1, which may cause vasoconstriction and plays an important role in the development of arterial hypertension. Insulin, by binding to its receptor and activated two difference pathway; 1) P13 kinase pathway: results in an increased capture and use of glucose, leads to an increase in NO synthesis, 2) MAP kinase pathway: result in the increase of pro-inflammatory proteins, cell proliferation to PAI-1.

Role of hyperglycemia

Hyperglycemia-induced endothelial dysfunction is mediated by the increase of oxidative stress. Five molecular mechanisms are involved in endothelial

dysfunction produced by hyperglycemia: 1) activation of protein kinase C by the diacyglycerol pathway, 2) enhancement of the hexosamine pathway, 3) increase in advanced glycation and formation of glycation end products, 4) enhancement of the polyol pathway, and 5) activation of the proinflammatory nuclear transcription factor.

Role of free fatty acid

The circulating levels of free fatty acids are high in Mets due to their excessive release by the adipose tissue and to their diminished use by skeletal muscle. Free fatty acids cause endothelial dysfunction through several mechanism, including; 1) increase in free oxygen radicals, 2) activation of protein kinase C, and 3) exacerbation of dyslipidemia. The increase in oxidative stress associated with cardiovascular risk factors leads to the appearance of vascular lesions and the increase in the permeability of endothelial cell dysfunction for low density lipoprotein (LDL) particles, followed by their oxidation in the arterial intima. And a number of cell growth and proliferation factor are released, which result in the stimulation of smooth muscle proliferation and excessive collagen production, with the initiation of the formation of the atheroma plaque (Hansson, 2005). In addition, this increased level of lipid associated with increased oxidative stress and pathophysiology of atherosclerosis in metabolic syndrome. Often times, Mets is characterized by oxidative stress. This is a condition that occurs due to imbalance results between the production and inactivation of reactive oxygen species. Even though they play an important role in multiple physiological systems, when under the condition of oxidative stress, they contribute to cellular dysfunction.

Role of oxidative stress

The oxidative stress caused by accumulated fat therefore plays an important role as a factor in metabolic syndrome predominantly through the dysregulation of adipocytokines. According to studies in humans and mice, the correlation between increased fat accumulation and systemic oxidative stress has been found. The reactive oxygen species (ROS) is a result from oxidative stress. Cultured adipocytes increased levels of FFA increased oxidative stress via NADPH oxidase activation and this caused dysregulated production of adipocytokines including adiponectin, PAI-1, and IL-6. This resulted in the decrease of dysregulation of adipocytokines moreover improving diabetes and hyperlipidemia. In addition, this increased level of lipid associated with increased oxidative stress and pathophysiology of atherosclerosis in metabolic syndrome. Often times, metabolic syndrome is characterized by oxidative stress. This is a condition that occurs due to imbalance results between the production and inactivation of reactive oxygen species. Even though they play an important role in multiple physiological systems, when under the condition of oxidative stress, they contribute to cellular dysfunction (Furukawa et al., 2004).

Lipoprotein-associated phospholipase A_2

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is produced largely by monocytes, macrophages, T lymphocytes, as well as mast cells. It is a member of the superfamily of A_2 phospholipases as well as a 50-kD and Ca^+-independent phospholipase. The enzyme was initially known as platelet activating factor acetylhydrolase (PAF-AH) due to its ability to degrade and additionally inactivate platelet activating factor (PAF). However, the more widely used nomenclature of Lp-PLA_2 is due to its association with lipoproteins in plasma and recognition of its

broader substrate specificity. Lp-PLA_2 binds to lipoprotein upon entering the circulation. Because of a specific protein-protein interaction between the N-terminus of Lp-PLA_2 and C-terminal portion of apolipoprotein B-100 (apoB-100), the major protein in low density lipoprotein (LDL), about 80% of the circulation enzyme is found on LDL. As for the rest, the majority (about 15%) is bound to high density lipoprotein (HDL) in an association that is dependent on the extent of N-linked glycosylation of Lp-PLA_2. With its propensity to bind to small dense LDL, a structural variant of the lipoprotein is likely to be found in subjects that has an increased plasma triglycerides levels. It is common that the altered conformation of apoB in small versus normal-sized LDL favors towards binding of the enzyme. Electronegative LDL which is a minor plasma species has been reported to have the greatest relative proportion of Lp-PLA_2 per particle and is enriched in the products of PLA_2 activity. Small dense as well as electronegative LDL is thought to be highly atherogenic. This is however due to the presence of increased amounts of Lp-PLA_2 per particle currently in conjecture. Lp-PLA_2 carried on LDL may contribute differently to atherogenesis than those bound to HDL. It is now obvious that plasma levels of Lp-PLA_2 are positively related to atherosclerosis as well as to the risk for a vascular event. Therefore, Lp-PLA_2 on LDL is thought to be pro-atherogenic. It is relatively direct to visualize a scenario in which Lp-PLA_2 is released all throughout the body and possibly under the influence of pro-inflammatory cytokines. Thereafter, it enters the circulation and bound to LDL which acts as a reservoir of the enzyme. LDL: Lp-PLA_2 complexes enter through the artery wall and later become trapped in the sub-endothelial space as the lipoprotein is retained by extracellular proteoglycans. LDL is subject to attack by free radicals which consist of nitric oxide, superoxide ion, and oxidation of its contained lipid ensues in the milieu of the artery wall. Lp-PLA_2 is then in prime position to release from the lipoprotein oxidized short-chain fatty acids and lysophosphatidylcholine (LysoPC). Both of which are

highly inflammatory thereafter affecting endothelial cell function as well as promote the recruitment of monocytes to the area. These monocytes become activated and release further Lp-PLA_2 upon entering the growing lesion (Figure 3) (Colley et al., 2011; Mallat, Lambeau, & Tedgui, 2010; Steen & O'Donoghue, 2013).

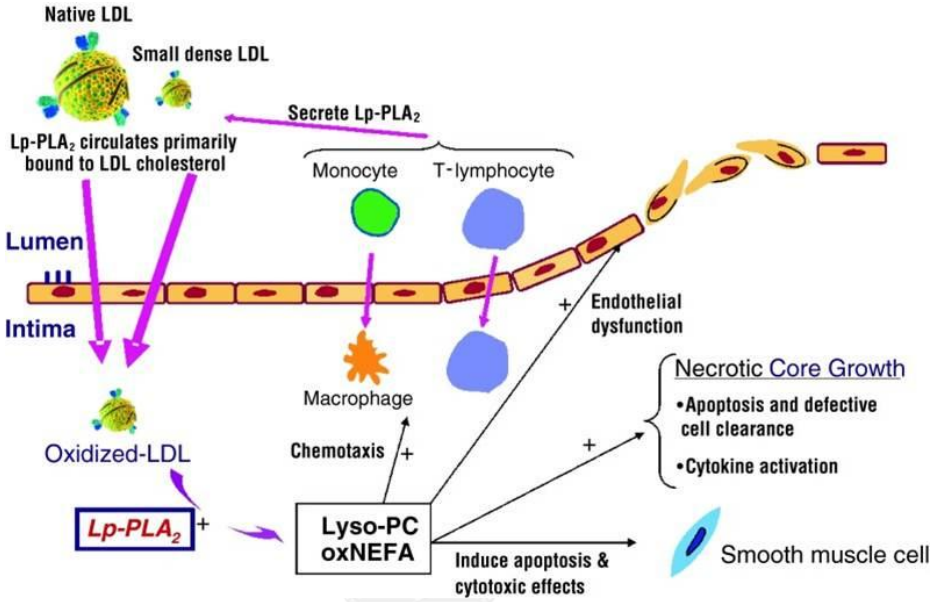


Figure 3 The mechanism of Lp-PLA2 and endothelial dysfunction (Steen & O'Donoghue, 2013)

Lipoprotein-associated phospholipase A_2 and vascular disease

A primary prevention trial by The West of Scotland Coronary Prevention Study (WOSCOPS) that showed Lp-PLA_2 compared in total of 580 cases and 1,160 age-matched controls. For the patients with the highest Lp-PLA_2 levels, it was found that they had twofold greater chance of coronary artery disease. In correlation with Lp-PLA_2, the highest level of hs-CRP was associated with a twofold increase in CHD risk as well. The risk associated with hs-CRP was attenuated but risk associated with

Lp-PLA$_2$ remained statistically significant on a running multivariate analysis (Figure 4) (Packard et al., 2000).

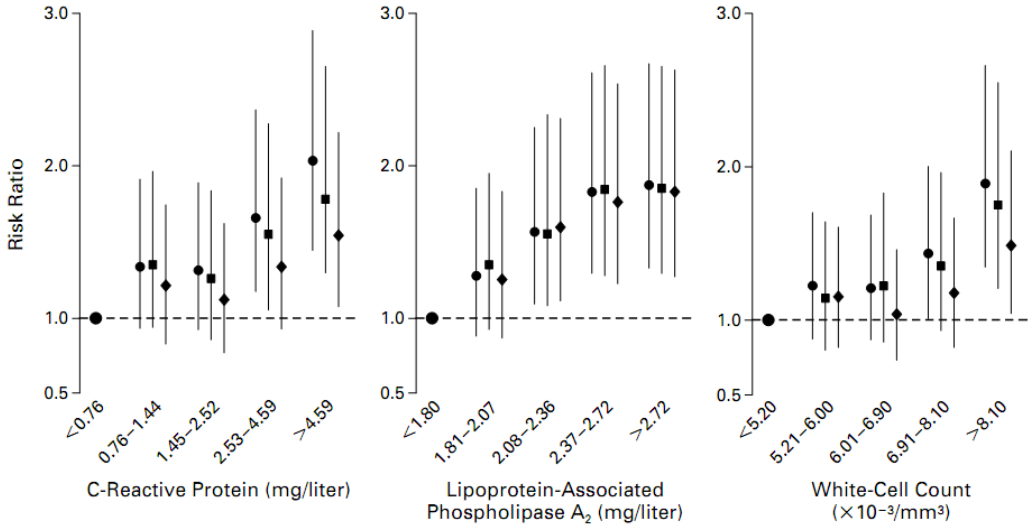


Figure 4 Association of the C-Reactive Protein Level, Lipiprotein-Associated Phospholipase A_2 Level, and the White-Cell Count with the Risk of a Coronary Event (Packard et al., 2000).

For WOSCOPS study, levels of C-reactive protein, lipoprotein-associated phospholipase A_2, and the white-cell count at base line in the patients were separated by the quintile values in the control subjects. The reference group (relative risk, 1.0) is classified by the group of patients with the lowest value in each case. The unadjusted relative risks were indicated with circles. In addition, the squares indicate relative risks adjusted for Lp-PLA$_2$ levels, the fibrinogen levels, as well as the white-cell count in the case of C-reactive protein; for C-reactive protein levels, the white-cell count, and fibriogen levels in the case of Lp-PLA$_2$; and for CRP levels, Lp-PLA$_2$ levels, and fibrinogen levels in the case of the white-cell count. The risk ratios adjusted for age, systolic blood presure, plasma triglyceride llevels, low-density lipoprotein cholesterol levels, and high-density lipoprotein cholesterol levels are indicated with diamonds. Vertical bars denote 95% confidence intervals. In

cinclusion, Lp-PLA_2 may risk factor for coronary heart disease, independent of traditional cardiovascular risk factors and C-reactive protein.

The Rotterdam Study demonstrated a cohort study in cases ≥ 55 years of age, inveatigated whether Lp-PLA_2 activity is an independent predictor of coronary heart disease and ischemic stroke. A total of 7,983 case by using Cox proportional-hazard models. Results of the comparison shows the first quartile of Lp-PLA_2 activity, multivariate-adjusted hazard ratios for coronary heart disease for second, third, and fourth quartiles were 1.39 (95% CI, 0.92 to 2.10), 1.99 (95% CI, 1.32 to 3.11), and 1.97 (95%CI, 1.28 to 3.2), respectively (p for trend=0.01). In addtion, Lp-PLA_2 activity was an inflammatory biomarker predictor for coronary heart disease in the general population according to this study (Oei et al., 2005).

The study of coronary heart disease in regards of production of Lp-PLA_2 and LPC in coronary circulation , the data represents that early coronary atherosclerosis in human is characterized by the production of Lp-PLA_2. Local coronary production of LysoPC as well as the active product of Lp-PLA_2 is associated with endothelial dysfunction which also supports the mechanism of regional vascular inflammation in concurrence with atherosclerosis in human (Figure 5)(Oei et al., 2005).

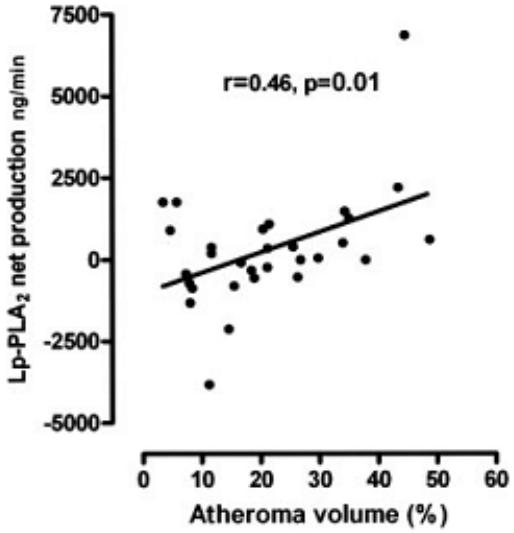


Figure 5 Correlation between Lp-PLA_2 net production in the left anterior descending artery territory and percent atheroma volume

In conclusion of Figure 5 showed that high level of Lp-PLA_2 associated with the increased of atheroma area in descending coronary artery, in left anterior descending artery related with the decreasing of coronary artery diameter (response of endothelial function to acetylcholine)

The review of the evidence for the clinical utility of Lp-PLA_2 as a cardiovascular risk factor, showed associated of Lp-PLA_2 level and survival rate that low level of Lp-PLA_2 without metabolic syndrome had high survival rate than high level of Lp-PLA_2 without metabolic syndrome. But, similarly survival rate in low level of Lp-PLA_2 with metabolic syndrome and high Lp-PLA_2 without metabolic syndrome. In addition, the prevention of low level of Lp-PLA_2 with metabolic syndrome to normal metabolic can increased survival rate (Figure 6) (Corson et al., 2008).

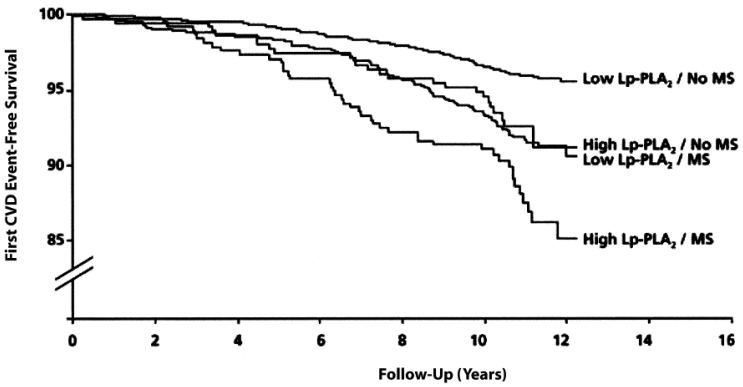


Figure 6 The association of Lp-PLA_2 level with The Event-Free Survival rate in first cardiovascular disease(Corson et al., 2008).

Oxidative stress and Endothelial Dysfunction (Roberts & Sindhu, 2009)

Oxidative stress is believed to be the key role in the pathogenesis of a variety of human disease, especially atherosclerosis. The potential role of oxidative stress in metabolic syndrome is rapidly changing. Evidence based results support the concept which states that an increased oxidative stress might play an important role in metabolic syndrome related manifestations. This may include atherosclerosis, hypertension, and type 2 diabetes. The association of oxidative stress with adiposity and insulin resistance in men, and in those with metabolic syndrome suggest that oxidative stress could be an early event in pathology of these chronic diseases.

Patients with metabolic syndrome have elevated oxidative damage as evidence show by decreased antioxidant protection in the form of depressed serum vitamin C and α-tocopherol concentrations, decreased superoxide dismutase activity, and increased lipidperoxidation malondialdehyde levels, protein carbonyls, and xanthine oxidase activity. In addition, the total body fat and waist circumference seem to be positively associated with oxidative stress-mediated endothelial dysfunction as well as vascular endothelial cell NAD(P)H oxidase activity.

Moreover, fat accumulation also correlates with plasma triobarbituric acid reactive substance (TBARS). The increase in ox-LDL in normal subjects, obese subjects, and obese subjects with metabolic syndrome were studied. As results show, a higher concentration of oxLDL was associated with an increased incidence of metabolic syndrome as well as components of abdominal obesity, hyperglycemia, and hypertriglyceridemia. In addition, the odd ratio for the highest oxLDL quintile was 3-fold higher than the lowest quintile and ox-LDL which was associated with the elevated C reactive protein (CRP) and insulin and inversely associated with adiponectin and HDL levels. The gathering of LDL in the sub-endothelial space and its subsequent oxidative modification is an important step in the development of a local inflammatory response, endothelial dysfunction, and in the end resulting in atherosclerosis. One of the main phospholipids of the LDL molecule is represented by Phosphatidylcholine. LysoPC is formed during the process of LDL oxidation as a result of phospholipase A_2-mediated hydrolysis of the sn-2-positioned fatty acid of phosphatidylcholine. Oxidized LDL might contain as much as 40% more LPC than native LDL which is present in high concentrations in the atherosclerotic wall. It is associated with numerous harmful effects which includes impairment of nitric oxide release, increase of cell adhesion molecules such as ICAM-1 and VCAM-1, monocyte chemotaxis as well as vascular smooth muscle cell proliferation.

The measurement of endothelial function

Endothelial dysfunction, a pathological condition, is mainly characterized by an impaired balance between substance with vasodilating, antimitogenic, and antithrombogenic properties (endothelium-derived relaxing factors) and substances with vasoconstricting, prothrombotic, and proliferative characteristics (endothelium-derived contracting factors). Nitric oxide (NO) is amongst the most important vasodilator molecules, especially in muscular arteries. It also inhibits other key

events in the development of atherosclerosis such as platelet adhesion and aggregation, leukocyte adhesion and migration, and smooth muscle cell proliferation. The active potential of modification of oxLDL is able to trigger inflammatory process, reduce NO level in endothelial cell.

Overall, losses of NO bioavailability indicate a widely dysfunctional phenotype across many properties of the endothelium. The assessment of its vasodilator properties which is a result of NO and other molecules might be able to provide information on the integrity and function of the endothelium. Fascinatingly, cardiovascular risk factors associate with endothelial dysfunction whereby risk factor modification can lead to improvement of vascular function. The method selected to detect endothelial function is venous occlusion plethysmography. It can detect reactive hyperemia which is the response of endothelial cell after venous occlusion and subsequently determine endothelial function. The underlying principle involves the arrest of venous outflow from the forearm with uninterrupted arterial blood flow. Therefore, the result of linear increase of forearm volume over time is proportional to arterial blood flow reflecting forearm vascular resistance which is a normal vascular endothelial function. A plethysmograph is used to measure the changes in the forearm volume after occlusion of blood circulation to the hand, though the method has good reproducibility (Lekakis et al., 2011; Wilkinson & Webb, 2001; Wythe, Davies, Martin, Feelisch, & Gilbert-Kawai, 2015).

Physical activity and Metabolic syndrome

Regular physical activity practice has been recommended for the prevention and rehabilitation for chronic disease, Mets is the group of vascular risk factor. The epidemiological and clinical studies demonstrated that the regular practice of

physical activity is importance factor for prevention and treatment in Mets (Pedersen & Saltin, 2015). The imbalance of calories uptake and sedentary life style are the most cause of Mets. The previous studies showed the benefit of exercise in Mets, and focus on many parameters that related with complication of Mets or the advantage effects to vascular disease such as, effects of physical exercise on blood pressure, plasma glucose, lipid profile, percentage of body fat, muscle mass, muscle strength, oxidative stress (Golbidi, Mesdaghinia, & Laher, 2012; Kojda & Hambrecht, 2005; Lin et al., 2015).

Moderate intensity exercise in metabolic syndrome

The moderate –intensity exercise range from 3-6 METs or have a relative intensity of 55-70% maximum heart rate that recommended to using in Mets group. This intensity of exercise was approved with many previous studies that can reduce risk of metabolic parameters. Many studies demonstrated amount and intensity that optimum benefit and recommended to used (Churilla, 2009).

The demonstrated of exercise training amount and intensity effects on Mets, determined how much exercise is recommended to decrease the prevalence of Mets , the results showed that, moderate intensity exercise in the absence of dietary change significantly improved Mets and thus supported the recommendation that adult get 30 minutes of moderate-intensity exercise every day. Although, the comparison of the amount and exercise intensity on the percentage change in ATPIII prevalence, +19% in control, -44% in the low-amount/moderate-intensity group, -12% in the low-amount/vigorous-intensity group and – 44% in the high-amount/vigorous-intensity group (Johnson et al., 2007).

Similarly with ACSM position stand on physical activity and weight loss, recommends that, in Mets the management to prescribe the long term risk of type 2

diabetes and cardiovascular disease are necessary. The combination of weight loss and exercise produces the beat effect, the previous data expressed that combination of weight loss and exercise can improve on blood glucose level, blood lipid, and blood pressure. In addition, reduce 29-68% risk of progressing T2DM. People with Mets can exercise safety by the program that begins slowly and progresses appropriately. The general recommendation for adults to participate in at least 30 minutes of aerobic exercise that used the large muscle group (brisk walking, jogging, cycling, swimming, etc.) are appropriate and effective. Moderate intensity aerobic exercise is probably best for overall improvement in Mets and may be more likely to be sustained than high intensity exercise. A simple role of exercise at a level that increase breathing and heart rate but still allows to maintained a conversation. The exercise period consist of 5-10 minutes of warm-up exercise (light aerobic activity), resistance exercise program at least twice a week, 2-3 set of 8-10 difference exercise, 12-15 repetition of each set (Grundy, Hansen, Smith, Cleeman, & Kahn, 2004; Jeon, Lokken, Hu, & van Dam, 2007; Norton, Norton, & Sadgrove, 2010).

Effects of moderate intensity exercise on Mets

The systematically evaluate expressed the evidence for an association between physical activity of moderate intensity and risk of type 2 diabetes [60]. The data showed that, moderate intensity and type 2 diabetes, including a total 301,221 participants and 9,367 incident cases. Five of these studies specifically investigated the role of walking. The summary RR of type 2 diabetes was 0.69(95% CI 0.58-0.83) for regular participation in physical activity of moderate intensity as compared with being sedentary. Similarly, the RR was 0.70 (0.58-0.84) for regular walking (typically $\geq$2.5 hour/week brisk walking) as compared with almost no walking. In conclusion, moderate intensity exercise such as brisk walking can reduce the risk of type 2 diabetes (Norton et al., 2010).

The investigate on effect of different intensities of exercise on endothelium-dependent vasodilation, role of endothelium-dependent nitric oxide and oxidative stress in hypertensive patients, which evaluated the forearm blood flow, serum oxidative stress before and after different intensities of exercise (Goto et al., 2003). The data showed that, both of exercise intensity increased FBF, but high-intensity exercise increases plasma concentrations of oxidative stress, whereas moderate exercise tended to decrease oxidative stress. In addition, moderate intensity aerobic exercise augments endothelium-dependent vasodilation through the increased production of nitric oxide and decrease oxidative stress.

Also found that moderate intensity along with moderate duration influence percentage of weight loss more than in metabolic disease with more vigorous intensity at 6 months training (Figure 7) (Church, 2011). Consequently, moderate intensity exercise effects on improvement of cardiorespiratory fitness and endothelial function. With the above-mentioned reasons, moderate intensity exercise can improve metabolic syndrome by improving metabolic marker and as a result increase quality of life.

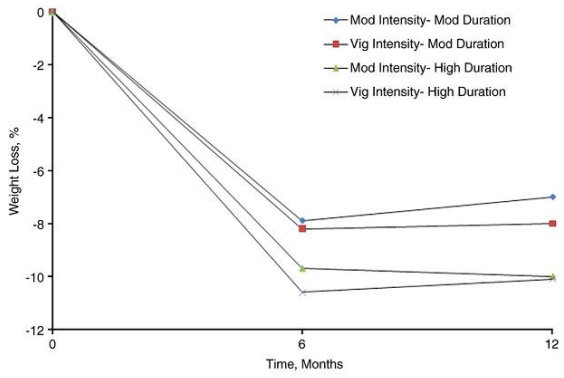


Figure 7 Weight lost at 6 and 12 months across group with different exercise durations and intensities in obese(Church, 2011).

In summary, moderate intensity exercise had benefit on reduced risk of vascular disease in Mets group, and useful to recommended for prevention, promotion, and rehabilitation in Mets.

The ACSM, the Centers for Disease Control, as well as Prevention guidelines suggest that 30 minutes or more of moderate physical activity on most, and preferably all, days of the week have beneficial adaptation effect on cardiorespiratory fitness, body composition, and muscular fitness. Fitness training complies with the fundamental physiologic principles of overload and specificity whereas physiologic adaptation requires a progressive increase stimuli specific to the muscles involved as well as the type of exercise. An exercise prescription comprises of the duration, intensity, mode, and in addition the frequency of exercise activities.

A broad fitness program involves all major muscle groups transfers the training effects to both vocational and recreational activities. Warm-up, conditioning phase, and cool down are important components of a particular training session. The 5 to 20 minutes of warm-up help prepare the muscle for more vigorous exercise and as a result prevent and reduce the risk of an injury. Thereafter, the 20 to 60 minutes of the conditioning phase involves a cardiorespiratory or resistance training session. Lastly, the cool down session, which might attenuate post-exercise hypotension, allow better dissipation of body heat, remove lactic acid, mitigate the rise in potentially arrhythmogenic catecholamines, and possibly reduce the risk of cardiac events during the recovery period.

The ACSM has recommended training with a frequency of 5-7 days per week. Each session lasting 45 to 60 minutes and longer-duration training at lower intensity is based on the premise that glycogen is the predominant fuel source during the first 20 minutes of exercise, followed by a shift to fat stores after 30 minutes. Several

studies of the moderate intensity have approved oxidative stress as well as metabolic parameter such as inflammatory cytokine, insulin resistance, blood glucose or Hb_{A1c} (Churilla 2009).

Principle of Qigong

Qigong is one modality of traditional Chinese medicine (TCM) believed to have a history dated back at least 4,000 years ago. The basis of this therapy is based on the traditional Chinese. It is believed that the human body contains a network of energy pathways through which vital energy called Qi circulates. The energy meridian system of TCM is what Qigong is based on. This interesting tradition identifies 18 energy meridians within the human body. Through these energy meridians flow the vital life energy, or Qi. Quality and Flow are the two important characteristics of qi. Illness is a result of stagnant, blocked, or impure qi according to the TCM .

The practice of Qigong cleanses the qi and improves the flow of qi throughout one's body, thereby resulting in an improved health state. The practice of Qigong combines gentle and slow body movements with breathing and the practice of calming down the mind. The main objective of practicing Qigong is to prevent ailments and to improve overall health as well as increase energy levels through regular practice (James 2015).

There are difference styles of Qigong, most Qigong are external forms of movement and exercise, which are known as "Wei Gong", others are internal, similar to meditation , which are known "Nei Gong".

Nei Gong is the basis of the accompanying set of practices call "Eight Extraordinary Meridian Qigong". The human energy system is one fundamental basis in all Qigong style.

The Meridians Energy System (James 2015)

The energy system of Qigong is most similarly through acupuncture. The energy system consists of pathways of energy which are call “Meridians or Channels”, and the energy pathway related to specific internal organ of functions, which are: heart, small intestine, bladder, kidneys, pericardium, triple heater, gall bladder, liver, lungs, large intestine, stomach and spleen. Along each meridian are points which have very specific function (Figure 2.8).

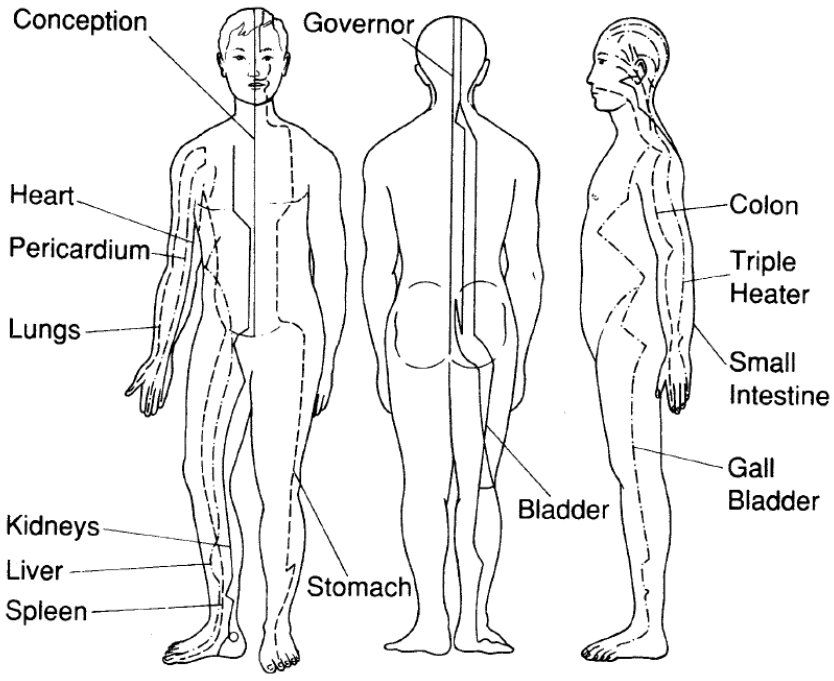


Figure 8 Main Meridian Channels (James 2015)

The eight of these channels are known as the Extraordinary Meridians and which 8 extras only two have points of their own, the others leapfrog and criss-cross over the points on the other channels (Figure 9).

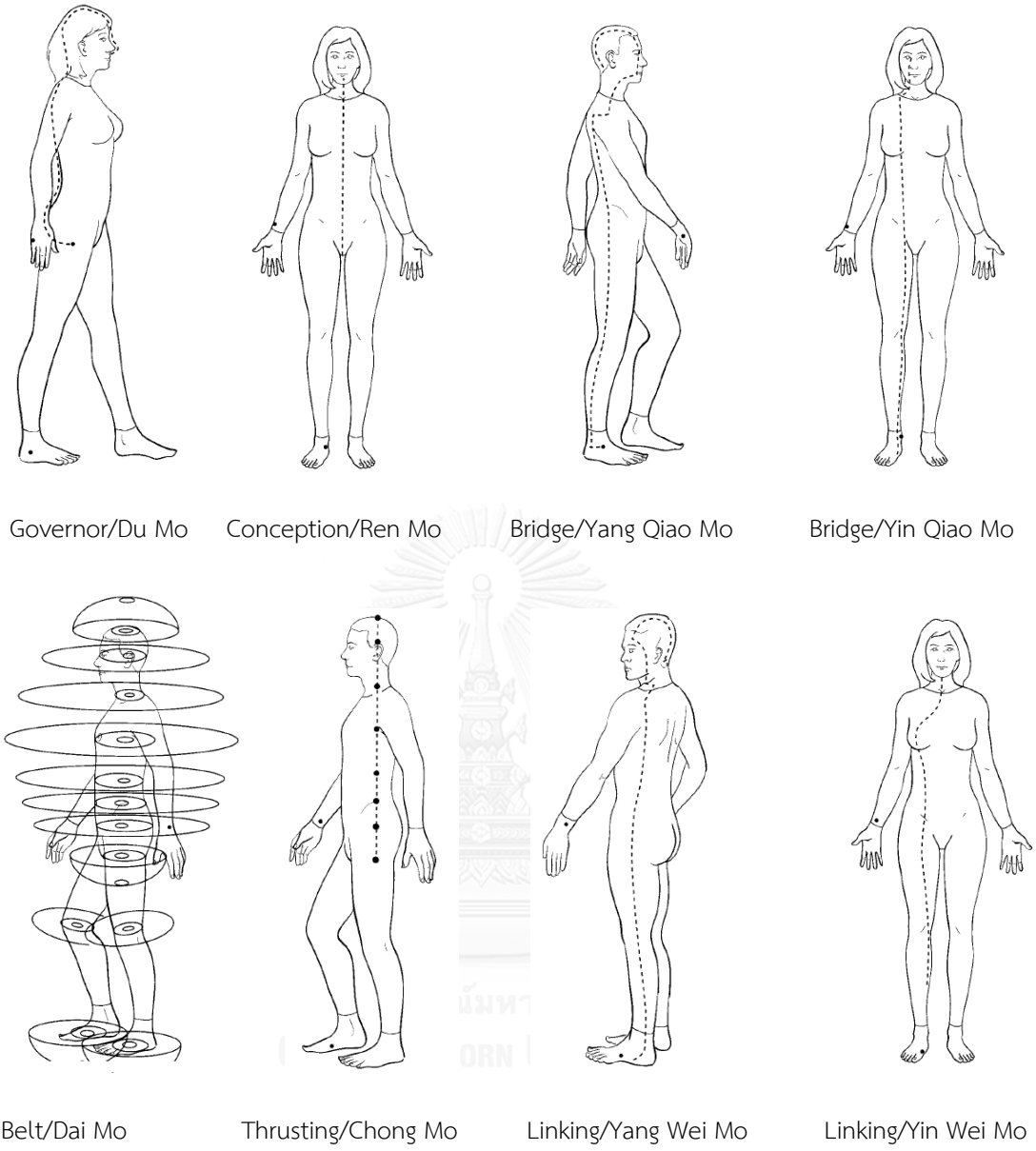


Figure 9 Eight Extraorinary Meridians

From 8 extra are separated into two sets of four each, primary set and secondary set.

The primary set operated within the torso, and are known as:

1. Governor channel/Du Mo
2. Conception channel/Ren Mo
3. Belt channel/Dai Mo
4. Thrusting channel/Chong Mo

The secondary set which back-up or support the primary set and run into the arms and legs are known as:

1. Yang Bridge/Yang Qiao Mo
2. Yin Bridge/Tin Qiao Mo
3. Yang Linking/Yang Wei Mo
4. Yin Linging/Yin Wei Mo

All of 8 extras may be accessed and controlled by a special group of points called the master and coupled points. Together with some sub-branches and connecting channels, these 20 meridians/channels/pathways constitute the full number of channels in the body familiar in acupuncture.

Another set of important centers used particularly in Qigong, call the Three Tan Tien, which can be translated as the three elixir fields or the three energy centers. There are located in the core of the body along the Chong Mo. They are located in the lower abdomen, the center of the chest, and the middle of the head. The Three Tan Tien contain the three treasures, which are known as Jing, Qi, and Shen, which can be translated as essence, energy, and spirit. The Three Treasures are considered to be the most important possession a person has, and are nurtured, cultivated and protected (Figure 10).

In addition, Qigong works by operating and affecting these channels and centers to increase the volume and heighten the frequency of energy, and put it under conscious control.

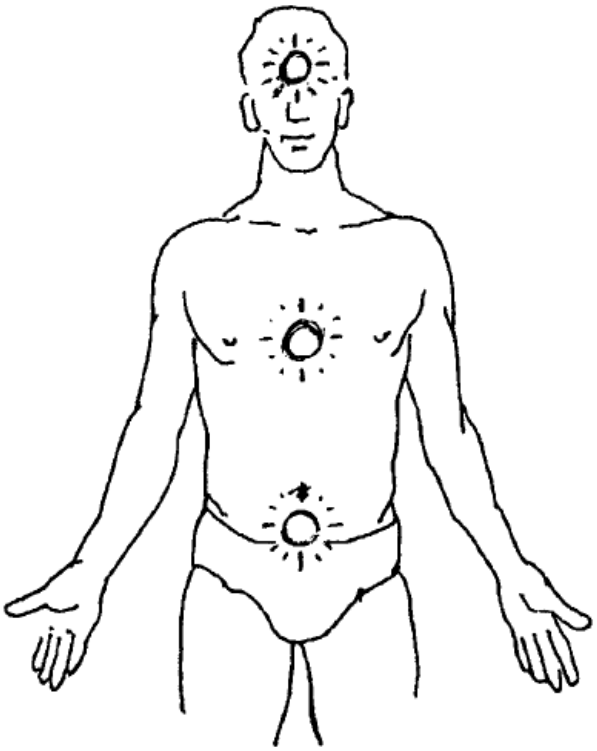


Figure 10 The Three Tan Tien

Qigong consists of two concepts: Qi which is the vital energy of the body whereas Gong is the training or cultivation of qi. In previous studies describe type of Qigong Therapies in 3 type.

1) Internal Qigong, employs prescribed postures and sequences of visualization, breathing techniques, and movements as a self-healing or health-promotion practice
2) External Qigong is the delivery of Qi stimulation by a healer or practitioners to a recipient, to influence circulation of Qi and the wellbeing in the recipient. This is usually done from several inches away from the recipient, with the practitioner sending Qi via the palms of the hands or the fingers point at the recipient

3) Medical Qigong is the application of either internal or external Qigong for healing from specific illness. There are many traditions of medical Qigong. A typical practice might include 5 steps: meditation, cleansing, recharging, strengthening, circulating, and dispersing Qi. Each step includes specific exercise, meditations, and sound.

The Meridian Theory

The meridian is one of major components of the traditional Chinese medicine (TCM) that given to explanation for a cause of disease and how treated a disease. Meridian is a network that existing in the body, its consist of three levels: meridians, collaterals, and sub-collaterals. The part of system is difference sizes of channels, meridian channels, collateral channels, and sub-collateral channels. The important knowledge related to the channel system is Qi-blood, the essential substance and energy of body. Qi flowed in the channels and spread throughout all organs and tissue. The obstruction of the energy conduced to the disease as showed in Figure 11, the pathway from keeping homeostasis in interstitial flow, to preventing edema, cell migration,, capillary pathogenesis and immunity (Zhang, Wang, & Fuxe, 2015).

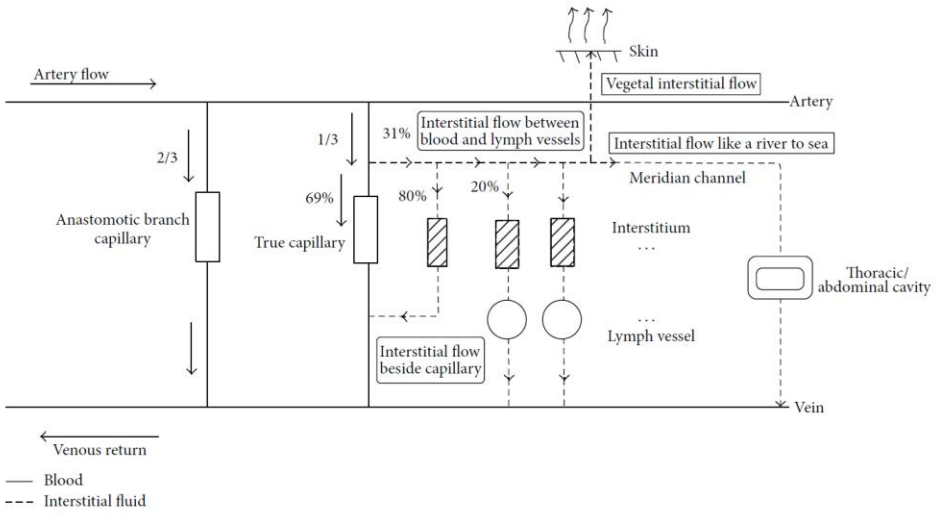


Figure 11 The general four kinds of interstitial flow on human body

The meridian associated with cardiovascular circulation of vital substances and effects on energy production within the cells involves oxidation of glucose to convert ADP to ATP which the fuels cellular processes. The branching vessel networks is the core pathway to conducts blood circulation, substances and energy through the body as showed in Figure 12 (E.Kendall, 2008)

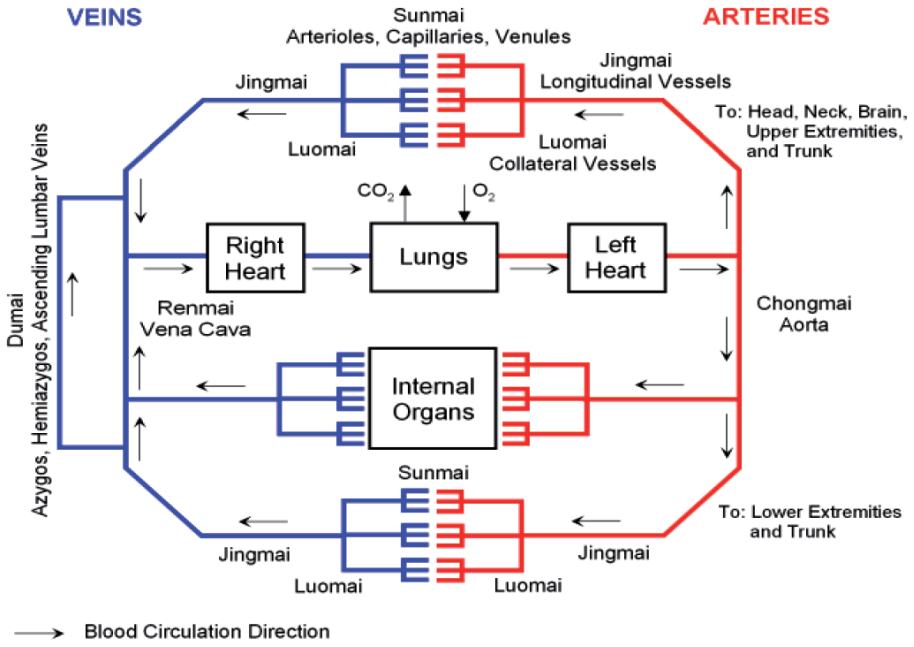


Figure 12 Schematic view of Chinese cardiovascular system and vessel branching

Intensity of Qigong

The demonstrated energy expenditure characteristics of 9 key components of Guo Lin Qigong in 40 cancer patients, found that MET value between 1.8-4.83 MET during perform Qigong and conclude that Qigong is a low-to-moderate intensity

aerobic exercise. With its unique breathing pattern, relative oxygen intake of several components is rather large considering the slow walking speed employed (Zhu, 2009)

The evaluated the cardiorespiratory response and energy expenditure during the practice of Tai-Chi-Qui-Gong (TCQG) in 47 TCQG practitioners, The result indicated that the estimated intensity of TCQG in elderly individuals approximated 50% of VO_{2max} for men, and 60%VO_{2max} for women, This findings that TCQG is a low intensity exercise and can be prescribed as an alternative exercise program for cardiopulmonary rehabilitation (Y.-F. C. Chao, et al. , 2002).

In addition, Qigong is a low intensity activity and benefits for rehabilitation in chronic disease such as cancer to improve physical and mental function.

In generally, the component of Qigong practice consist of meditation, deep breathing, relaxation, and stretching & resistance exercise, from these component may benefit on the psychological and physiological change (Ulbricht C, 2010).

Effects of meditation on physiological change

The demonstrated post mindfulness meditation practice found the changing in gray matter concentration within hippocampus, posterior cingulate cortex of temporo-parietal junction and cerebellum. And, conclude that meditation training associated with change in gray matter concentration in brain regions involved learning and memory processes, and emotion regulation (Holzel et al., 2011).

The studied effects of meditation on sympathetic nervous system found that regular practice of meditation initially blunted the sympathetic activity such as decreased in blood pressure and heart rate (Deepak, 2012).

In addition, meditation practice can improved both behavioral and physiological.

Effects of deep breathing on physiological change

For the skill of deep breathing technique explained that deep breathing had the effect on autonomic nervous system resulting in the decreased blood pressure and increased oxygen consumption by activated parasympathetic activities as follows (Figure 13) (Jerath, Edry, Barnes, & Jerath, 2006):

1) Activated of slowly adapting stretch receptors (SARs) and generated the inhibitory impulse in neural tissue then decreased action potential in neural tissue.
2) Stretch of fibroblasts surrounding lungs to generate of hyperpolarization current then synchronized of neural tissue including hypothalamus and brainstem, increasing resting membrane potential polarity in surrounding tissue then decreasing metabolic activity.

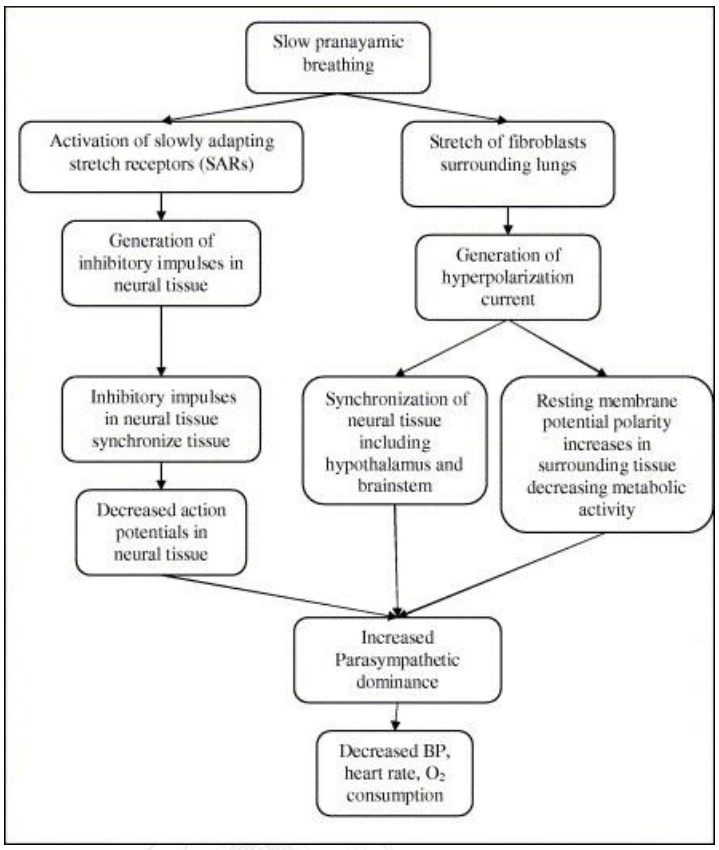


Figure 13 Diagram of the series of events that occur during the autonomic shift present in pranayamic slow breathing (Jerath et al., 2006)

Effects of imagination and relaxation on physiological change

The imagination and relaxation technique of Qigong reduced the level of stress hormone such as cortisol and ACTH and increased beta-endophin then effects on cardiovascular response adaptation after training period.

The demonstrated effects of qigong on anxiety and physiological stress function in transverse flute music schoolchildren found that, reductions of subjective perception of anxiety, and salivary cortisol levels and heart rate after seven week of training (Sousa et al., 2012).

The investigate of the acute effects of Qigong training on the levels of human endogenous opioid peptides, beta-endorphin, and adrenocorticotrophic

hormone (ACTH), cortisol, the results found significantly increased beta-endorphin level related to decreased ACTH level. And suggest that Qigong training, affects and plays a role in hormonal regulation related to the maintenance of homeostasis in man (Ryu et al., 1996).

Effects of stretching and resistance training on physiological change

In generally, the postures of Guang-Im-Ju-Jai-Gong Qigong consist of;

1) standing position with resistance and 2) upper limb movement with stretching (Figure 14)

Figure 14 The posture of Guang-Im-Ju-Jai-Gong Qigong

The resistance exercise, also know as strength training, is established as an effective strategy to increase muscle mass and strength in healthy and patients. It involves muscle exerting a force againt a resistance contraction, and usually repetitively at a workload well above the aerobic capacity of muscle(Murton & Greenhaff, 2013).

The metabolic effects of reduced muscle mass, engendered by normal aging or decreased physical activity, lead to a risk for metabolic syndrome. Skeletal muscle

is the primary metabolic source of glucose and triglyceride disposal and is an important determinant of resting metabolic rate. The previous study showed that increased in muscle can reduce risk for cardiovascular disease(Braith & Stewart, 2006). In addition, Qigong consist of resistance training with low intensity aerobic training which a benefit on cardiovascular and muscle.

CHAPTER III

METHERIAL AND METHODS

Research Design

The design of this study can be classified as an experimental study with randomized control trial. The study protocol was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University. Prior to consenting to participate in the study, all participants gave written informed consent.

Population and Sample

Target population: Thai female between 40-65 years of age, diagnosed with metabolic syndrome, and lead a sedentary life style.

Study population: Thai female between 40-65 years of age, diagnosed with metabolic syndrome with eligible criteria, lead a sedentary life style, and living in Bangkok and perimeter area.

Sample: Thai female between 40-65 years of age, diagnosed with metabolic syndrome with eligible criteria, lead a sedentary life style, living in Bangkok and perimeter area, qualify with eligibility criteria, and willing to participate in the study.

Eligibility Criteria

Inclusion Criteria

The inclusion criteria are as follows:

(1) According to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATPIII) the criteria for metabolic syndrome is defined by the following. The participant eligible must obtain at least 3 criteria:

1.1) Elevated waist circumference; Men:>102 cm (>40 in), Women:>88 cm (>35 in)

1.2) Elevated TG; ≥ 150 mg/dL or drug treatment for elevated TG

1.3) Reduced HDL-C; Men < 40 mg/dl, women < 50 mg/dL or drug treatment for reduced HDL-C

1.4) Elevated blood pressure; ≥ 130 mmHg systolic blood pressure or ≥ 85 mmHg diastolic blood pressure or drug treatment for hypertension

1.5) Elevated fasting glucose; ≥ 100 mg/dL or drug treatment for elevate glucose

(2) Sedentary life style, participate in a regular exercise program less than 30 minutes per week

(3) Living in Bangkok and perimeter area

(4) Non Smoker or quit smoking for more than 6 months

Exclusion Criteria

The exclusion criteria are as follows:

(1) Serious symptomatic cardiac disease, including previous myocardial infarction, angina, or heart failure

(2) Previous history of transient ischemic attacks or stroke

(3) Taking medications that affect the central nervous system

(4) Concomitant illnesses that includes cancer, infectious disease, renal failure

(5) Pregnant or suspected to be pregnant

(6) Poor DM control with macro and micro vessel complications such as diabetes nephropathy, diabetes retinopathy, diabetes foot, diabetes neuropathy; use insulin injection ($HB_{A1C} \geq 7$, able to control FPG 3 months before and during 3 months of intervention, adjust dosage and type of medication during 3 months of intervention)

(6) Positive exercise stress test, myocardial infarction, and ischemic heart disease

(7) Stage 2 hypertension with SBP $\geq$ 160 mmHg, DBP $\geq$ 100 mmHg (following JNC7 criteria), uncontrolled blood pressure before and during 3 months of intervention, adjust dosage and type of medication during 3 months of intervention)

(8) Morbid obesity with BMI greater than or equal to 40 kg/m^2

(9) Uncontrolled lipid profile with and without usage of medication before and during 3 months of intervention, adjust dosage and type of lipid lowering drug during 3 months of intervention (following NCEP III criteria: high LDL-C ($\geq$ 190 mg/dL), low HDL-C ($\leq$ 40 mg/dL), high TG ($\geq$ 350 mg/dL)

(10) Menopause with hormonal therapy

(11) Rapid atrial fibrillation, atrial flutter, ventricular arrhythmia, secondary to complete heart block, valvular heart disease, and cardiomyopathy

(12) Use of anticoagulant therapy

(13) Inability to comply with the study instructions

(14) Adherence rate less than 70%

Sample size estimation

The sample size was calculated by using the data of a pilot study with same Qigong training protocol. The study compared the level of area under the curve (AUC) of forearm blood flow in 10 participants. They were separated to 2 groups; control and Qigong group respectively. After 12 weeks of Qigong for 1 hour per day, 4 days per week, AUC of Qigong group and control group were 1074.435±17.77369 and 455.835±26.7005 mL/100 mL tissue respectively. The data was used to calculate sample size with the equation as follows:

n/group = $2[\frac{(Z_{\alpha/2}+Z_{\beta})\sigma}{\Delta}]^2$

n = number of study population

$Z_{\alpha/2}$ = alpha error = 1.96

Z_{β} = beta error = 1.28

σ = the variation from pilot study was calculated from the equation as follows:

$$\sigma = \frac{SD_1^2+SD_2^2}{2} = \frac{26.7005^2 + 17.77369^2}{2} = 514.41$$

Δ = absolute mean difference from the pilot study was calculated from the equation as follows:

$$\Delta = (\overline{X}_1 - \overline{X}_2)^2 = (455.835-1074.435)^2 = (618.6)^2$$

n/group = $2[\frac{(Z_{\alpha/2}+Z_{\beta})\sigma}{\Delta}]$

$$= \frac{2(1.96+1.28)^2(514.41)^2}{(618.6^2)}$$

= 14.5

The actual calculation is 15 n/group. However, we chose to use 21 n/group in order to subsidize for the estimated drop out of 40%.

Randomization and allocation concealment

The participants were randomly allocated into 3 groups; control (usual activity with health education), Qigong, and moderate intensity exercise. Participants were randomized by block randomization (block of 6). Allocation concealment was ensured. The randomization code would not be revealed until the participants are recruited into the trial after all baseline measurements are complete.

Blinding

Outcome assessors were blinded to group allocation of participants and are not involved in providing intervention.

Procedure

Three parallel groups, randomized, repeated measures, and control trial was employed. During the first visit, the participants were medically screened and measured the anthropometric data. At the second visit, the participants underwent a progressive diagnostic treadmill test to evaluate their VO_{2peak} at baseline and again after the 12^{th} week of the training program, measured endothelial function, CAVI, ABI, and fasting blood samples were collected to determine the biochemical parameters. Then, the participants were randomly assigned to 3 groups; control group, moderate intensity exercise group, and Qigong group. The moderate intensity exercise group performed brisk walking on treadmill 1 hour per day, 4 days week for 12 weeks at an intensity in the comparison with the initial vo2 peak at 70-75%. Qigong group performed Guang-Im-Ju-Jai-Gong for 1 hour per day, 4 days per week for 12 weeks and blood samples were collected at baseline, 4^{th}, 8^{th}, and 12^{th} week of the exercise program (Figure 15). FBF, CAVI, and ABI were measured the same weeks respectively.

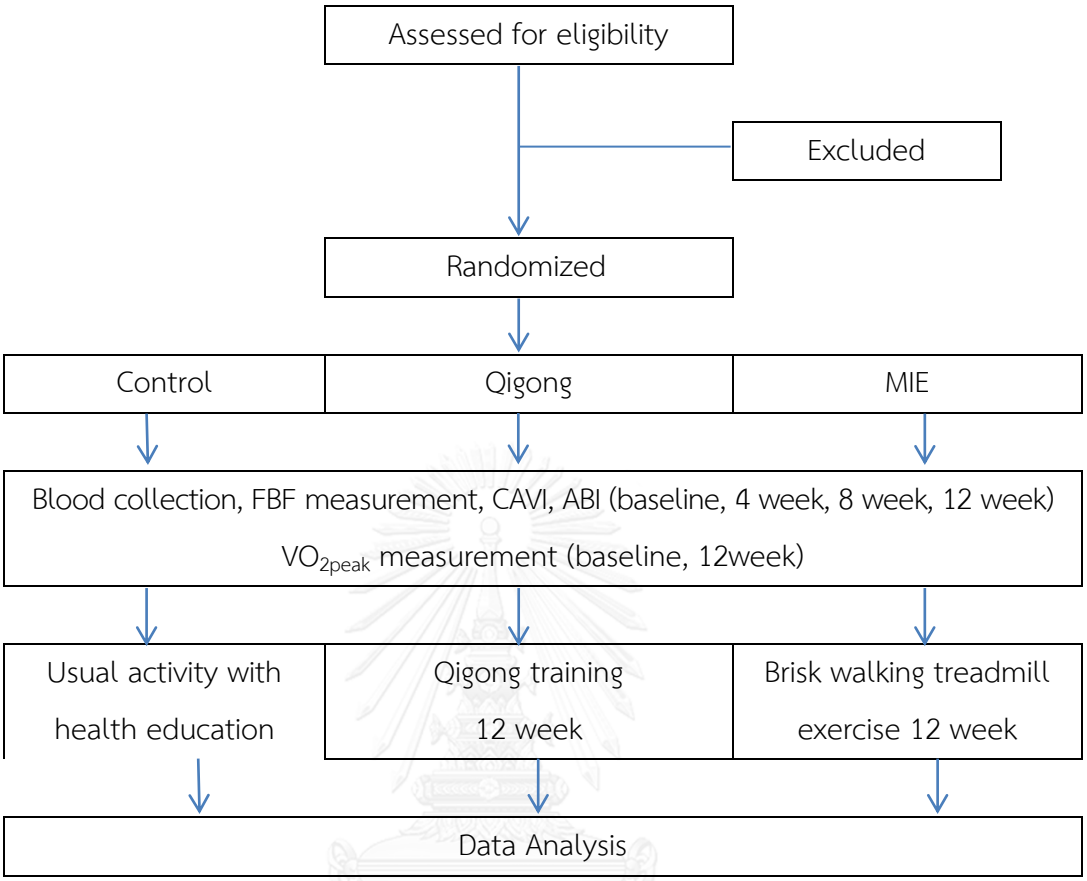


Figure 15 Experimental Procedure

Chemical and Instrument

Chemical substance for Malondiadehyde (MDA)

- Trichloroacetic acid (TCA) (Sigma Chemical®, USA)
- Thiobarbituric acid (TBA) (Sigma Chemical®, USA)
- Malondiadehyde tetrabutylammonium (Sigma Chemical®, USA)

Chemical substance for Ferric reducing antioxidant power (FRAP)

- Sodium acetate.3H_2O (Sigma Chemical®, USA)
- Acetic acid (Merck®, Germany)
- Hydrochloric acid (HCl) (Mollinckrodt Chemical®, USA)
- 2,4,6-tripyridyl-s-striazine (TPTZ) (Sigma Chemical®, USA)
- Ferric chloride ($FeCl_3.6H_2O$)
- Ferrous sulphate ($FeSO_4.7H_2O$)

ELISA kit for $LpPLA_2$ (Quantikine®ELISA, USA)

Indirect calorimetry pulmonary gas exchange system : Oxycon® (USA)

Treadmill: Nautilus T518® (USA)

Venous occlusion plethemography: Hokanson® (USA)

- Rapid Cuff Inflation System E20
- RD2 Rapid Cuff Deflator
- Contoured Cuffs
- EC6 Strain Gauge Plethysmograph
- Strain Gauge Forearm Sets (from 16 to 30 cm. in 2 cm. increments)
- DS400 Aneroid Sphygmomanometer

Vascular screening system Vasera 1500® (Fukuda Denshi, Japan)

Bioelectrical Impedance Analysis: Inbody230® (Korea)

Heart Rate Monitor: Polar® (Finland)

Blood and specimen collection tube

- EDTA tube : BD Vacutainer®
- Plan tube : BD Vacutainer®
- Microcentrifuge tube : BD Vacutainer®

Multi Channel pipette

Single Channel pipette

Digital Dry Bath (Labnet®,USA)

Microplate Spectrophotometer (Biotek®,USA)

Vortex mixer (Scientific Industry®, USA)

Refrigerated Centrifuge

96 well plate (Greniner bio-one, Germany)

Anthropometric and body composition measurements

1. The height was measured by the participant standing barefoot with heels together, in stretching upward position to the fullest extent. Heels, buttocks, and upper back fully touch the wall. The chin not lifted. All measurements recorded in centimeters.
2. The waist circumference (WC) was measured by the minimum circumference (narrowest part of the torso, above the umbilicus) and the maximum hip circumference while standing straight with their heels together.

3. The body weight, fat free mass, body fat mass, muscle mass, body fat percentage, waist to hip ratio (WHR) were measured by using bioelectrical impedance analysis (InBody230®Korea).

Bioelectrical impedance analysis (BIA)

Bioelectrical impedance analysis (BIA) uses a direct segment multi-frequency bioelectrical impedance analysis method. It uses impedance measurements by using 2 different frequencies (20 kHz, and 100 kHz) with an excitation current of 330 μA with contact points (Figure 16).

BIA Measurement Protocol

1. Participants were advised to wear the same clothing for each measurement. All jewelry removed.
2. The measurement done after 12 hours of fasting.
3. The participant void prior to each measurement.
4. The participant stands on the machine barefoot with both feet contacting directly with metal contact points. Both hands grip loosely on hand grips.
5. The assessor enters name, date of birth, height, and sex into the machine's database.
6. The participant stand still on the machine until it completes the analysis.

Figure 16 A body composition analysis device and measurement of body composition

Blood Samples collection protocol

Prior to all blood sample collection, 12 hours of fasting and no exercise for 48 hours is required. Ten milliliters of blood is required to be taken from antecubital vein at baseline, 4^{th}, 8^{th}, and 12^{th} week of the exercise training program.

Endothelial function measurement

Venous Occlusion Plethysmography Technique

Endothelial function was measured by the use of forearm blood flow of reactive hyperemia (RH) during venous occlusion plethysmography technique following the protocol below:

1. Plethysmography is performed early in the morning and after fasting of 12 hours at room temperature between 25°C and 27°C.

2. Plethysmography is performed in accordance to previously describe by Alomari, 2004. It was done on the upper limb with a raised arm on a flat surface at an angle of 30° to allow spontaneous emptying of the veins.
3. Before any measurement, the participant remains in supine and relaxed position for a period of 15-20 minutes.
4. An inflating cuff or upper cuff (SC10D, Hokanson, USA) was placed around the participant's bicep to occlude venous blood flow and connected to a rapid cuff inflator (E20, Hokanson, USA) which was set above venous but below arterial pressure (50mmHg) (Figure 3.3).
5. A strain gauge (Hokanson USA) was placed around the widest part of the forearm and connected to a plethysmograph (EC6, Hokanson USA). The forearm circumference allowed for correct size mercury-silastic strain gauge (maximum forearm circumference determined by using a tape measure minus 2 cm) (Figure 17).
6. To exclude the hand circulation which contains a large number of arterio-venous shunts, a segmental pressure cuff (TMC7, Hokanson) or lower cuff was placed around the wrist and inflated to supra-arterial pressure immediately before testing. The wrist cuff manually inflated to 250 mmHg.
7. The rapid cuff inflator was set to inflate the bicep cuff to 50 mmHg for 10 seconds at time. During each 10 second inflated interval, the change in forearm circumference was detected as well as change in the electrical resistance in the strain gauge.
8. Data was recorded on a computer using the NIVP3 arterial inflow studies software (Hokanson ®USA).
9. Baseline blood flow was measured in 5 minutes.

10. Ischemia was induced in the examined limb by inflating the cuff on the upper arm to a pressure of 50 mmHg higher than the baseline systolic pressure of the participant under study for a period of 5 minutes.
11. After the cuff is deflated, the automatic compressor immediately starts with intermittent occlusion at 50 mmHg for another 5 minutes where the pressure is kept at the same level as the baseline pressure.

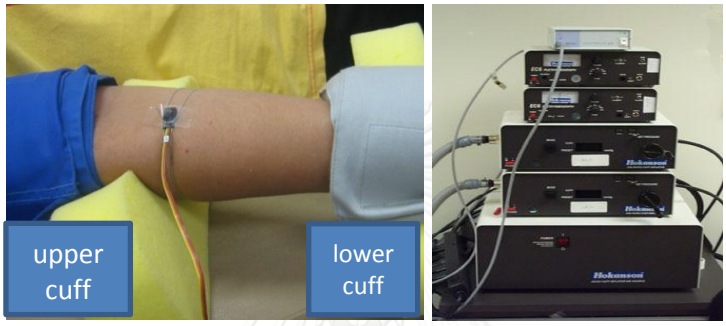


Figure 17 Venous Occlusion Plethysmography device

12. The tracings were recorded, stored, and analyzed. Maximum forearm blood flow will be obtained from the first 5 recordings in hyperemia were used to calculate area under the curve of post-hyperemia blood flow, measured after 1 minute at the end of ischemia (Figure 18) (Alomari et al., 2004)

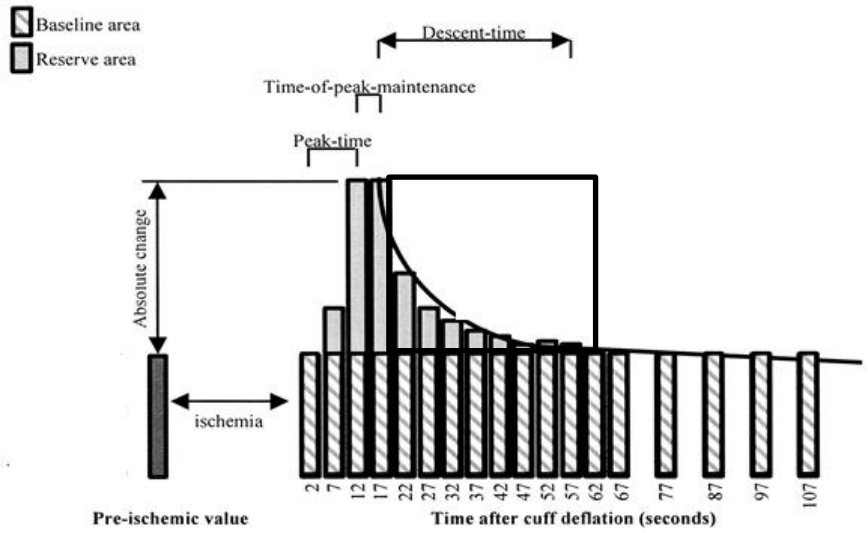


Figure 18 Area of post-hyperemia after forearm occlusion

Cardio Ankle Vascular Index (CAVI) and Ankle Brachial Index (ABI) Measurement Protocol

The participants were measured CAVI and ABI to detect arterial stiffness and arterial stenosis respectively by using a VaserVS-1000 vascular screening system (Fukuda,® Japan).

1. Before the testing, the participants were advised to refrain from caffeine, alcohol, and heavy activities.
2. The testing was performed in a quiet, warm environment to prevent vasoconstriction of the arteries (21-23$\pm$1°C). Remove socks, shoes, and tight clothing to permit placement of pressure cuffs and access to pulse sites by Doppler.
3. Place the participant in a flat, supine position and place pressure cuffs at the bottom of the cuff approximately 2-3 cm above the cubital fossa on the arm and malleolus at the ankle.

4. ECG electrodes were placed on both wrists. A microphone was placed to detect heart sounds on the sternum.
5. Ensure the participant's comfort and rest for a minimum of 5 minutes prior to the test to allow heart rate and pressure to normalize.
6. After automatic measurements, data was analyzed by software resulting in the automatic value of CAVI. Right CAVI and right ABI for analysis were chosen for the analysis.
7. The procedure and device shown in Figure 19

Figure 19 Cardio Ankle Vascular Index (CAVI), Ankle Brachial Index (ABI) device and measurement

Cardiopulmonary fitness measurement

Measurement of Peak Oxygen Consumption (VO_{2peak})

VO_{2peak} of each participant was measured during the incremental exercise test by using oxygen and carbon dioxide gas analyzer (Oxycon, USA). Oxygen consumption (VO_2), Carbon dioxide production (VCO_2), minute ventilation (VE), and other derived parameter were continuously monitored breath-by-breath by a computerized system (Oxycon, USA). Data were expressed in a standard condition at standard temperature pressure dry (STPD). The Naughton's protocol for treadmill was used in the exercise test (Figure 20).

VO_{2peak} criteria as follows;

1) the highest value of VO_2 attain while conducting the test

Criteria to stop testing as follows;

1. cannot tolerate further testing
2. uncomfortable to test
3. show signs of low cardiac output and complications after testing such as chest pain, cyanosis, loss of conscious

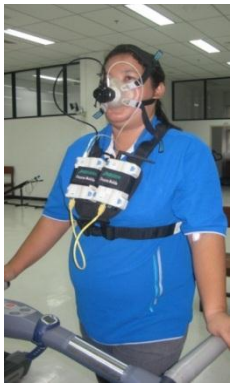


Figure 20 A Peak Oxygen Consumption measurement and device

The Incremental treadmill by Naughton's protocol adjusted speed and grade every two minutes was used to perform VO_{2peak} testing (Table 2). The heart rate and rate of perceive exertion (RPE) was continuously monitored during exercise to ensure safety and the investigator team was certified with basic life support for the emergency case.

Table 2 Naughton's protocol for treadmill testing

Stage	Time (min)	Speed (mph)	Grade (%)	METs
Rest	00.00	1.0	0.0	1.8
1	02.00	1.0	0.0	1.8
2	02.00	2.0	0.0	2.5
3	02.00	2.0	3.5	3.5
4	02.00	2.0	7.0	4.4
5	02.00	2.0	10.5	5.4
6	02.00	2.0	14.0	6.4
7	02.00	2.0	17.5	7.3
8	02.00	2.0	21.0	8.3
9	02.00	2.0	24.5	9.2
10	02.00	2.0	25.0	9.4

Moderate Intensity Exercise

In the participants who have been allocated to the moderate intensity exercise group performed brisk walking on treadmill at 70-75%of VO_{2peak} for 1 hour (warm-up 10 minutes, exercise 40 minutes, and cool down 10 minute) by using the treadmill. The heart rate and RPE were continuously monitored during exercise for safety and to ensure that the subject exercise at the intended intensity, shown in Table 3.

Table 3 Moderate Intensity Exercise Protocol

week/ visit	Activity	Duration	rational
1/1	- introduction to exercise protocol, exercise instrument, and self-monitoring during exercise training	10 min	Preparation for exercise training, instrument, and self- monitoring of exercise program
	- instruction of treadmill demonstration	5 min	
	- brisk walking at 50%VO_{2peak}	30 min	
1/2	- brisk walking at 50%VO_{2peak}	30 min	
1/3	- brisk walking at 50%VO_{2peak}	30 min	
1/4	- brisk walking at 50%VO_{2peak}	40 min	
2/1	- brisk walking at 60%VO_{2peak}	30 min	Increase intensity to be adjusted to higher intensity of exercise
2/2	- brisk walking at 60%VO_{2peak}	30 min	
2/3	- brisk walking at 60%VO_{2peak}	40 min	
2/4	- brisk walking at 60%VO_{2peak}	40 min	
3/1	- brisk walking at 70%VO_{2peak}	30 min	Increase intensity to be adjusted to higher intensity of exercise
3/2	- brisk walking at 70%VO_{2peak}	30 min	
3/3	- brisk walking at 75%VO_{2peak}	30 min	
3/4	- brisk walking at 75%VO_{2peak}	30 min	
week 4-15	- warm up	10 min	Maintain intensity and increase exercise duration
	- walking at 70-75%VO_{2peak}	40 min	
	- cool down	10 min	

*week 1-3 of the training program allow participants to prepare and become familiar with protocol and adhere to the program

Qigong Group

Qigong group performed Guang-Im-Ju-Jai-Gong Qigong and the training program consist of three parts; 1) 500 repetitive arm swings (backward and forward), 2) 18 postures of body movements, and 3) 3 sets of finger movements at standing position. Qigong was performed for 1 hour per day, 4 days a week, and for a total of 12 weeks in a quiet and good ventilation environment without air conditioning. During those trainings, the participants concentrated on inspiration and expiration of breathing, relaxation, and keep the body alignment at standing position. Between each part of training, the participants performed energy storage postures in lower Tan Tien site, 3 finger base lower of the umbilical, by male put the right hand on the left hand and female put the left hand on the left hand. After that, turn both hands 36 rounds clockwise and 36 rounds counter-clockwise respectively. The heart rate and RPE was continuously monitored during exercise for safety and ensure that the subject exercise at the intended intensity, shown in Table 4

Table 4 Guang-Im-Ju-Jai-Gong Qigong Protocol

week/ visit	Activity	Duration	Rational
1/1-4	- introduction to Qigong protocol, and self-monitoring during Qigong training	10 min	Preparation for exercise training, instrument, and self-monitoring of Exercise program
	- practice standing posture and keep body alignment of Qigong	10min	
	- practice arm movements	10 min	
	- practice finger movements	20 min	
2/1-4	- 500 repetitive arm swings	10 min	Gradually increase postures then duration
	- 10 postures of body movement	20 min	
	- 1 set of finger movement	10 min	
4-15	- 500 repetitive arm swings	10 min	
	- 18 postures of body movement	30 min	
	- 3 sets of finger movement	10 min	

*week 1-3 of the training program allow participants to prepare and be familiar with protocol and adhere to the program

Lipoprotein associated phospholipase A2 assay (ELISA kit)

This assay employs the quantitative sandwich enzyme immunoassay technique (Figure 21). A monoclonal antibody specific for PLA2G7 has been pre-coated onto a microplate. Both Standard and samples are pipetted into the well. Any unbound substance, an enzyme-linked polyclonal antibody specific for PLA2G7 is added to the wells. After that, a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. In the initial step, color will develop in proportion to the amount of PLA2G7 bound. The intensity of the color is measured when the color development stops.

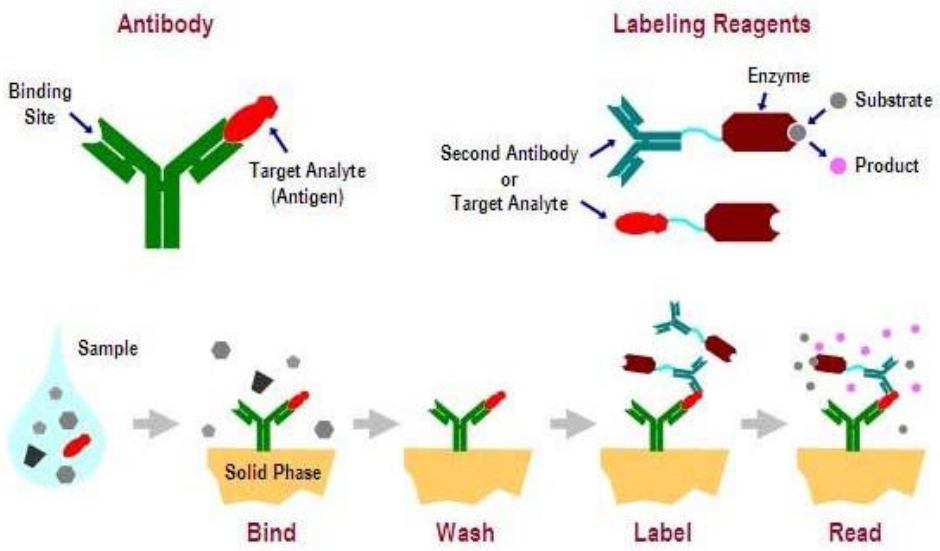


Figure 21 The principle of ELISA technique (Koivunen, 2006)

Sample collection, storage, and sample preparation for Lp-PLA_2

Using EDTA as anticoagulant, plasma was collected then centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay is done immediately or aliquot and store samples at -80ºC. Cautiously avoid repeated freeze-thaw cycles. 20-fold dilution prior to assay is required of plasma samples.

PLA2G7 Standard

Reconstitute the PLA2G7 standard with 1.0 mL calibrator diluent RD5-17 (1x). This reconstitution produces a stock solution of 100 ng/mL. After that, use the stock solution to produce a dilution series as shown in Figure 22

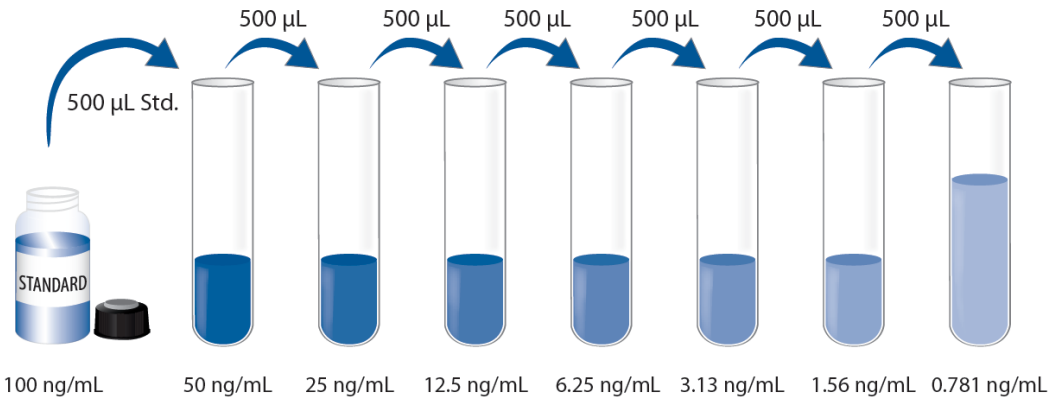


Figure 22 Standard Preparations for Lp-PLA_2

Assay procedure

1. Add 100 µL of assay diluent RD1-9 each well
2. Add 50 µL of standard and sample per well. At room temperature, cover and incubate for the duration of 2 hours on a horizontal orbital microplate shaker.
3. Aspirate each well and wash, repeat the process three times for a total four washes with wash buffer.
4. Add 200 µL of PLA2G7 conjugate to each well and incubate for 2 hours at room temperature on the shaker.
5. Repeat the aspiration and wash again.
6. Add 200 µL of substrate solution to each well, protect from light, and incubate for 30 minutes at room temperature on the benchtop.
7. Add 50 µL of stop solution to each well. The color in the wells change from blue to yellow.
8. Determine the optical density of each well within 30 minutes by using a microplate reader set to 450 nm.

Malondialdehyde (MDA) assay

This assay employs oxidizing agents which can alter lipid structure, creating lipid peroxides that result in the formation of malondialdehyde (MDA). This therefore can be measured as Thiobarbituric acid reactive substances (TBARS). Lipids that are multi-unsaturated are both most likely to form peroxides and the most reactive in the TBAR assay. Free MDA is typically quite low, requiring release of MDA by acid treatment of protein and breakdown of peroxides by heat and acid to facilitate color development in the TBAR reaction. Removal of protein by precipitation eliminates potentially interfering amino acids that may react with thiobarbituric acid (Shen, Chen, & Wang, 2007).

In the presence of heat and acid, MDA react with TBA to produce a colored and product that absorbs light Figure 23. The intensity of the color at 532 nm corresponds to the level of lipid peroxidation in the sample. Unknown samples are compared to the standard curve.

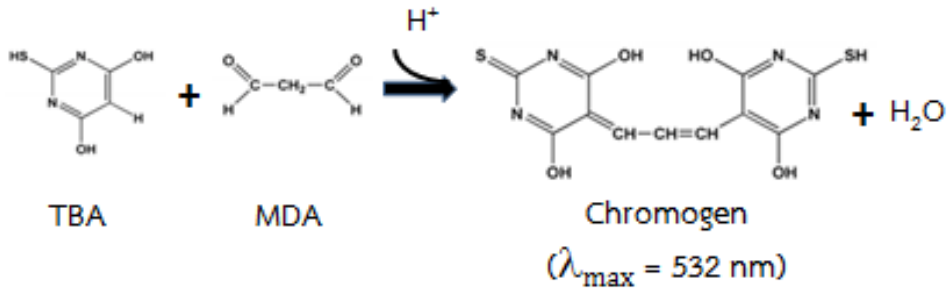


Figure 23 The principle of MDA assay

Sample collection, storage, and sample preparation for MDA

Plasma was collected by using EDTA as an anticoagulant then centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay is done immediately or aliquot and store samples at -80ºC. Avoid repeated freeze-thaw cycles.

Acid treatment of all samples is required prior to assay to clarify the samples by precipitating interfering proteins and other substances for removal by centrifugation. Following the protocol, it catalyzes the TBARS reaction.

1. Add 300 µL of sample and 600 µL cold TCA (0.7 mol/L) to a micro-centrifuge tube and mix well.
2. Incubate for 15 minutes at room temperature
3. Centrifuge at ≥12,000 x g for 4 minutes
4. Carefully remove and retain the supernatant

   MDA standard: Reconstitute the MDA standard with a stock solution of 30 µM of MDA. Then use the stock solution to produce a dilution series follow Figure (3.10). Add 1 mL of cold TCA (0.7 mol/L) with 0.5 mL of standard MDA then mix well and used this solution for determine standard with used PBS buffer for the diluent.

.

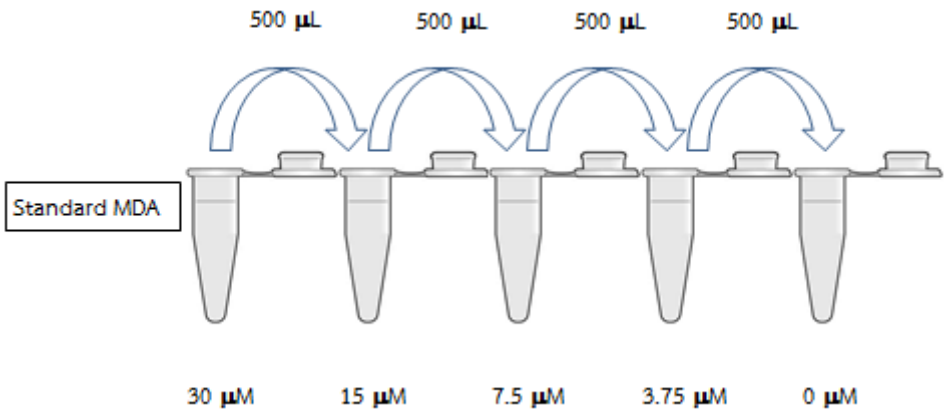


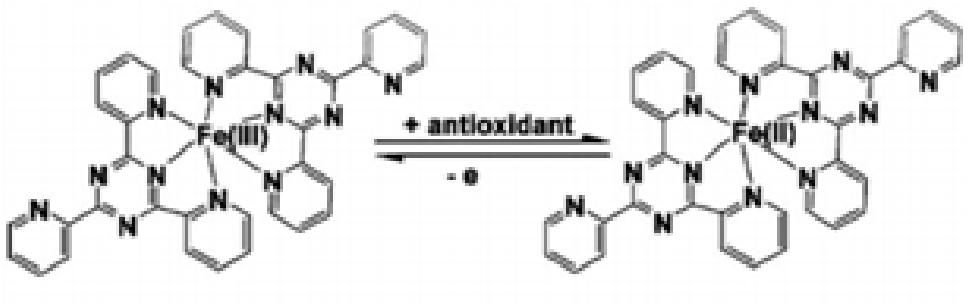
Figure 24 Standard preparation of MDA

Assay procedure

1. Add 600 µL of MDA standard or supernatant of plasma with 600 µL TBA (46.8 mM)
2. Boil with dry bath at 95 ºC for 10 minutes (the color of standard changes to pink).
3. Determine the optical density of each well within 30 minutes by using a microplate reader set to 532 nm.

Ferric reducing antioxidant power (FRAP) assay

The Ferric reducing antioxidant power (FRAP) assay measures the antioxidant capacity. In a redox-link colorimetric method, FRAP assay use antioxidants as reductants. Ferric tripyridyl triazine (Fe III TPTZ) complex reduces to ferrous form (Fe III to Fe II) ion formation at low pH. The absorbance change is straightforwardly related to the combination or total reducing power of the electrodonating antioxidants present in the reaction mixture (Figure 25) (Benzie & Strain, 1996).



$[Fe(III)(TPTZ)_2]^{3+}$ $[Fe(II)(TPTZ)_2]^{2+}$

(λ_{max} = 593 nm)

Figure 25 The principle of FRAP assay

Sample collection, storage, and sample preparation for FRAP

Using EDTA as an anticoagulant, plasma was collected and then centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay was done immediately or aliquot and store samples at -80ºC. Avoid repeated freeze-thaw cycles.

Preapration FRAP reagent by following this protocol:

1. Sodium acetate buffer solution (0.3M, pH3.6) by mixture of 0.15 g of sodium acetate trihydrate with acetic acid 800 µl and added DW until total solution to 50 mL
2. 10 mM 2,4,6-tripyridyl-s-striazine (TPTZ) by adding 10 mL of 40 mM HCl with 31.23 mg of TPTZ
3. 20 mM Ferric chloride ($FeCl_3.6H_2O$) by adding 10 mL of DW with 32.44 mg of $FeCl_3$
4. Mix FRAP reagent by using solution ratio 10:1:1 (Sodium acetate buffer solution: TPTZ: $FeCl_3$)

FRAP Standard: Reconstitute the FRAP standard with a stock solution of 2000 μM of $FeSO_4.7H_2O$ (mix 5.56 mg of $FeSO_4$ with 10 mL DW) And then use the stock solution to produce a dilution series with 500 μl of DW followed Figure 26

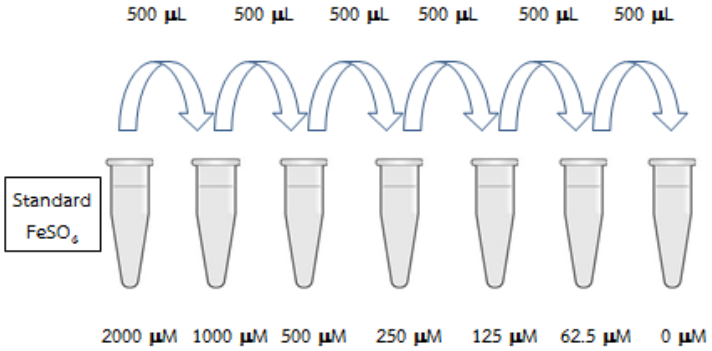


Figure 26 Standard preparation of $FeSO_4$ for FRAP

Assay procedure

1. Add 10 μL of $FeSO_4$ standard or 10 μL to sample with 90 μL FRAP reagent
2. At room temperature, incubate with light protection for 30 minutes
3. Determine the optical density of each well within 30 minutes by using a microplate reader set to 595 nm

Venue

All of participants were collected blood sample, evaluated physical fitness, endothelial function, and arterial stiffness/stenosis at Sports Medicine Laboratory on the 4th floor of Patthayaput building, Faculty of Medicine, Chulalongkorn University. During the training period, they performed at several sites including Health Promotion Center, Faculty of Nursing, Thammasat University, Public Health Center Bangsue District, and Sports Medicine Laboratory on the 4th floor of Patthayaput building, Faculty of Medicine, Chulalongkorn University.

Data Analysis

Average values and standard deviations were computed. The distribution of data was tested to determine if it was normal distribution or not. Normal distribution was used to repeat the measurement of ANOVA to compare the value between groups. Post Hoc analysis was used to compare the difference at time point.

All data were analyzed using the Statistical Package for Social Science (SPSS). Difference at significant level of $p<0.05$ were considered significant.

CHAPTER IV
RESULTS

A total of 127 participants were eligible for this study. All of they were recruited from the Bangkok and perimeter by advertisement posted in poster, social media. And after assess eligibility, 36 of the participants were refused consent study. Ninety one participants were randomized to group allocated. In control group, four participant lost follow up in 4 week. In Qigong, two participant lost follow up in 4 week of exercise session. And for Moderate Intensity Exercise (MIE), two participant lost follow in 4 week. Only 83 participants completed the study. The consort flow diagram as showed in Figure 27

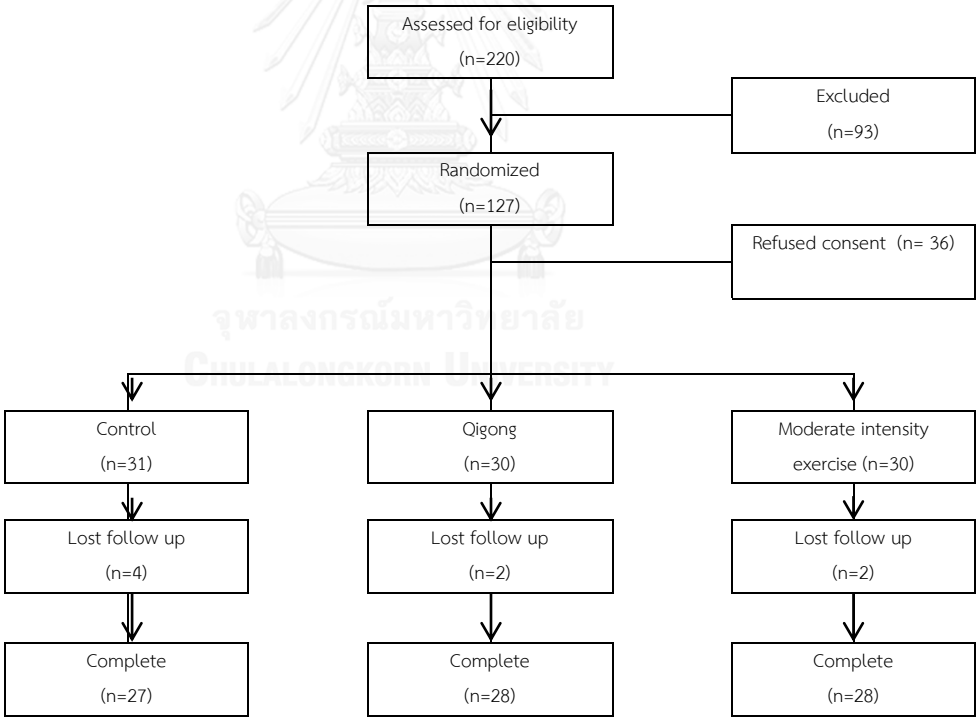


Figure 27 The consort flow diagram of study

The baseline characteristics data of control, QE, MIE group such as body mass index (BMI), wrist circumference (WC), fat mass, muscle mass, blood pressure, fasting plasma glucose, triglyceride, high density lipoprotein (HDL-C), Cardio Ankle Vascular Index (CAVI) , and Ankle Brachial Index (ABI) were showed in Table 4.1. The average age of control, Qigong, and MIE were 53.44±3.18, 51±3.18, and 48.18±5.93 years, respectively, all of baseline characteristics data no significant difference between group (Table 5).

Table 5 Participants Characteristic Data

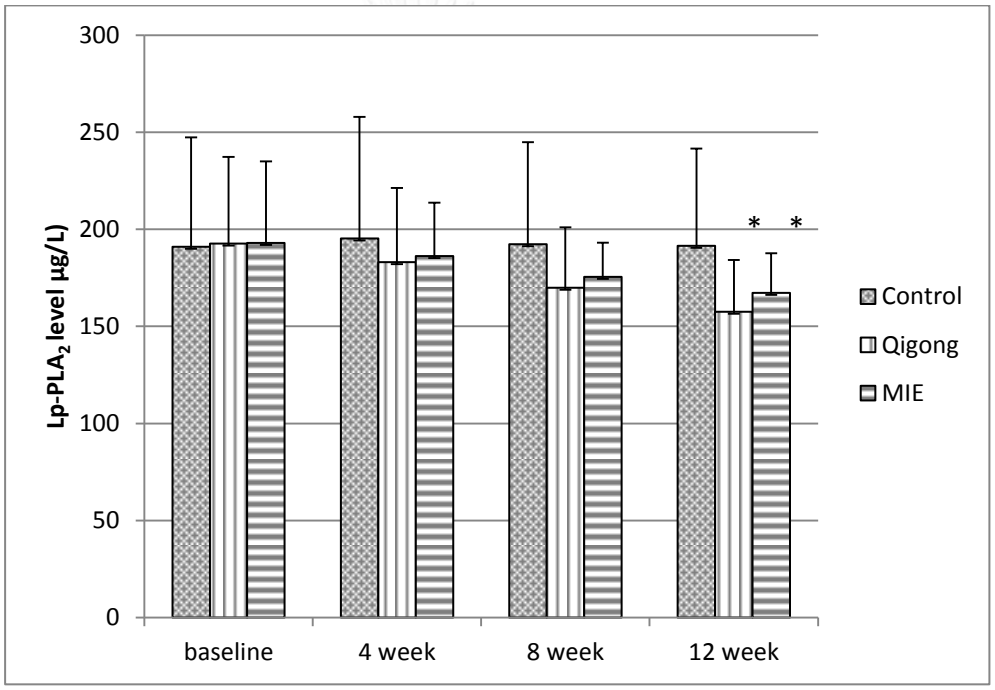
Characteristic	Control (n=27)	Qigong (n=28)	MIE (n=28)
Age (years)	52.56±2.56	51±2.56	48.178 ±5.93
BMI (kg/m^2)	26.24±5.55	25.96±4.17	28.30±3.24
WC (cm.)	88.18±8.51	89.17±10.43	91.64±5.43
SBP (mmHg)	134.26±10.30	134.89±15.73	131.18±9.53
DBP (mmHg)	81.04±18.06	79.04±10.19	80.21±9.48
FPG (mg%)	86.57±14.08	89.96±13.89	80.64±9.75
Triglyceride (mg%)	173.96±28.46	173.89±41.62	171.44±25.23
HDL-C (mg%)	47.64±5.19	46.14±8.48	45.29±5.81
Fat mass (kg)	23.11±8.53	23.66±7.50	26.22±6.65
Muscle mass (kg)	21.35±3.25	21.75±3.94	23.97±3.40
CAVI	7.73±1.15	7.45±1.2	6.69±1.26
ABI	1.06±0.06	1.04±0.10	1.00±0.08

Data were expressed as mean±SD

The average attention rate in both groups was 90%, ranging from 80% to 100% of training program.

The Effects of Qigong and Moderate intensity exercise on Lipoprotein associated phospholipase A_2 ($Lp\text{-}PLA_2$)

Effect of training program on $Lp\text{-}PLA_2$ was determined at baseline, 4th week, 8th week, and 12th week in all subjects. The result showed that in Qigong, significant decreased when compared with control group at 12th week (157.53±26.57 vs. 191.39±50.23 μg/L) ($p<0.05$). Similarly with moderate intensity exercise, significant reduced when compared with control group at 12th week (167.25±20.37 vs. 191.39±50.23 μg/L) ($p<0.05$) (Figure 28).



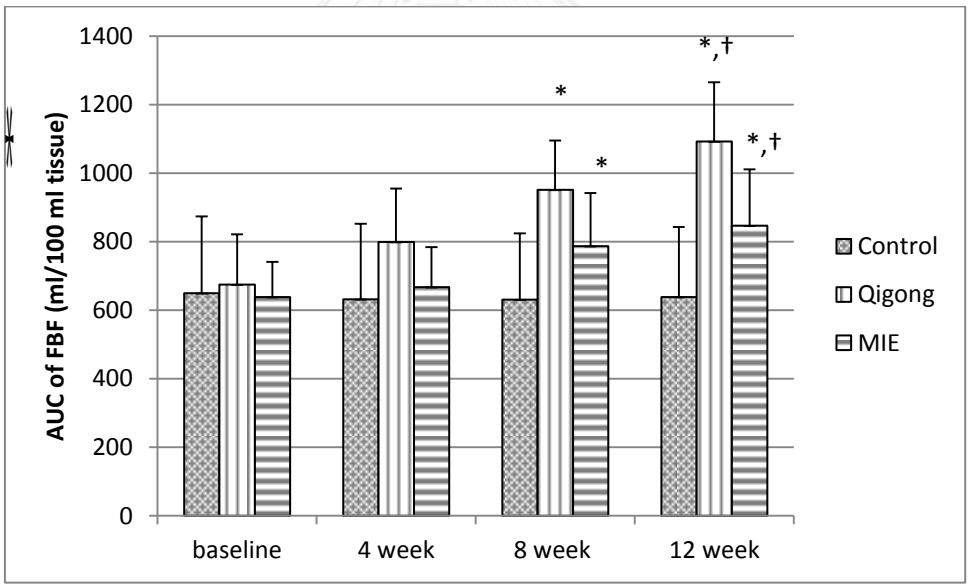
Significant difference by repeated measure ANOVA

* $p<0.05$ compared with control

Figure 28 The comparison of $Lp\text{-}PLA_2$ (μg/L) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

The Effects of Qigong and Moderate intensity exercise on area under the curve (AUC) of forearm blood flow (FBF)

Comparison the area under the curve (AUC) of FBF, the results showed that significant difference increased of AUC in Qigong group when compared with control group at 8th week (951.16±144.19 vs. 630.97±193.24 ml/100 ml tissue) and 12th week (1092.07±172.79 vs. 638.61±203.81 ml/100 ml tissue) ($p<0.05$). For moderate intensity exercise group, significant increased when compare control at 8th week (786.57±155.58 vs. 630.97±193.24 ml/100 ml tissue) and 12th week (846.47±164.14 vs. 638.61±203.81 ml/100 ml tissue) ($p<0.05$). Furthermore, at 12th week found the significant difference between Qigong and moderate intensity exercise (1092.07±172.79 vs. 846.47±164.14 ml/100 ml tissue) ($p<0.05$) (Figure 29).



Significant difference by repeated measure ANOVA

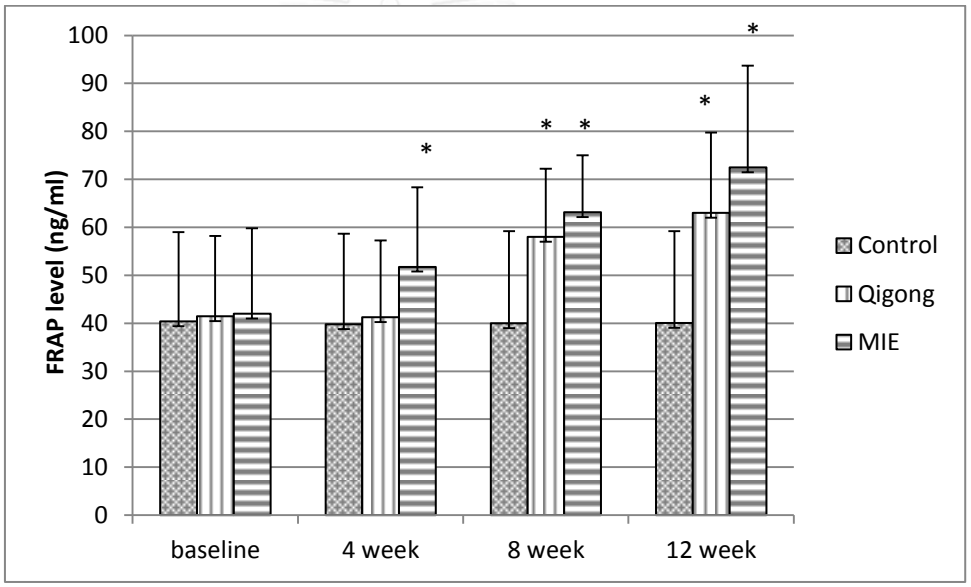
* $p<0.05$ compared with control,

†$p<0.05$ compared between Qigong and MIE

Figure 29 The comparison of FBF (ml/100 ml tissue) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise.

The Effects of Qigong and Moderate intensity exercise on Ferric reducing antioxidant power (FRAP)

The anti-oxidative capacity was demonstrated by FRAP. After 8th week and 12th week of Qigong, significant enhanced when compared with control group 58.14±14.23 vs. 39.97±19.24 (ng/L) and 63.03±16.71 vs. 40.08±19.12 (ng/L) respectively ($p<0.05$). But for moderate intensity exercise group effects on the increasing of FRAP level at 4th week (51.76±16.6 vs. 39.78±18.9 ng/L), 8th week (63.11±11.88 vs. 39.97±19.24 ng/L), and 12th week (72.45±21.26 vs. 40.08±19.12 ng/L) (Figure 30).



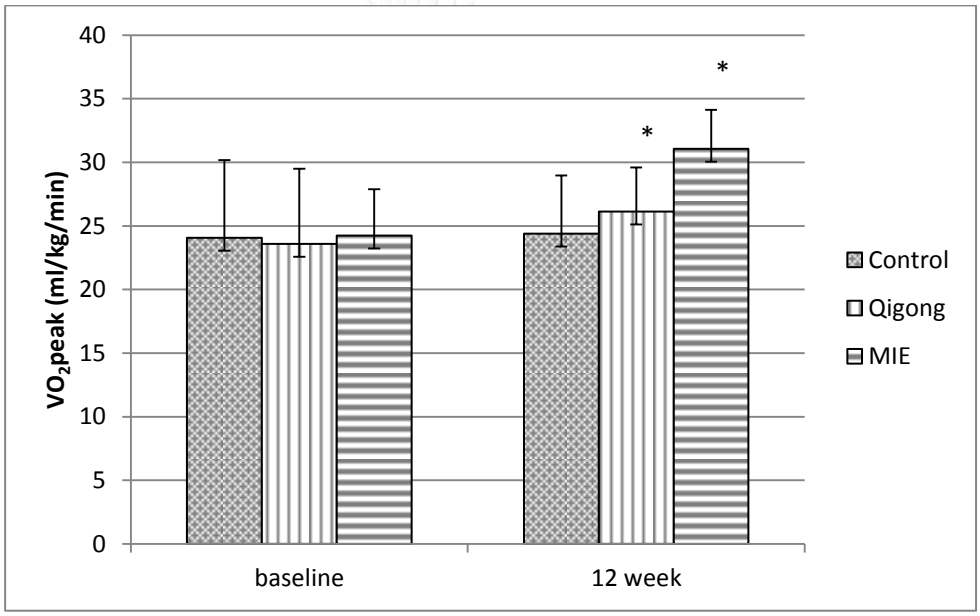
Significant difference by repeated measure ANOVA

* $p<0.05$ compared with control

Figure 30 The comparison of FRAP (ng/ml) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise.

The Effects of Qigong and Moderate intensity exercise on The Peak Oxygen Consumption (VO_{2peak})

The training effects of Qigong and moderate intensity exercise were determined by used peak oxygen consumption (VO_{2peak}). The results expressed that after 12th week training session, significant increase of VO_{2peak} ($p<0.05$) when compared with control group in both Qigong (26.12±3.49 vs. 24.39±5.91 ml/kg/min) and moderate intensity exercise group (31.05±3.06 vs. 24.39±5.91 ml/kg/min) (Figure 31).



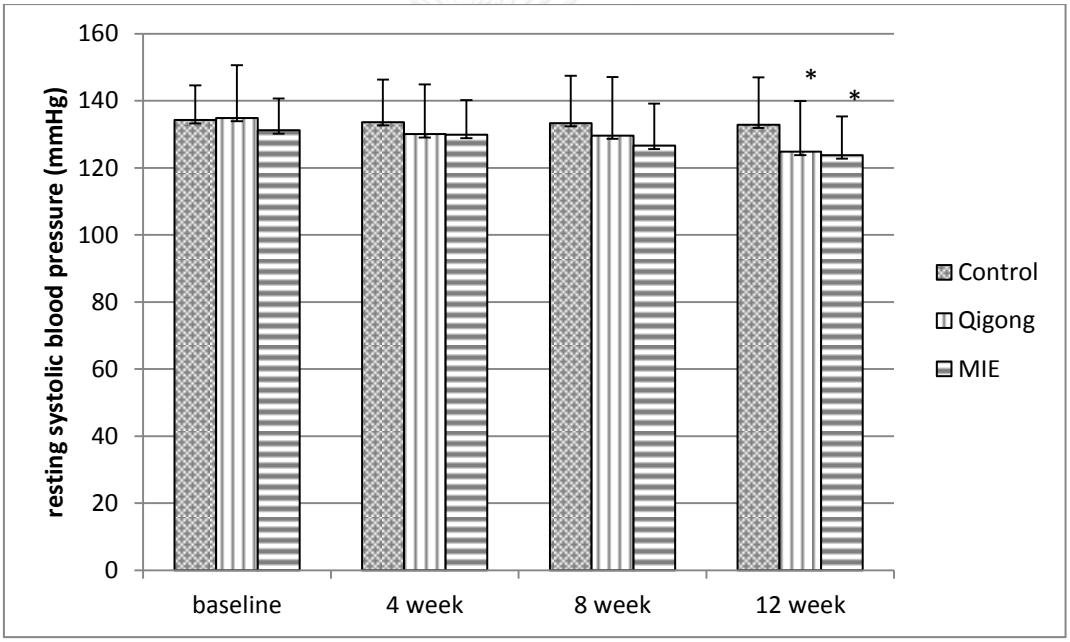
Significant difference by repeated measure ANOVA

* $p<0.05$ compared with control

Figure 31 The comparison of VO_{2peak} (ml/kg/min) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise.

The Effects of Qigong and Moderate intensity exercise on resting systolic blood pressure

Twelve week training period of Qigong and moderate intensity exercise, resting systolic blood pressure were significant difference reduced when compared with control group at 12th week, Qigong exercise 124.82±15.13 vs. 132.89±14.04 mmHg and moderate intensity exercise 123.75±11.58 vs. 132.89±14.04 mmHg (Figure 32).



Significant difference by repeated measure ANOVA

* p<0.05 compared with control

Figure 32 Comparison of resting systolic blood pressure (mmHg) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

The Effects of Qigong and Moderate intensity exercise on resting diastolic blood pressure

No significance difference on diastolic blood pressure after 12 week exercise training in both type of exercise (Figure 33).

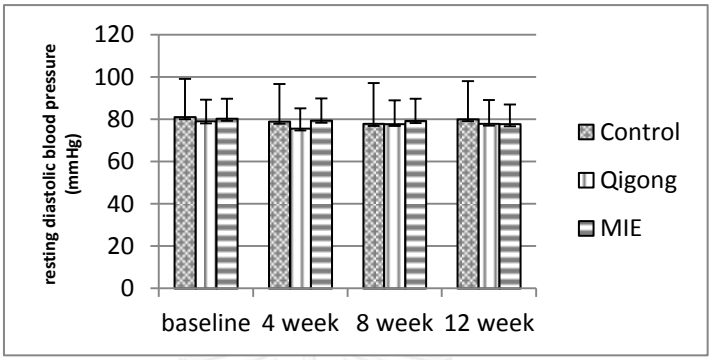


Figure 33 Comparison of resting diastolic blood pressure from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

The Effects of Qigong exercise and Moderate intensity exercise on resting heart rate

After 12 week exercise training, no significance difference on resting heart rate in both Qigong and moderate intensity exercise (Figure 34).

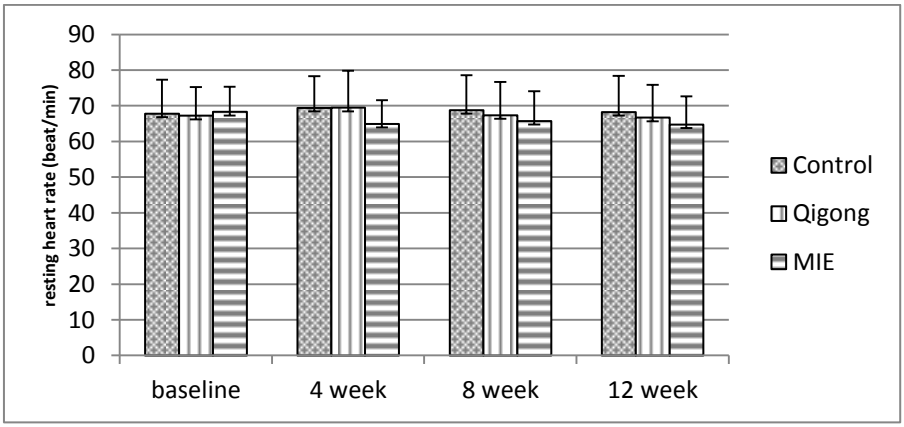


Figure 34 Comparison of resting heart rate from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

The Effects of Qigong and Moderate intensity exercise on Cardio Ankle Vascular Index (CAVI)

No significance difference with control group on CAVI after exercise training both of Qigong and moderate intensity exercise after 12 weeks of training session (Figure 35).

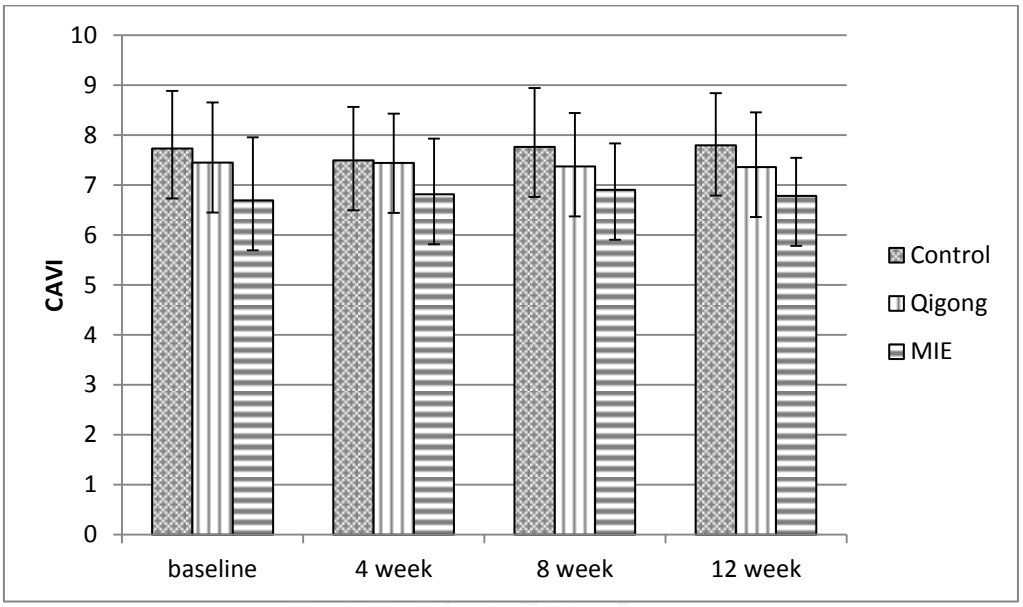


Figure 35 Comparison of Cardio Ankle Vascular Index (CAVI) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

The Effects of Qigong and Moderate intensity exercise on Ankle Brachial Index (ABI)

After 12 week of training session, the data demonstrated that no statistical significance when compared with control group on the change of ABI in both Qigong and moderate intensity exercise group (Figure 36).

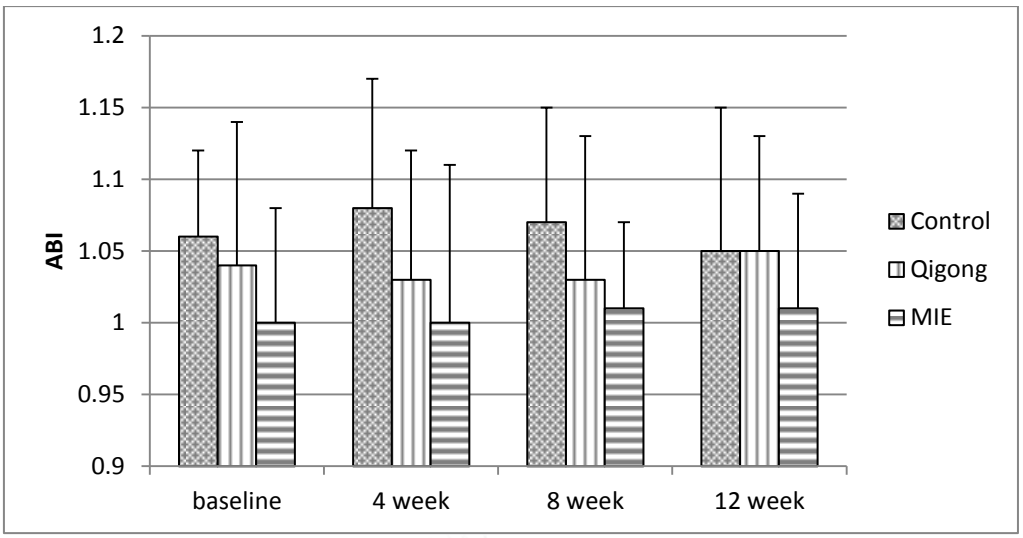


Figure 36 Comparison of ABI from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

The Effects of Qigong and Moderate intensity exercise on malondialdehyde (MDA)

Twelve months of Qigong exercise and moderate intensity exercise, no change in lipid peroxidation which demonstrated by malondialdehyde (MDA) level. The results showed in Qigong exercise 12 week, decreased MDA from 1.21±1.41 to 0.93±0.68 (ng/L), for moderate intensity exercise decreased from 11.36±1.71 to 1.02±1.00 (ng/L) no statistical difference (Figure 37).

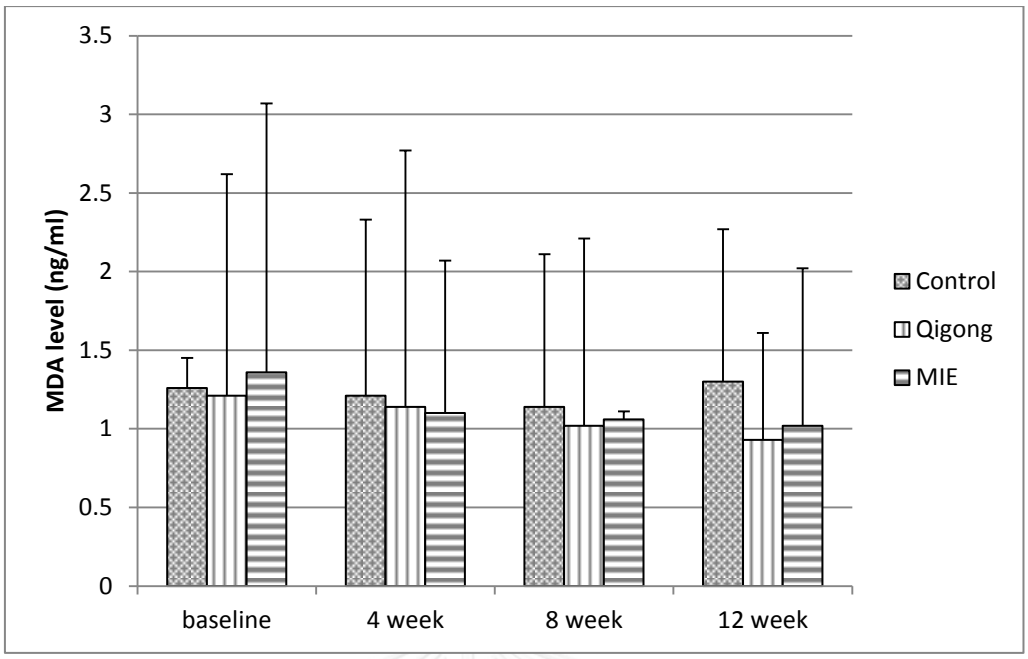


Figure 37 The Effects of Qigong and Moderate intensity exercise on malondialdehyde (MDA) (ng/L)

CHAPTER V
DISCUSSION

Metabolic syndrome (Mets) is the world health problem and developed to chronic disease such as diabetes, hypertension, coronary artery disease and stroke (Zimmet et al., 2005). The pathogenic mechanism of Mets early effects on arteries' endothelial cell and progress to dysfunction of the endothelial cell by various mechanisms but the previous study had approved that oxidative stress and inflammation mechanism were the major pathway of endothelial dysfunction (Ahirwar et al., 2015; Mahalle, Garg, Naik, & Kulkarni, 2014). National policy on resolving this problem was exercise(Tjonna et al., 2008).

Generally, the people who have Mets were invited to do the moderate intensity exercise as the first priority since it effects on the adaptation of metabolic pathway, especially lipid metabolism, a crucial pathway of metabolic syndrome. Previous study showed that Qigong, the Traditional Chinese Medicine (TCM) training, and the practice of moderate intensity aerobic exercise in the people with Mets effect on the prevention or improvement of this problem but there is no study reporting its effects on the changes of endothelial function, and Lipoprotein associated phospholipase-A_2 (Lp-PLA_2).

This study was first investigation and comparison of Qigong and moderate intensity aerobic exercise on lipoprotein associated phospholipase A_2 and endothelial function in the people with Mets. From 127 eligible participants, 36 were declined from study protocol. Ninety one participants were randomized allocated to three groups: 1) control with usual activity and health education group (n=31), 2) Qigong training group (30), and 3) moderate intensity exercise group (n=30). After 4 weeks training session, 8 participants were lost follow up (4 from control, 2 from Qigong

exercise, and 2 from moderate intensity exercise group). Thus, the data were analyzed from 83 participants.

General characteristic data

Female participants were enrolled to the study. The average age of control, Qigong, and moderate intensity exercise were 53.44±3.18, 51±3.18, and 48.18±5.93 years, respectively. The baseline characteristics data of control, Qigong, moderate intensity exercise group such as body mass index (BMI), wrist circumference (WC), blood pressure, fasting plasma glucose, triglyceride, and HDL-C, fat mass, muscle mass, Cardio (CAVI) and (ABI) were no significant difference between groups. All participants were overweight with abdominal obesity, dyslipidemia, pre-hypertensive stage, and high fat mass.

Effect of Moderate intensity exercise and Qigong on functional aerobic capacity

This study determines training effects between moderate intensity exercise and Qigong with peak oxygen consumption (VO_{2peak}). After 12 week of training period, VO_{2peak} were increased in both training when compared with control group. The finding showed that moderate intensity exercise and Qigong achieved the enhancement of functional aerobic capacity of cardiorespiratory system in Mets. Similarly with the previous studies found that moderate intensity exercise at 19 km/wk were adequate to increased aerobic fitness in overweight men and women with mind-to-moderate dyslipidemia (Duscha et al., 2005). The other studies showed that three months of moderate-intensity exercise in patients with coronary artery disease, the Mets complication, can improvement the VO_{2peak} and after 16 week of resistance and endurance training in sedentary men, the results showed increased VO_{2peak} in both group but in endurance training better than resistance exercise (Libardi, De Souza, Cavaglieri, Madruga, & Chacon-Mikahil, 2012). Similarly with this study which revealed that there was the increased $VO_{2\ peak}$ after 12 weeks of training

in the Mets group. Although Qigong has been defined as a low intensity training, this study showed its effects on increasing the VO_{2peak} as same as moderate intensity exercise (Murton & Greenhaff, 2013). The explanation of this findings focused on the isometric and isotonic contraction of legs' gross motor, the type I fibers (slow oxidative muscle), during practice Qigong in standing position with upper limbs movement. The effects of this Qigong practice result as same as resistance training which can also increase VO_{2peak}.

The effect of Qigong and moderate intensity exercise on Ferric reducing antioxidant power (FRAP)

The result found that both of the Qigong increased FRAP level at 8th week, and 12th week and moderate intensity exercise increased FRAP level at 4th week, 8th week, and 12th week when compared with control group. In the metabolic syndrome group, the previous data showed only the increasing of malondialdehyde (MDA) levels while ferric reducing ability of plasma (FRAP) were significantly decreased (Powers, Ji, & Leeuwenburgh, 1999). In conclusion, the people with metabolic syndrome presence the oxidative stress and decrease anti-oxidant capacity to protect cardiovascular risk. The effects of exercise on oxidative stress were demonstrated in many studies. For example, the regular endurance exercise resulting in skeletal muscle antioxidant adaptation and prevents extensive cellular damage from cellular oxidants by increased metabolic rate during skeletal muscle contraction (Otocka-Kmiecik, Lewandowski, Szkudlarek, Nowak, & Orlowska-Majdak, 2014; Souza-Junior et al., 2014). The anti-oxidant capacity actions to protect injury from ROS at the time of exercise and immediately after exercise.

The data of this study showed Qigong and moderate intensity exercise effects on adaptation of function aerobic capacity (increased VO_{2peak}). The effects of both type of training trigger anti-oxidative capacity in metabolic syndrome group and

reduced oxidative stress stage. In this study, the training program did not effect on increased lipid peroxidation or ROS but enhanced only anti-oxidative capacity. In conclusion, the moderate intensity exercise and Qigong effects on the adaptation in the skeletal muscle antioxidant, the moderate and low intensity, and dynamic muscle movement improve the anti-oxidative capacity in metabolic syndrome.

The effect of Qigong and moderate intensity exercise on Lp-PLA_2

The results revealed that the two groups of training had statistically significant reduced the level of Lp-PLA_2 compared with control group at 12^{th} week. The result from previous study demonstrated the significantly reduced the Lp-PLA_2 after 4 months of aerobic physical activity and diet control (Tzotzas et al., 2008). Other study showed the relation of Lp-PLA_2 level with oxidative stress level such as a case-control study, investigated the association of Lp-PLA_2 activity in coronary artery disease and markers of oxidative stress in 799 patients with coronary artery disease and 925 healthy controls and found that Lp-PLA_2 activity significantly higher in coronary artery disease cases than healthy control(Kim et al., 2008). The elevation of Lp-PLA_2 activity level associated with the oxidative stress biomarker, urinary excretion concentrations of 8-epi-prostaglandin $F_{2\alpha}$(8-epi-$PGF_{2\alpha}$). Thus, exercises had the effects on the reduction of Lp-PLA_2 and the oxidative stress level affects the Lp-PLA2 level. Similary with this study, Qigong and moderate intensity exercise resulted in functional capacity improvement and developed adaptation after exercise in terms of increasing the anti-oxidative capacity and decreasing the oxidative stress of the body that effects on decrease the Lp-PLA_2 level.

The effect of Qigong and moderate intensity exercise on the endothelial function

Qigong and moderate intensity exercise resulted in improving the area under the curve (AUC) of forearm blood flow (FBF) compared with baseline and control group. Both of the Qigong and moderate exercise group showed the increased FBF at 8^{th} week, and 12^{th} week of training period. In this study found the effects of training on increasing the anti-oxidant capacity to improve oxidative stress in metabolic syndrome. Similarly with the study of Edward in year 2004 investigated the training effects on oxidative stress and endothelial function and found that after 12 weeks of standard cardiac rehabilitation, the endothelial function, oxidative stress, and antioxidant of the patients with coronary artery disease were improved. The results showed the brachial artery flow-mediated dilation from 7.9% at baseline to 11.1% at 12 week and related with increased plasma nitrite and nitrate levels, increased plasma superoxide dismutase activity and decreased oxidative stress (Edwards et al., 2004). In addition, the results also demonstrated the effects of difference intensity of exercise on endothelium-dependent vasodilation. In human found that before and after difference intensity of exercise (mild, moderate, high, bicycle ergometers, 30 minutes, 5 to 7 times per week for 12 week) in 26 healthy young man , moderate intensity exercise, significantly augmented endothelial-dependent vasodilation (Goto et al., 2003). For endothelial function, nitric oxide (NO) is a bioavailability to control vascular tone. Exercise training showed the improvement of endothelial function in the people with hypertension who have endothelial dysfunction. The findings suggested that endothelial dysfunction in hypertension is reversible (Niebauer & Cooke, 1996). Thus, lifestyle modifications including exercise are expected to prevent cardiovascular complications through an augmentation of endothelial function in hypertension patients. Through the exercise, there was the increasing of NO production and decreasing NO inactivation. Exercise induced the increasing of blood

flow which appear to have the direct effects on vascular function and structure. The flow enhances endothelium dependent vasodilation by increasing the vascular expression of NO synthase and by enhancing the NO release and prostacyclin. Both of the NO and prostacyclin inhibit the multiple processes involved in atherogenesis.

In conclusion, the exercise effects on the improved endothelial function by stimulated the nitric oxide production. The moderate intensity exercise had the beneficial effects to improve the endothelial function and the increasing of endothelial function related with oxidative stress status.

In this study found Qigong and moderate intensity exercise effects on increased aerobic functional capacity and enhanced anti-oxidative level in metabolic syndrome. The improvement of anti-oxidative capacity related with the decreased of Lp-PLA_2 the importance bio-marker of vascular inflammation. Moreover, the increased of anti-oxidative capacity and decreased of Lp-PLA_2 level effects on increased of endothelial function in metabolic. These changes affected the decreasing of the resting systolic blood pressure resulted as there was the reduction of vascular resistance.

The explanations of the training effects on the decreased resting systolic blood pressure from this study were as follows. One was the moderate intensity exercise, the endurance training, effecting on the reduction of blood pressure. The meta-analyses of randomized controlled trials on the effects of chronic dynamic aerobic endurance training on blood pressure demonstrated that aerobic endurance training can decrease blood pressure through a reduction of vascular resistance and sympathetic nervous system (Inder et al., 2015) and the renin-angiotensin system appear to be involved, and favorably affects concomitant cardiovascular risk factor (Hsu et al., 2015). The study of effects of endurance moderate aerobic exercise

training on the decreased blood pressure concluded that a reduction in systemic vascular resistance, in which the sympathetic nervous system and the renin-angiotensin system appear to be involved (Cornelissen & Fagard, 2005).

In Qigong training, not only the effect of physical training but also the mindfulness occurring from meditation, relaxation, and deep breathing component were effected on the induction of function of parasympathetic nervous system (Jerath et al., 2006) and the depletion of stress hormone (Ryu et al., 1996; Sousa et al., 2012). The systematic review of Qigong the reducing of blood pressure showed, after Qigong training lowered systolic blood pressure (Xiong, Wang, Li, & Zhang, 2015). The study of meridian theory of TCM found that during Qigong practice, there was the effects on Qi circulation follow the major meridian of the body (Chang, 2015). Each of the meridian points enhanced the blood circulation including capillary, artery, venous and it resulted in the vasodilation. Thus, the total peripheral resistance were decreased and presented the decreased of the blood pressure (Zhang et al., 2015).

In addition, this study demonstrated that the traning effects resulted in the endothelial function improvement. Consequently, the depletion of vascular resistance effected to the decreased blood pressure. Moreover, this study found that there was the reduced of the stress hormone and decreased the sympathetic activity in the Qigong training group and resulted in the elevation of the blood pressure. This might be from the body and mind training during Qigong practice differently from the moderate intensity exercise.

This study were demonstrated effect of Qigong and moderate intensity exercise on CAVI and ABI in metabolic syndrome, the results found that no difference of CAVI and ABI after 12 week of training. The results showed normal CAVI and ABI in

this study population at the baseline evaluation and may be the intensity and duration of training program not effected on the change of the elastic property of vessel, in the previous study investigated effects of body weight lost program on CAVI showed improved CAVI related with body weight lost(Nagayama et al., 2013), but in this study not change of body weight may be effects of non CAVI change.

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APPENDIX

APPENDIX A

Comparison of Lp-PLA_2 (μg/L) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

group / time	control (n=21)	Qigong (n=25)	MIE (n=28)
Baseline	191.00±56.39	192.65±44.55	192.93±42.04
4 week	195.29±62.62	183.00±38.35	186.14±27.59
8 week	192.33±52.48	169.80±31.15	175.38±17.67
12 week	191.39±50.23	157.53±26.57*	167.25±20.37*

Data were express as mean±SD,

significant difference by repeated measure ANOVA

* $p < 0.05$ compared with control group

Comparison of Area under the curve (AUC) of FBF (ml/100ml tissue) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

group / Time	Control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	649.63±223.95	674.43±146.49	638.13±102.55
4 week	631.67±220.12	799.10±155.68	667.04±117.01
8 week	630.97±193.24	951.16±144.19*	786.57±155.58*
12 week	638.61±203.81	1092.07±172.79*,†	846.47±164.14*,†

Data were express as mean±SD

significant difference by repeated measure ANOVA

* $p < 0.05$ compared with control group

† $p < 0.05$ compared between Qigong and moderate intensity exercise

Ferric reducing antioxidant power (FRAP) (ng/L) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

group / time	control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	40.41±18.62	41.48±16.72	41.98±17.82
4 week	39.78±18.9	41.25±16	51.76±16.6*
8 week	39.97±19.24	58±14.23*	63.11±11.88*
12 week	40.08±19.12	63.03±16.71*	72.45±21.26*

Data were expressed as mean±SD

Significant difference by repeated measure ANOVA

* $p < 0.05$ compared with control

The Effects of Qigong and Moderate intensity exercise on The Peak Oxygen Consumption (VO_{2peak})

group / time	Control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	24.06±6.1	23.58±4.57	24.23±3.66
12 week	24.39±5.91	26.12±3.49	31.05±3.06

Data were expressed as mean±SD

Significant difference by repeated measure ANOVA

* $p < 0.05$ compared with control

Comparison of Cardio Ankle Vascular Index (CAVI) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

group / time	Control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	7.73±1.15	7.45±1.2	6.69±1.26
4 week	7.49±1.07	7.44±0.99	6.81±1.12
8 week	7.76±1.18	7.37±1.07	6.90±0.93
12 week	7.79±1.05	7.36±1.09	6.78±0.76

Data were expressed as mean±SD

Comparison of ABI from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

group / time	Control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	1.06±0.06	1.04±0.10	1.00±0.08
4 week	1.08±0.09	1.03±0.09	1.00±0.11
8 week	1.07±0.08	1.03±0.10	1.01±0.06
12 week	1.05±0.10	1.05±0.08	1.01±0.08

Data were expressed as mean±SD

Comparison of resting systolic blood pressure (mmHg) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

group / time	control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	134.26±10.30	134.89±15.73	131.18±9.53
4 week	133.63±12.69	130.04±14.85	129.86±10.38
8 week	133.37±14.04	129.64±17.40	126.61±12.53
12 week	132.89±14.04	124.82±15.13*	123.75±11.58*

Data were expressed as mean±SD

Significant difference by repeated measure ANOVA

* $p < 0.05$ compared with control

Comparison of resting diastolic blood pressure (mmHg) from baseline, 4 week, 8 week, and 12 week of Qigong and moderate intensity exercise

group / time	control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	81.04±18.06	79.04±10.19	80.21±9.48
4 week	78.89±17.73	75.64±9.53	79.36±10.4
8 week	77.81±19.24	77.75±11.13	79.21±10.44
12 week	80.00±17.96	77.89±11.19	77.64±9.24

Data were expressed as mean±SD

The Effects of Qigong and Moderate intensity exercise on resting heart rate(beat/min)

group / time	control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	67.78±9.56	67.21±8.02	68.29±7.04
4 week	69.41±8.84	69.46±10.37	64.93±6.63
8 week	68.78±9.77	67.36±9.36	65.71±8.40
12 week	68.26±10.15	66.68±9.21	64.71±7.93

The Effects of Qigong and Moderate intensity exercise on malondialdehyde (MDA) (ng/L)

group / time	control (n=27)	Qigong (n=28)	MIE (n=28)
Baseline	1.26±.19	1.21±1.41	1.36±1.71
4 week	1.21±1.12	1.14±1.63	1.10±0.97
8 week	1.14±0.97	1.02±1.19	1.06±0.05
12 week	1.3±0.97	0.93±0.68	1.02±1.00

Data were expressed as mean±SD

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