EFFECT OF INTENSE AEROBIC EXERCISE ON FOREARM BLOOD FLOW AND POSTPRANDIAL LIPEMIA IN NORMOTENSIVE OFFSPRING OF HYPERTENSIVE PARENTS

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ผลของการออกกำลังกายแอโรบิกระดับสูงต่อการไหลของเลือดไปเลี้ยงแขนและระดับไขมันในเลือดภายหลังมื้ออาหารในทายาทของผู้ที่เป็นความดันโลหิตสูง

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	EFFECT OF INTENSE AEROBIC EXERCISE ON FOREARM BLOOD FLOW AND POSTPRANDIAL LIPEMIA IN NORMOTENSIVE OFFSPRING OF HYPERTENSIVE PARENTS
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ชนนิกานต์ อริยะกุล : ผลของการออกกำลังกายแอโรบิกระดับสูงต่อการไหลของเลือดไปเลี้ยงแขนแล ระดับไขมันในเลือดภายหลังมื้ออาหารในทายาทของผู้ที่เป็นความดันโลหิตสูง. (EFFECT OF INTEN AEROBIC EXERCISE ON FOREARM BLOOD FLOW AND POSTPRANDIAL LIPEMIA IN NORMOTENSIVE OFFSPRING OF HYPERTENSIVE PARENTS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.พญ. อรอนงค์ กุละพัฒน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร.วิไล อโนมะศิริ, 71หน้า

ภาวะความดันโลหิตสูง ถือเป็นสาเหตุสำคัญของการเกิด โรคหัวใจและหลอดเลือด ซึ่งภาวะความดันโลหิตสูงสามารถถ่ายทอดได้ทางพันธุกรรม ส่งผลให้ทายาท (offspring of hypertensive parents: OHT) มีความเสี่ยงต่อการเกิดภาวะความดันโลหิตสูงและอาจพัฒนาเป็นโรคหัวใจและหลอดเลือดได้ในอนาคต

วัตถุประสงค์ : เพื่อศึกษาผลของการออกกำลังกายแอโรบิกระดับสูง (Ex) ก่อนมื้ออาหาร ต่อการไหลของเลือดไปเลี้ยงแขน (forearm blood flow: FBF) ในขณะ reactive hyperemia และคำนวณเป็นค่า area under curve (AUC_{RH}) และระดับไขมัน (triglyceride : TG) ในเลือดภายหลังมื้ออาหารในทายาทของผู้ที่เป็นความดันโลหิตสูง

ระเบียบวิธีการวิจัย : ในงานวิจัยนี้ทำการศึกษา 2 กลุ่ม คือ ทายาทของผู้ที่มีภาวะความดันโลหิตสูง (OHT) และกลุ่มควบคุม (offspring of normotensive parents: ONT) ในภาวะหลังมื้ออาหาร (Postprandial lipemia: PPL) โดยการวัดการไหลของเลือดไปเลี้ยงแขน โดยใช้ venous occlusion plethysmograghy ในขณะ reactive hyperemia (AUC_{RH}) และระดับ TG ที่เปลี่ยนแปลงในภาวะที่ได้รับการออกกำลังกายระดับสูง

ผลการทดสอบ: ผู้ชายสุขภาพดีจำนวน 30 คน (อายุระหว่าง 20-28 ปี) แบ่งเป็นกลุ่ม OHT จำนวน 15 คน และ ONT จำนวน 15 คน พบว่าในภาวะที่ไม่ได้รับ Ex กลุ่ม OHT มีค่า AUC_{RH} ที่ต่ำกว่า กลุ่ม ONT (802.1±28.3 vs 780.3±18.5 ml/100ml, p = 0.014) แต่มีระดับ TG ที่มากกว่า (179±57 vs 233±84 p<0.01 mg/dl, P = 0.003) อย่างมีนัยสำคัญทางสถิติที่ 4 ชั่วโมงหลังอาหาร และเมื่อเปรียบเทียบกับภาวะ Ex พบว่ากลุ่ม ONT และ OHT มีค่า AUC_{RH} สูงขึ้น (780.3±18.5 vs 800.1± 28.2 ml/100ml, p< 0.01 ในกลุ่ม OHT และ 802.1±28.3 vs 808.4±26.3 ml/100ml, p< 0.02 ในกลุ่ม ONT) อย่างมีนัยสำคัญทางสถิติ ที่ 4 ชั่วโมงหลังอาหารแต่ ระดับ TG ลดลงในกลุ่ม OHT เท่านั้น (233±84 vs 167±58 mg/dl, p<0.05 ในกลุ่ม OHT และ 179±57 vs 158±58 mg/dl, p=0.21 ในกลุ่ม ONT)

สรุปผลการทดลอง : กลุ่ม OHT ตอบสนองต่ออาหารไขมันสูงมากกว่ากลุ่ม ONT อาจเกิดจากภาวะ endothelial dysfunction และ ปริมาณTG ที่คั่งค้างมากกว่า แต่ในภาวะ Ex พบว่าระดับ TG ลดลง ทั้งกลุ่ม ONT และกลุ่ม OHT ซึ่งสรุปได้ว่า การออกกำลังกายระดับสูงก่อนมื้ออาหารทำให้การทำงานของหลอดเลือด (vascular function) ดีขึ้น ในทายาทของผู้ที่มีภาวะความดันโลหิตสูง

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

ปีการศึกษา.....2555.....ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEYWORDS :POSTPRANDIAL LIPEMIA/FOREARM BLOOD FLOW/OFFSPRING/ EXERCISE
CHONNIKAN ARIYAKUL : EFFECT OF INTENSE AEROBIC EXERCISE ON FOREARM BLOOD FLOW AND POSTPRANDIAL LIPEMIA IN NORMOTENSIVE OFFSPRING OF HYPERTENSIVE PARENTS. ADVISOR : ASSOC.PROF. ONANONG KULAPUTANA, M.D., Ph.D. CO-ADVISOR : ASSOC.PROF. WILAI ANOMASIRI, Ph.D.,71 pp.

High blood pressure is a common risk factor for cardiovascular disease. Due to its heredity in nature, offspring of hypertensive parents (OHT) are at high risk of impairment of vascular function and development of hypertension according to their parental history of hypertension.

Objective : To study postprandial forearm blood flow and blood lipid levels in response to a high intensity aerobic exercise in young individuals whose parents are hypertensive.

Methods : Normotensive young men were categorized into OHT and ONT (offspring of normotensive parents) groups. All subjects underwent randomized cross over 2 experimental conditions: standard meal without exercise (control; NoEx) and standard meal with exercise (Ex). In both occasions, forearm blood flow during reactive hyperemia using venous occlusion plethysmography and blood lipids were determined at baseline and 2 and 4 hours postprandially.

Results : Fifteen OHT (age 20-28 years) and 15 ONT (age 21-28 years) participated in this study. In control condition , the OHT group had a significantly lower AUC_{RH} (802.1±28.3 vs 780.3±18.5 ml/100ml, $p < 0.014$), but a higher triglyceride level (179±57 vs 233±84 mg/dl, $p<0.01$) than the ONT group, at 4-hour postprandially, However, when exercise was performed prior to meal ingestion, both groups had a significant increase in 4-hr postprandial AUC_{RH} (780.3±18.5 vs 800.1± 28.2ml/100ml, $p<0.01$ for OHT and 802.1±28.3 vs 808.4±26.3 ml/100ml, $p< 0.02$ for ONT). However, a significant decrease in postprandial TG with exercise was demonstrated only in OHT group (233±84 vs 167±58 mg/dl, $p<0.05$ in OHT and 179±57 vs 158±58 mg/dl, $p=0.21$ in ONT).

Conclusion : OHT was affected from postprandial lipemia more than ONT. The evidence include increasing of endothelial dysfunction and delayed clearance of TG. The high intensity aerobic exercise was an effective measure to reduce lipemia induced impairment of vascular function in normotensive young individuals with or without a family history of hypertension.

Field of Study…Sports Medicine…..Student's Signature……

Academic Year……2012……Advisor's Signature……

Co-advisor's Signature……...

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LIST OF ABBREVIATIONS

ABBREVIATIONS

AUC_{RH0}	Area under the Curve of reactive hyperemia at fasting
AUC_{RH2}	Area under the Curve of reactive hyperemia at 2 hour postprandially
AUC_{RH4}	Area under the Curve of reactive hyperemia at 4 hour postprandially
CVD	Cardiovascular Disease
Ex	Exercise experimental
FBF	Forearm Blood Flow
FFA	Free Fatty Acid
HDL-C	High-Density-Lipoprotein cholesterol
LDL-C	Low- Density-Lipoprotein cholesterol
HTG	Hypertriglyceridemia
LPL	Lipoprotein Lipase
NO	Nitric Oxide
NoEx	Non-Exercise (control experimental)
O_2^-	Superoxide
OHT	Normotensive Offspring of Hypertensive Parents
ONT	Normotensive Offspring of Normotensive Parents
$ONOO^-$	Peroxynitrite
PPL	Postprandial Lipemia
SMC	Smooth Muscle Cell
TAG	Triacylglycerol
TG	Triglyceride
Resting FBF	Resting (baseline) Forearm Blood Flow

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CHAPTER I

INTRODUCTION

Background and Rationale

Cardiovascular disease (CVD) has become the leading cause of death of the world population (1). It is acknowledged that atherosclerosis, which is the vascular deterioration owing to diseases such as high blood pressure, diabetes mellitus, dyslipidemia, obesity, is the major cause the vessel stenosis. Due to the blockage of blood and oxygen to cardiac muscle, vessel stenosis is the main reason of acute myocardial infarction and die suddenly (2). Thereby the development of atherosclerosis is a primary cause of CVD (1).

Considering the factors that cause atherosclerosis, it is normally accepted that the high blood pressure is the main factor leading to changes of the structural vessel. Such alterations include the imbalance of production of the relaxing and contracting elements and also the diminished production of nitric oxide amid the endothelial dysfunction. As a result, the patients with high blood pressure are more susceptible to develop atherosclerosis (3). The high blood pressure can be caused by a genetic inheritance from either genetic factors or genetic environmental interactions (3). Then, offspring with high blood pressure parents will be at a risk to develop the endothelial dysfunction condition as well. This is due to the impaired process of endothelium-dependent vasodilatation which is the significant character of defective work of the

vessel before changing the level of blood pressure. Such phenomenon can be found in persons with high blood pressure and their offspring. Thus, the offspring of parents with high blood pressure may encounter the risk of endothelial dysfunction as well. It is because of not only the decrease the nitric oxide production, the offspring may also have an abnormal transfer of the substrate of the nitric oxide (4).

The vascular endothelium plays a central role in mediating the atherosclerotic process. The endothelium is located at the interface between the vascular compartment and the surrounding tissues and therefore is ideally situated to regulate several functions involving fluid and solute exchange between the plasma and interstitial fluid, the regulation of vascular tone, the control of smooth muscle (SMC) proliferation, the inflammatory process, and mediating the balance between pro- and anti-coagulant factors in the blood. Furthermore, in response to various stimuli, endothelial cells synthesize and release various vascoactive substances (5-9), which function to maintain cardiovascular homeostasis. Endothelial dysfunction found in the offspring of the high blood pressure person has been related to the error of a substrate transportation of nitric oxide, L- arginine, in the process of L- arginine - NO pathway. This defect causes the loss of bioavailability of nitric oxide.

Numerous studies have consistently shown that, in the hours following a fatty meal, plasma triglyceride (TG) concentration is higher in individuals with hypertension than without hypertension (10-12). Moreover, the patients with hypertension have an

exaggerated response and delayed clearance of plasma TG concentration after fat loading (10). In today's society, individuals spend the majority of their time in a postprandial relatively sedentary state. High caloric meals rich in carbohydrates and saturated fats can yield increased levels of blood glucose, free fatty acids, and triglycerides (13). A high-fat meal is a direct source of oxidative stress (14). Thus, higher oxidative stress and greater endothelial dysfunction induced by high fat meal can be worsening for each consecutive meal (15). As recurring postprandial oxidative stress initiates a nearly continuous cycle of endothelial dysfunction (16), thereby postprandial lipemia may represent an independent risk factor for atherosclerotic CVD (17).

Many studies (18, 19) have shown that acute exercise mitigates the elevation of plasma TG concentration after a fatty meal, adding to the potential health benefits of physical activity. Many forms of exercise have been shown to improve endothelial function during postprandial lipemia and to reduce postprandial lipemia as well (18, 20). Previous studies found that a single bout of aerobic exercise performed before a high fat meal reduced area under curve of TG (18). Recently studies showed benefit of intense aerobic exercise that not only reduce the level of postprandial lipemia (20, 21), but also preserve the efficiency of the endothelial function in healthy men (21). In normotensive offspring of hypertensive parents participating in an exercise program, the result has been shown to reverse hemodynamic, metabolic and hormonal alterations that are involved in the pathophysiology of hypertension (22). However, it remains unknown

whether or not impaired endothelial function also occurs during postprandial lipemia in healthy young men who have a family history of hypertension. If so, further interest would be the issue on protecting lipemia induced endothelial dysfunction in this population.

The evidence showed closely relation of endothelium-dependent nitric oxide-mediated vascular function in conduit and resistance vessels (23). Forearm blood flow reflected resistance vessel function in the forearm and responsed to vasoactive agents (24). Furthermore, the vasodilatory responsed to reactive hyperemia such as the evaluation of the maximum hyperemic flow and the overall time–flow curve during reactive hyperemia has been shown to offer significant information in evaluating resistance vessel endothelial function (25).

Due to the fact that intense aerobic exercise is an important part in preservation of the endothelial function on postprandial lipemia in healthy men (20, 21), with a lesser extent than in those with hypertension. It is hypothesized that in offspring of hypertensive parents, when exposed to postprandial lipemia, vascular function is affected and such function is improved with a single exercise session. The main purpose of this study is to search for these changes using a noninvasive method of blood flow measurement called venous occlusion plethymography. The technique is relatively easy and safer as there is no chemicals injected into the blood vessels to assess their function.

Research questions

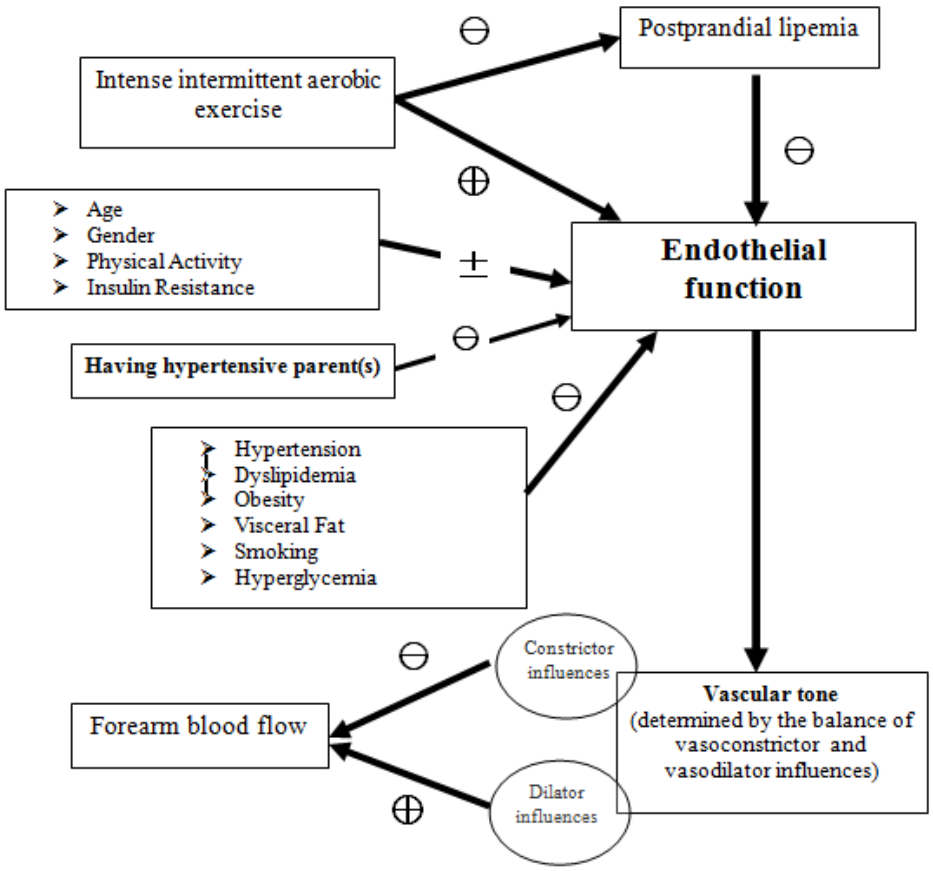
Primary question: What are the effects of the high level of aerobic exercise prior to a high fat meal on postprandial forearm blood flow and the lipid profile of the offspring of hypertensive parents?

Secondary question: Are the effects of high level of aerobic exercise prior to the high fat meal on postprandial forearm blood flow and the lipid profile different between the offspring of hypertensive parents and those of normotensive parents?

Objective

To study postprandial forearm blood flow and lipid profile in response to a high intensity of aerobic exercise in young individuals whose parents are hypertensive.

Conceptual framework



Hypotheses

1. With the presence of the preceeding bout of intense aerobic exercise, the forearm blood flow determined after a meal of the offspring of hypertensive parents (OHT) is greater compared to no exercise.
2. With the presence of the preceeding bout of intense aerobic exercise, the blood level of triglyceride determined after a meal of the offspring of hypertensive parents (OHT) is lower compared to no exercise.

3. With the presence of the preceeding bout of intense aerobic exercise, the forearm blood flow determined after a meal of the offspring of hypertensive parents (OHT) differs from that of the offspring of normotensive parents (ONT).
4. With the presence of the preceeding bout of intense aerobic exercise, the blood triglyceride level determined after a meal of the offspring of hypertensive parents (OHT) differs from that of the offspring of normotensive parents (ONT).

Scope of research

This is a cross over analytical design study in which the offspring of normotensive parents (ONT) and the offspring of hypertensive parents (OHT) participated as subjects.

The study approval was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from the subjects after the experimental details and risk involved were explained, and they were reminded of their right to withdraw at any stage.

Assumptions

1. The equipment was calibrated to meet the standard of accuracy and reliability.
2. Forearm blood flow measured by strain gauge venous occlusion plethymography truly reflects in *vivo* endothelial function.

Limitation

1. This study had been done in healthy men with 18-30 years of age, and normal blood pressure. They volunteered to join the research and pass the recruitment criteria. The results of this study may be specific to the population similar to sampling participants.
2. Although it was not perfect, the daily activities and types of food consumed by the subjects several days before the experiment were controlled. The subjects were explained, and asked to follow the information about physical activities and food details during the research participation.

Operational definition

1. *Forearm blood flow* is defined as the rate of blood flow measured at the non-dominant forearm by venous occlusion plethysmography with mercury - in- rubber circumference gauge.
2. *Reactive hyperemia* is the transient increase in organ (forearm) blood flow that occurs following 5 minutes of arterial occlusion.

3. *Postprandial lipemia* is the condition of the increment of the lipid level in the blood after a meal. The lipid levels in the blood were measured after the meal for 2 hours and 4 hours.
4. *Normotensive offspring of hypertensive parents* is the healthy person who denies his underlying disease history and especially has normal blood pressure but his parent(s) has the high blood pressure records (BP ≥140/90 mmHg.) examined by the physician; the father has the diagnosis before 55 years and mother before 65 years of age.
5. *Endothelial function* is the ability of endothelial cell to control the functions of the blood vessel particularly the constricting/dilating function by which is controlled through chemical substance created by endothelial cell.
6. *Intense aerobic exercise* is the combination of high level of aerobic exercise above 85-95% of HRmax alternating with moderate level at 70% of HRmax performed on a motorized treadmill in 38 min duration.

Expected Benefits and Applications

1. To address the effect of high level of aerobic exercise to the forearm blood flow and lipid profile after a meal of the offspring of hypertensive parents as initial information and potential exercise recommendation to reduce the future risk of high blood pressure and cardiovascular disease.

2. To address the different effect of the high level of aerobic exercise on the forearm blood flow and the lipid profile after a meal of the offspring of hypertensive parents and those of normotensive parents. This ground information may provide important insight onto future research regarding lifestyle intervention for specific population.

CHAPTER II

REVIEW LITERATURES

Atherosclerosis is the leading cause of death in many countries. It is an inflammatory vascular disease characterised by the progressive accumulation of lipids and fibrous elements in the sub-endothelial space of large arteries (26). It frequently affects the coronary and cerebral vessels, leading to myocardial infarction and stroke, respectively. The initiating event in atherosclerosis appears to be injury to the arterial endothelium. Risk factors such as hypertension, smoking, genetic factors, advanced age, type II diabetes mellitus, obesity and a sedentary lifestyle are linked by a common mechanism through their deleterious effects on endothelial function. Structural damage to the endothelium can impair permeability, interfere with anti-thrombotic properties, and alter the release of vasoactive substances (27, 28).

Hypertension of unknown cause accounts for more than 90% of cases (29). It tends to cluster in families and represents a collection of genetically based diseases or syndromes with several resultants inherited biochemical abnormalities (29-31). One of the pathophysiologic factors has been implicated in genesis of essential hypertension: deficiency of vasodilators, mainly nitric oxide (NO). Recently, there are many innovative concepts that may antedate the hypertension and contribute to its pathogenesis. One that have already gained the support include the concept of structural and functional

abnormalities in vasculature, the endothelial dysfunction, the increased oxidative stress, the vascular remodelling, and decreased compliance (32).

Endothelial function

Intima or the innermost layer of blood vessels is composed with a layer of the endothelial cells which act as the selective semi-permeable membrane in the directions between circulating elements in blood and tissue. Endothelium mediates vascular tone and actively suppresses thrombosis, vascular inflammation, smooth muscle cell (SMC) migration, and proliferation (26, 33, 34). Endothelial cells can both synthesize and release various vasoactive substances, for instance, NO, free radicals, and platelet-activating factors (35), altogether to maintain the cardiovascular homeostasis. NO was originally described as an endothelium-derived relaxing factor. It is a free radical generated by oxidation of L-arginine to L-citrulline. The reaction is catalysed by endothelium-derived nitric oxide synthase (NOS). Though the action of NO, endothelium can effectively relax vascular smooth muscle cell in vasodilation and increase blood flow.

Reactive hyperemia and endothelial function

The '*Reactive hyperemia*' is the transient raise of organ blood flow (hyperemia) that occurs following a brief period of ischemia like arterial occlusion. The traditional concept is that both metabolic, or substances that accumulate during period of circulatory arrest, and myogenic auto-regulation contribute to reactive hyperemia.

Several studies have suggested that, the endogenous NO only plays a minor role in vasodilation during reactive hyperemia. The reactive hyperemia is mostly caused by endothelium related mechanisms, such as, adenosine, prostaglandins, and endothelium derived hyperpolarizing factor (36). Nevertheless, some reports suggest that NO may provide a significant effect to the late stage of reactive hyperemia (12), including the peak hyperemic flow (37).

An early study showed that, if the periods of ischemia were increased up to 5 minutes, the peak forearm blood flow would response after the restoration of flow increased. In addition, they also found that during reactive hyperemia, forearm blood flow can be increased up to 10-15 times compared with baseline measurement. Increasing the period of occlusion, result in an increase of metabolic stimulus for vasodilation which will also lead to increase the peak reactive hyperemia and duration of hyperemia too (38). Each organ would response to the maximal vasodilation as predicted by peak blood flow differently. The reactive hyperemia might occur within less than a minute of the complete arterial occlusion (e.g., coronary circulation), or it may require up to several minutes of an occlusion (gastrointestinal circulation) (39-41). This implies that, the concentration of vasodilator substances would be increasing with time. The reactive hyperemia subsides exponentially, in keeping with a wash out or conversion of vasodilator metabolites. In addition, the venous blood metabolities which is collected during the reactive hyperemia is having vasodilator properties too (41). During the

arterial occlusion, the tissue hypoxia plus the accumulation of vasodilator metabolites (e.g. adenosine) dilate arterioles and also decrease vascular resistance.

Recently, there are some studies showing that, inhibition of NO produced by NG-monomethyl-L-arginine (L-NMMA) infusion significantly decreases peak hyperemic flow as well as the total hyperemic flow (determined by inflow-time curves) by 30-50% (42). In addition to endothelial mediated mechanisms, myogenic mechanisms may also contribute to reactive hyperemia in some tissues. With this mechanism, an arterial occlusion will be resulting in a reduction of pressure downstream in arterioles, which would contribute to myogenic-mediated vasodilation. Furthermore, the mechanical forces originated by rapid cuff inflation to obstruct blood flow may cause an increasing in BP; though, it may result in an increased level of total peripheral resistance also. The increasing BP could activate the baroreceptors and consequently restrain the muscle sympathetic nerve activity (MSNA) (43). When perfusion pressure is restored, which is normally would be immediately after the release of the occlusion cuff, blood flow will rapidly elevated (the peak or maximum forearm blood flow). It could stay up to several minutes because of the reduction of vascular resistance (minimum forearm vascular resistance). During the hyperemia, the tissue becomes re-oxygenated and vasodilator metabolites are washed out of the tissue. This would cause the resistance vessels to recover to their normal vascular tone, thereby returning flow to control level. Interestingly, it was also demonstrated that the overall blood flow during the hyperemic

period (total flow) was far in excess of demand to repay any metabolic debt (flow debt) which occurred during the ischemia (reactive hyperemia) (44).

Endothelial function in hypertensives and their offspring

In a normal condition, the endothelial cell will secrete both the relaxing and contracting chemical groups to control the performance of the smooth muscle wall of blood vessel (2) (figure 2.1) and the vascular tone. A phenomenon controlled by NO is called “endothelium-dependent vasodilatation”. Defective work of the endothelial cell will cause pathology of blood vessels and high blood pressure (45).

High blood pressure can be caused by a genetic inheritance from either genetic factors or genetic environmental interactions (3). Then, offspring with high blood pressure parents will be at a risk to develop the endothelial dysfunction condition. This is due to the impaired process of endothelium-dependent vasodilatation which is the significant character of defective work of the vessel before changing the level of blood pressure. Such phenomenon is observed in persons with high blood pressure and their offspring is observed (4).

Endothelial dysfunction found in the offspring of the high blood pressure person has been related to the error of a substrate transportation of nitric oxide, L- arginine, in the process of L- arginine - NO pathway. This defect causes the loss of bioavailability of nitric oxide which let the offspring of the high blood pressure person gain differed endothelial function from the controlled group (4). Therefore, the study of endothelial

function in various conditions and subject groups is regarded a significant area of study with the ultimate goal to find the measure for the high blood pressure risk reduction in the future and also reduce the risk of atherosclerosis condition in people owing a high blood pressure and their offspring as well (3).

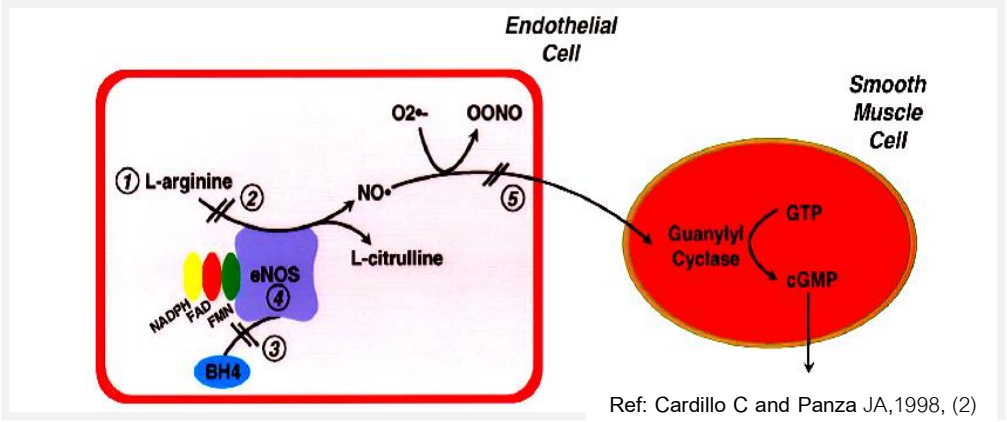
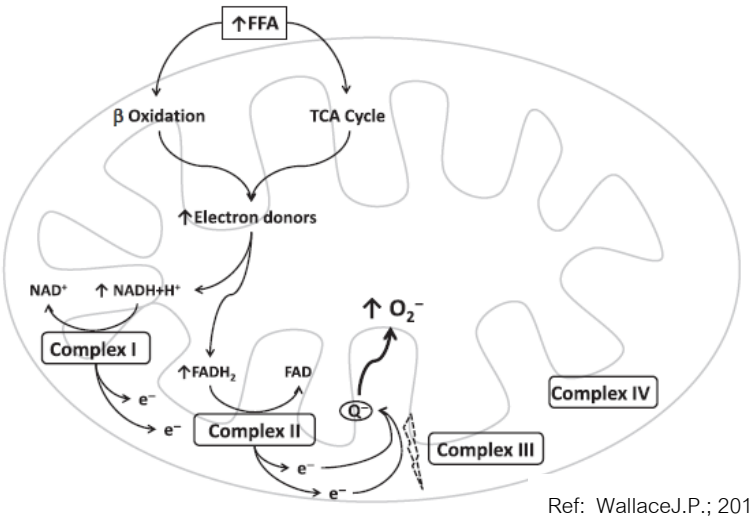


Figure 2.1 Mechanism of vascular relaxation by endothelial-dependent vasodilation in hypertension.

Endothelial function on postprandial lipemia

The postprandial lipemia may consider one of the risk factors of the cardiovascular disease which is the result of the atherosclerosis (17). The consumption of high-fat food may cause the oxidative stress (46). In the postprandial lipemia stage, a large amount of free fatty acids will be added in the muscle, adipose tissue, hepatic tissue and vascular endothelial cell (47, 48). The increased level of free fatty acids in the blood affects the process of β-oxidation and tricarboxylic acid cycle in mitochondrion. A higher rate of fatty acid oxidation produces excessive number of electrons (NADH

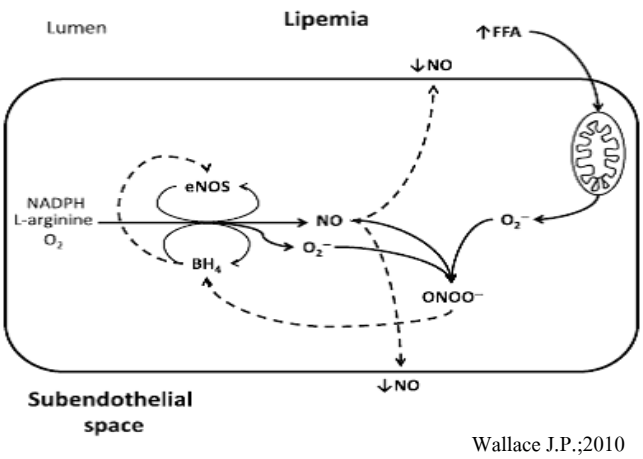
and $FADH_2$). As a result, the overloading of electron transport chain (ETC) increases O_2^- (46, 49). (figure2.2)



Ref: WallaceJ.P.; 2010

Figure 2.2. Mitochondrial superoxide production during postprandial lipemia lipemia

O_2^- matches with nitric oxide to produce the peroxynitrite ($ONOO^-$) and the bioavailability of nitric oxide reduces temporarily due to $ONOO^-$and O_2^-. These molecules can oxidize the tetrahydrobiopterin (BH_4) which is the co-factor of the production process of nitric oxide. Subsequently, the bioavailability of nitric oxide can resume to normal condition (50). (figure 2.2)



Wallace J.P.;2010

Figure 2.3 Nitric oxide function in the postprandial lipemia state leading to endothelial dysfunction

The link between physical activity and cardiovascular disease (CVD) was first characterized in 1953 (37) and well confirmed later in 1978 in the Paffenbarger Harvard Alumni study (51). These groundbreaking studies laid the foundation for many studies to follow and demonstrate the central role of physical activity in the prevention and treatment of CVD as well as a reduction in morbidity and mortality from CVD (52). It is of interest to note that the reduction in cardiovascular morbidity and mortality achieved through exercise training has comparable results to pharmacological interventions (53).

A number of factors contribute to the beneficial effects of physical activity and are most notably related to its effect on the vascular formation and regulation of NO. Early studies on the effect of exercise training on muscle blood flow used handgrip training exercise (54). The study showed an increase in forearm muscle blood flow with exercise training. The authors concluded that the increase in blood flow was the influence of local mechanism rather than alteration in autonomic control. Later, Green

and colleagues (55) conducted four weeks of handgrip training in male subjects. They demonstrated a reduced vascular resistance in the trained limb following a ten minute ischemic challenge. To determine if exercise training has a positive systemic effect on vascular function, Kingwell and colleagues (56) trained the subjects for four weeks on cycle ergometers and examined NO production in the forearm. They found that following training there was evidence suggesting enhanced forearm NO production. More recently, whole body exercise studies supported the increase in NO production through an upregulation of eNOS (57-59).

Exercise and postprandial lipemia

Exercise affects the increment of energy expenditure. Due to insufficiency of pre-existing intramuscular energy, muscles need other sources of energy in order to have enough energy during exercise. The requirement for external sources causes exercise to affect the change of the lipid levels in postprandial condition (60).

The effect of aerobic training has been reviewed (61). It has been well accepted that exercise training increased plasma HDL-C and lower the plasma TAG level in fasting condition.

The interest in the blood lipid response to exercise has been paid not only to exercise training but also to acute exercise in postprandial condition. Acute exercise caused the change in cholesterol level as reported in the study of Cohen and Goldberg (62). The study showed that 10 km. distance of walking reduced the plasma turbidity

after a meal of high fat containing food, indicating that the elimination of high fat ingested had been increased (62). The result was conformed with the study of McDonald and Fullerton (63), which determined the plasma turbidity after a meal of high fat containing food in comparison between the ambulation treated patients and the bed ridden patients. The authors found that the ambulation treated patient has lower of plasma turbidity at the significance level (64).

However, one reason of exercise having an effect to the change of postprandial lipemia level may be due to the fact that exercise help increasing of the lipoprotein lipase (LPL) level. The impact on LPL will appear after a period of time post exercise; thus, the TAG concentrations will be seen in the next fasted state when performing the prolonged exercise on the day before (65). The same results were found in the study of plasma TAG in healthy teens who performed a 2-hr walking exercise at 40% VO_2max for 16 hours before consuming a high fat containing meal (66). The exercising subjects reduced the plasma TAG level roughly 1/3 times compared to the subjects with no exercise (66). On the other hand, there were studies showing the influence of an hour of exercise at 30% VO_2max for 12 hours prior to a meal on lipemia level in sedentary subjects and found few changes of lipemia (60, 67). As a result of exercise, changes were found on the elevated concentrations of TAG-rich lipoproteins as well as the high capacity of the TAG clearance process. Additionally, the increment of changing rate of

TAG and cholesterylesters from HDL and the reduction of the cholesterol level in the postprandial lipemia condition were reported (62).

In summary, the effect of exercise on lipid change of the postprandial lipemia condition depends on factors such as the character of the participants (trained/untrained/active/sedentary etc.), time period of exercise (before-after a meal), type of exercise and intensity of exercise. However, there is no clear conclusion regarding which exercise characteristics will serve best to modify the condition of postprandial lipemia.

Exercise, postprandial lipemia and endothelial function

The beneficial effect of regular physical activity on postprandial lipid metabolism has received extensive attention (68), whereas the paradoxical effects of physical activity on endothelial function have yet to be elucidated. However, physical activity may have a beneficial role in postprandial endothelial dysfunction, yet the interaction between the acute affects of physical activity and postprandial lipid metabolism on endothelial function remains unclear. In an attempt to determine whether the timing of the exercise bout is important, a number of investigation have performed exercise 24 hours (18), 16 or 4 hours (69), and 1 hour (70) prior to the ingestion of a high fat meal. When exercise was performed prior to ingestion of the high fat meal, microvascular function (18) and indices of arterial compliance (pulse-wave velocity, PWV) (70) were improved. Gill and colleagues (18) demonstrated that 90 minutes of

treadmill walking performed the day before a high fat meal improved the endothelium-dependent vascular function in response to acetylcholine by almost 25% (pooled data for lean and obese subjects). Similarly, Clegg and colleagues (70) reported that exercise performed one hour before the meal prevented the increase in brachial artery PWV observed in the control visit suggesting an endothelium-dependent mechanism as well. Moreover, recently studies showed that a single bout of high intensity aerobic exercise performed prior a high fat meal reduced TG and preserved flow- mediated dilatation (FMD) in healthy men (20, 21). Hence, exercise reduce postprandial lipemia and preserves endothelial function particularly in healthy individuals. It seem likely such benefit of exercise can be expected when it is performed before a meal.

CHAPTER III

Research Methodology

Research design

This study is a cross over analytic research which examined the effect of intense aerobic exercise on changes of forearm blood flow in response to postprandial lipemia in healthy offspring of normotensive and hypertensive parents. All subjects underwent a control condition in which a high fat meal was given and a serial blood lipid levels and forearm blood flow were determined at immediately before (0hr), 2 hours (2hr) and 4 hours (4hr) postprandially. At random order within 7 days apart, the subjects also underwent another experimental condition in which an intermittent exercise session on a treadmill was performed prior to a standard high fat meal was given and all the measurements were repeated accordingly. Venous occlusion strain gauge plethysmography was employed to evaluate forearm blood flow. The study protocol was approved by The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University.

Population

Target population

In this study, the target groups were apparently healthy men whose age were between 18-30 years, and blood pressure were normal. All subjects met the inclusion and exclusion criteria of the study.

Study subjects

1. Men whose age were between 18-30 years, who were healthy and particularly having normal blood pressure and who were offspring to the persons with high blood pressure.

2. Men whose age were between 18-30 years, healthy and had normal blood pressure and who were offspring to the persons with normal blood pressure.

The study participants were recruited according to the following criteria and all were initially contacted by telephone to determine their initial qualification before including in this study.

Inclusion Criteria

1. **Offspring of hypertensive parents (OHT) group**

1.1 Men whose ages are between 18-30 years.

1.2 Being healthy: normal blood pressure (BP = 120±10/80±10 mmHg.); normal fasting blood sugar (FBS < 100mg/dl); normal blood lipid levels (cholesterol < 200 mg/dl, triglyceride < 200mg/dl, LDL < 150 mg/dl); normal resting cardiac electrical signals.

1.3 Performing physical activity less than 30 minutes at a time and less than 3 times per week.

1.4 BMI $\leq$ 25 kg/m^2

1.5 Father and /or mother having the high blood pressure records (the diagnosis made before : 55 years for father, 65 years for mother) (3), or currently on antihypertensive medication.

1.6 No abnormalities in nerve and upper limb muscles.

2. **Controlled group: offspring of normotensive parents (ONT)**

2.1 Men whose ages were between 18-30 years.

2.2 Healthy: normal blood pressure (BP = 120±10/80±10 mmHg.); normal fasting blood sugar (FBS < 100 mg/dl); normal blood lipid levels (cholesterol < 200 mg/dl, triglyceride < 200 mg/dl, LDL < 150 mg/dl); normal resting cardiac electrical signals.

2.3 Performing physical activity less than 30 minutes at a time and less than 3 times per week.

2.4 BMI $\leq$ 25 kg/m^2

2.5 Father and mother having normal blood pressure records (BP$\leq$140/80mmhg).

2.6 No abnormalities in nerve and upper limb muscles.

Exclusion Criteria

1. Conditions that put the subjects at risk or are exercise limitation
2. An open wound or muscle inflammation in the arm and forearm area.

3. Difficulties of provided meal consumption

4. Incomplete participation of all experimental conditions

Subjects

Subjects recruitment and screening

Subjects were recruited by flyers and personal contact. The study participants were recruited according to the following criteria and all subjects were initially contacted by telephone to determine their qualification and explain there search project before included in the study. Subjects in OHT group were initially interviewed by phone regarding family history (who it was in family having high blood pressure) which was diagnosed by doctor. Moreover, this study provided a standard automatic blood pressure monitor (OMRON, HEM-7200®) with instruction for measuring by themselves at home before participation.

Sample size determination

In this study, the sample size determination was obtained from a pilot study.

Three normotensive offspring of hypertensive parents (OHT) and three normotensive offspring of normotensive parents (ONT), who consumed a standard breakfast containing high fat food resulting in a change of blood lipid level in 4 hours. Both groups were assigned for two experiments: for an exercise day (Ex) and a no exercise day (NoEx). The average AUC of forearm blood flow in response to transient

ischemia (reactive hyperemia) was calculated. When comparing the fasting state with the postprandial state at 4 hours (AUC_{RH4}) and Ex with NoEx which the mean± standard deviation of OHT is 143.1±8.7 ml/100ml tissue (890.6±30.7 [fasting] – 747.5±22 [4hr]) and ONT is 103.2 ± 42.06 ml/100ml tissue (888.5±50.7 [fasting] – 785.3±8.64 [4hr])

Then, calculate the sample size of formula n/group = $2(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2 / (x_1 - x_2)^2$

Where α = 0.05

$Z_{\alpha/2} = Z_{0.05/2}$ = 1.96 (two tail)

β = 0.10

$Z_{\beta} = Z_{0.10}$ = 1.28

$$\sigma^2 = \text{Pooled variance} = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}$$

$$\text{Pooled variance} = \frac{(3 - 1)(8.75)^2 + (3-1)(42.06)^2}{3 + 3 - 2}$$

$$= 1037.6$$

X_1 = mean of AUC_{RH4} of OHT group

X_2 = mean of AUC_{RH4} of ONT group

$$\text{n/group} = \frac{2(1.96 + 1.28)^2 \, 1037.6}{(143.1 - 103.2)^2}$$

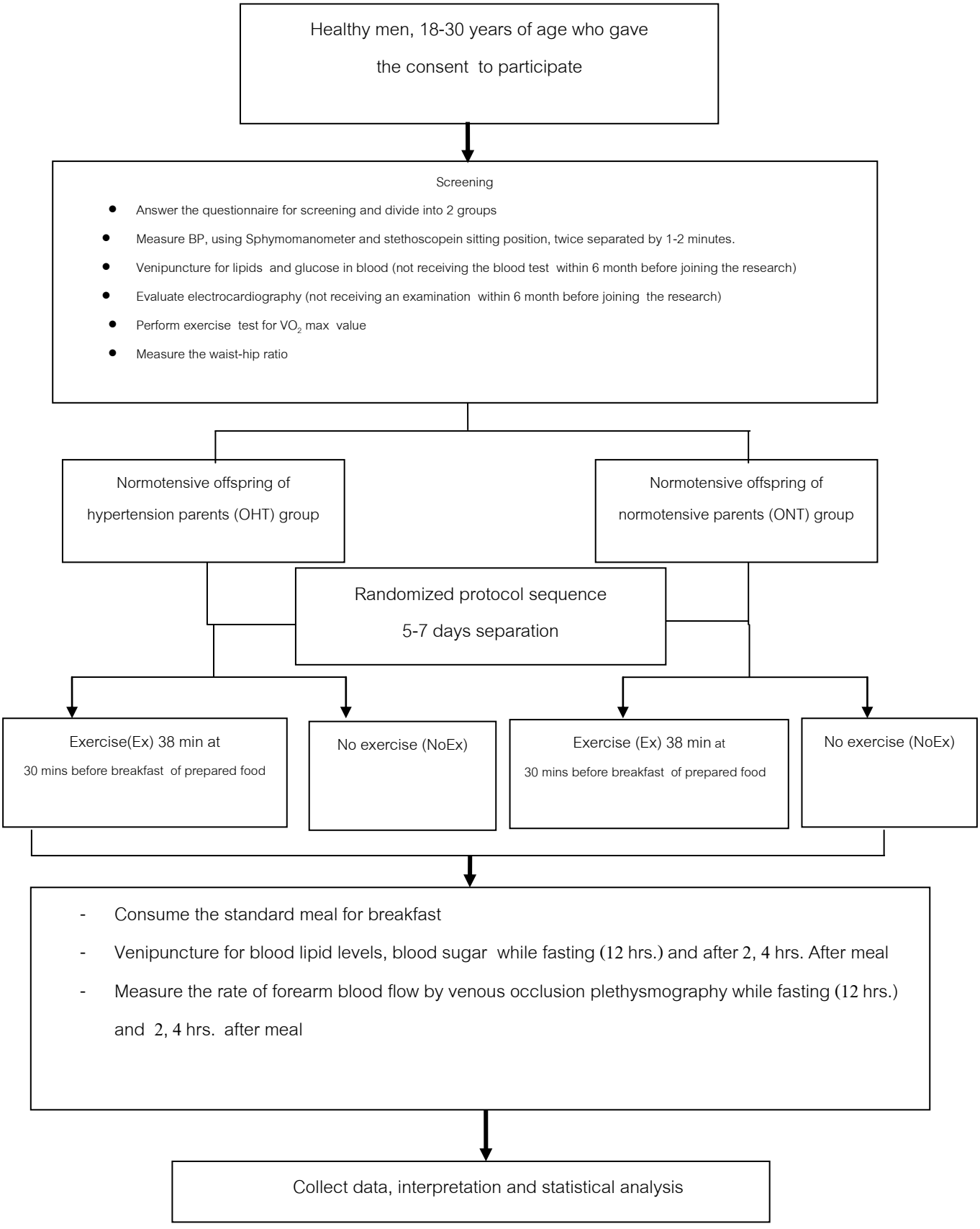
= 13.6 ~ 14 persons

To ensure a reliable result of the study and cover the questions of this research, each group of 14 persons, total 28 persons were recruited. A dropout rate of 5% was estimate, and then recruit 15volunteers per group, total 30persons were recruited.

Instruments

1. Venous occlusion strain gauge plethysmogragphy (EC6, DE HokansonInc, WA, USA) and equipment.
2. Mercury-in-rubber strain gauge (size 16,20,24,28 cm.)
3. Arterial occlusion cuff , 2 sets.
4. Treadmill (Nautilus™model: T 518).
5. Medical supplies for venipuncture
6. Stopwatch
7. Computer for data recording
8. Heart rate monitor (FS2c, Polar Watch)
9. Standard high fat meal

Figure 3.1 Flowchart of experimental procedure



Timeline of experimental protocols'

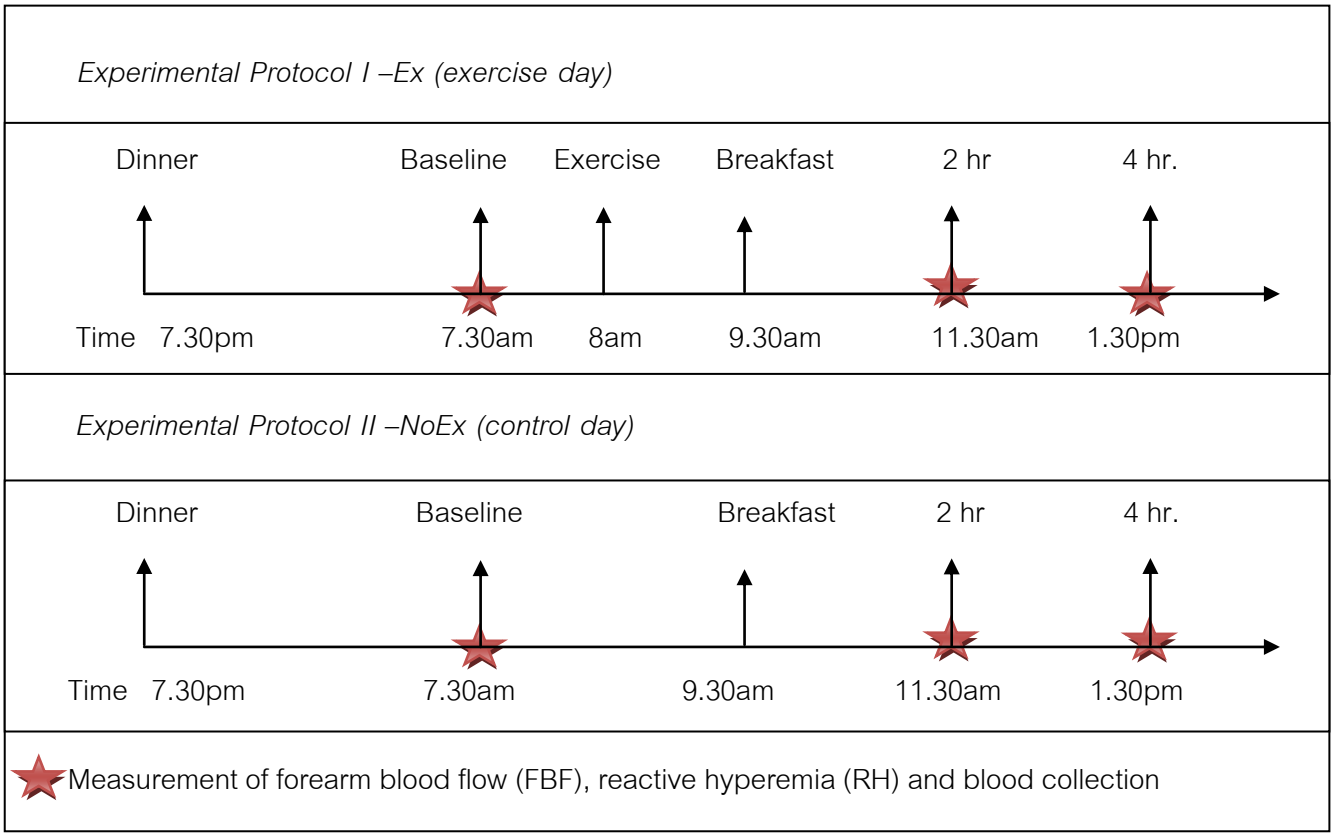


Figure 3.2 Timeline of experimental protocols

Preparation of the research participants

For stabilizing the blood lipid level and blood flow, prior to the experimental day, the volunteers were asked to:

- Refrain from supplements such as vitamins with antioxidant properties at least 7 days

- Refrain from food with a high proportion of fat (more than 1 gm. fat/1 kg. of bodyweight) at least 3 days
- Refrain from alcohol containing beverages at least 48 hours
- Refrain from containing beverages at least 12 hours
- Refrain from food (except water) consumption at least 12 hours
- Avoid vigorous activity at least 24 hours

Food preparation for the experiment (Fig3.3)

Breakfast consisted of Double Sausage Mac Muffin with egg, corn pie, and soybean milk that provided a total energy of 980 Kcal. The meal has 513 Kcal as energy from fat. The nutrition information is shown in Table 3.1. This meal had been tested during the pilot study and found that it successfully induced an illuviation the blood lipid level. There was a large increment of triglycerides causing the postprandial lipemia in both groups (OHT and ONT). The mealtime lasted no later than 15 minutes. (21)

Double Sausage Mac Muffin with egg, corn pie,and soybean milk

Table 3.1 The nutrition information of test meal

Food lists	Quantity (g.or ml)	Energy (Kcal)	Fat (g)	Carbohydrate (g)	Protein (g)	Sodium (mg)	Cholesterol (mg)
Double Sausage Mac Muffin with egg	170	560	34	27	36	2700	285
Corn pie	80	220	12	23	3	220	10
Soybean milk	300	200	11	23	8	110	-
Total	550	980	57	73	78	3030	295

Data from http://www.mcthai.co.th/nutrition and http://www.thaiunbox.com/lactasoy

Measurement of resting FBF and reactive hyperemia by venous occlusion plethysmography

Subject setup

Subject was relaxed in the supine position on the treatment bed. The non dominant arm were extended, slightly external rotated and supported approximately 10 cm. above the heart level. This position was set in order to lower the initial venous pressure, ensure adequate venous emptying and facilitate outflow during the period of venous cuff deflation (71), which is usually achieved by resting the elbows on foam pads and supporting the hand with pillows (Figure 3.4). Consequently, the appropriate size strain gauge was selected and placed around the largest part of the forearm, without touching anything other than the forearm. Then the gauge was fixed to the skin by a piece of masking tape putting over the head of the gauge. Subjects were asked to hold the position without hand and forearm movement to prevent gauge disturbance during measurement.

Basic concept

Venous occlusion plethysmography measures arterial inflow by abruptly stopping venous outflow with an upper arm cuff (venous occlusion cuff) inflated to above venous pressure but below arterial pressure (about 50 mmHg (72)), while arterial inflow is unaltered and blood can only enter the forearm but cannot escape. This results in a linear increase in forearm volume over time, which is proportional to arterial blood inflow. A mercury – in – rubber gauges around the forearm is used to measure the volume change in the forearm. The rate of change of the volume, in percent per minute, is the arterial flow rate (forearm blood flow) at the moment of venous occlusion. Often the flow rate is reported as being ml/100 ml/minute or % change/minute.

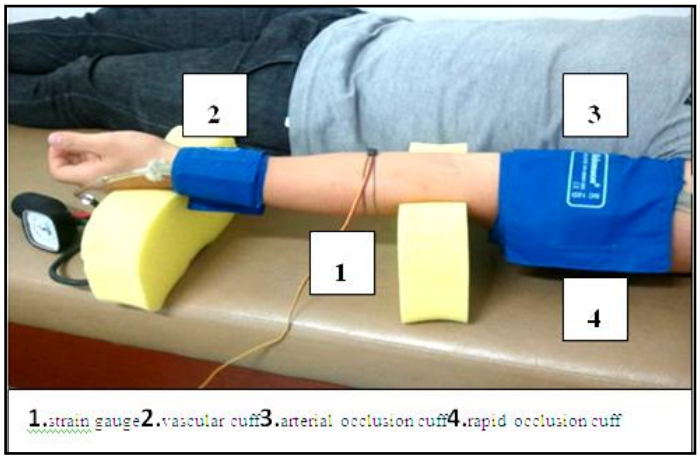


Fig 3.4 Subject starting position and instruments

Instrumentation

This arterial inflow or venous occlusion plethysmography (Fig 3.5) measurement system requires; an E20 rapid cuff inflator to abruptly occlude the upper arm cuff (venous cuff). The cuff pressure was maintained at 50 mmHg for 5 seconds (inflow time) in each 15 seconds cycle (reading interval) to occlude venous outflow from the arm (according to the manufacturer guidelines). The plethysmograph was automatically set to inflate and deflate the venous cuff at the specified interval. The noninvasive Vascular Program (NIVP3) software that interfaces the laptop computer was compatible with the plethysmography. The NIVP3 software stores patient information and waveforms for arterial inflow measurements (Fig 3.6). The program very accurately set the slope according to the chosen intercept points and displays the flow rate as the slope is edited. The ideal sensitivity will give an inflow slope of approximately 45 degrees. So, strain gauge plethysmography measures the rate of change (slope) of the circumference of a forearm. This represents a change in the volume of the limb segment over a brief period of time rather than the change in blood flow. However, because the change in volume is assumed to be the result of arterial inflow of blood, the convention is to report the change as blood flow (73). The DC (vein) mode was set on the EC6 plethysmography in accordance with the other settings. In this mode, the signal from the strain gauge is directly coupled to the recorder without any filters to distort the signal, which allows the instrument to record continuous changes in blood flow (every 15 seconds). Since hand

blood flow was predominantly through skin blood vessels rather than skeletal muscle and thus had different control mechanisms than FBF (74), a second wrist cuff is placed distal to the strain gauge. Therefore, the hand was excluded from the circulation by inflating cuffs around the wrist to above systolic pressure (about 220 mmHg) at least 1 minute before each measurement and throughout measurement of FBF.

Fig 3.5 EC6 strain gauge plethysmography and E20 rapid cuff inflator

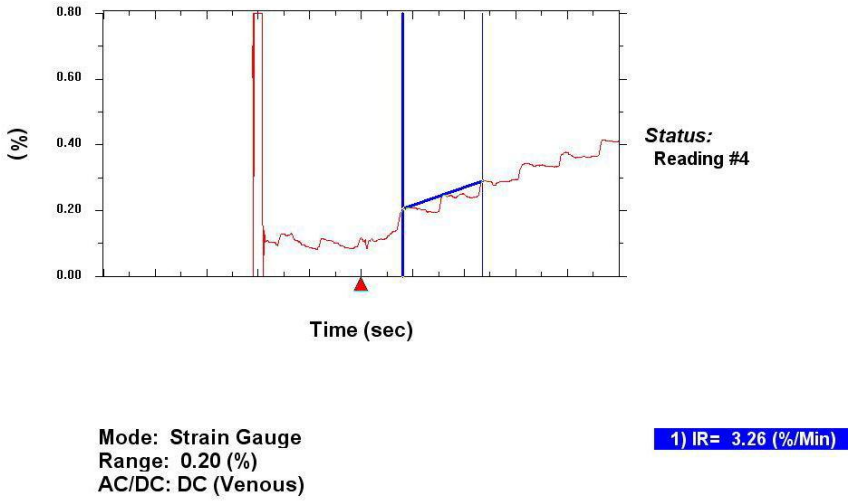


Fig 3.6 The waveforms for arterial inflow measurement

Resting forearm blood flow measurement

As mentioned above, a strain gauge was placed around the forearm at the point of greatest circumference and two cuffs were placed around the upper arm and the wrist. FBF was obtained from the mean of the all reading in every 15 sec cycle. Resting FBF was recorded for 5 minutes before the reactive (defined as baseline FBF)

Reactive hyperemia measurement (25, 39, 40, 74)

Following limb ischemia there is a rapid increase in forearm blood flow, which slowly returns to baseline values and is termed reactive hyperemia. In this study reactive hyperaemia was induced by manually inflating another occlusion cuff on the non-dominant upper arm (above venous cuff) to at least 60 mmHg above the systolic blood pressure (220 mmHg in most subjects) for a period of 5 min to occlude the FBF. After the cuff was released, FBF was measured for a 5-minute duration. There were a total of 20 data points of FBF sequentially recorded during reactive hyperemia.

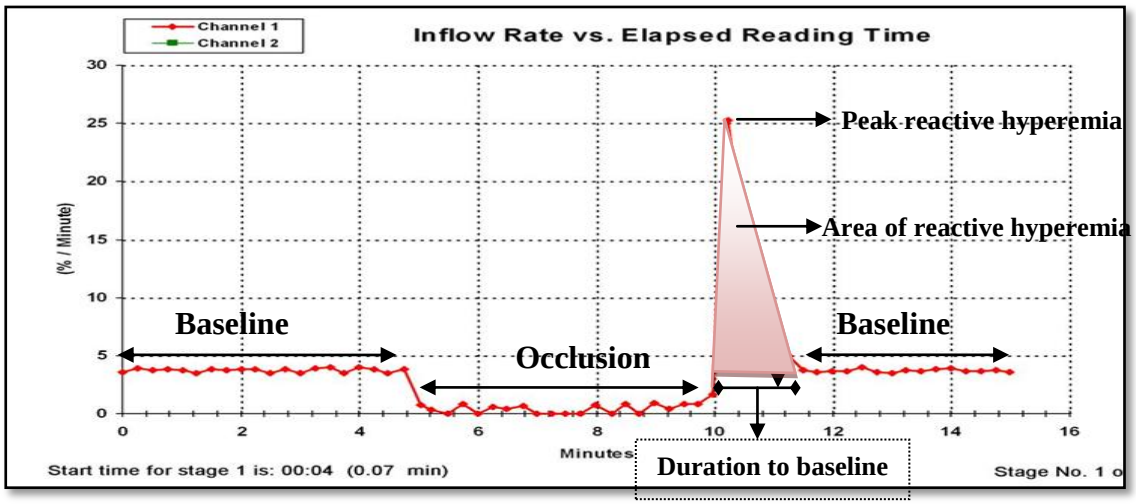


Fig 3.7 The inflow rate and elapsed reading time graph of forearm blood flow measurement

Blood collection

The collection of blood from a cubital or dorsal hand vein was done by a registered nurse using sterile equipment. The needle puncture was performed just one time and the catheter was kept securely in the vein until completing 3 times of blood collection. Each time of the blood about 5cc. (total 15 cc). The separated drown to measure the level of lipid (3 cc.) and for glucose (2 cc.). An amount of about 2 cc. of saline was injected to the catheter prevent the coagulation of blood after each draw. Blood sample for lipid profile was kept as the clotted blood and sample for measuring glucose was kept in the sodium fluoride tube in the temperature of 2-8°C before the analysis in the laboratory. The lipid profile measurement consisted of total cholesterol, triglyceride, HDL-C, LDL-C and blood sugar level during after 12 hours of fasting (0 hr.) and after 2 hours of meal (2 hr.) and 4 hours of meal (4 hr.).

Biochemistry assays

All of specimens carried to standard private laboratory (BANGKOK R.I.A. Lab) for analysis.

Blood levels of *triglycerides* were measured using Roche Diagnotics®, Germany kits. Test principle was enzymatic, colorimetric method (GPO/PAP) with glycerol phosphate oxidase and 4-aminophenazone. Triglycerides were hydrolysed by lipoprotein (LPL) to glycerol and fatty acids. Glycerol was then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalysed by glycerol kinase (GK). The oxidation of glycerol-3-phosphate was catalysed by glycerol phosphate oxidase (GPO) to formdihydroxyacetone phosphate band hydrogen peroxides (H_2O_2).

LPL

Triglycerides ----------> glycerol + fatty acids

glycerokinase

Glycerol + ATP ----------> glycerol-3-phosphate + ADP

glycerophosphate oxidase

Glycerol-3-phosphate + O2 ---------->dihydroxyacetone phosphate + H2O2

peroxidase

H_2O_2 + 4-aminophenazone + 4-chlorophenol ---> 4-(p-benzoquinone monoimino) phenazone + $2H_2O$ +HCl.

Intra-assay coefficients of variation was 1.9%

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyzecholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

cholesteryl ester hydrolase

Cholesteryl ester + H_2O ----------->cholesterol + fatty acid

cholesterol oxidase

Cholesterol + O_2 ----------> cholest-4-en-3-one + H_2O_2

peroxidase

$2H_2O_2$ + 4-aminophenazone + phenol ----------> 4-(p-benzoquinone-monoimino)-phenazone + 4 H_2O

High density lipoprotein cholesterol (HDL-C)

HDL is measured directly in serum. The basic principle of the method is as follows. The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-chol is detected under the assay conditions. The reagents are purchased from Roche/Boehringer-Mannheim Diagnostics. The method uses sulfated alpha-cyclodextrin in the presence of Mg^{+2}, which forms complexes with apoB

containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement. The reactions are as follows:

(1) ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 --->

soluble non-reactive complexes with apoB-containing lipoproteins

(2) HDL-cholesteryl esters PEG-cholesteryl esterase > HDL-unesterified

cholesterol + fatty acid

(3) Unesterifiedchol + O_2 PEG-cholesterol oxidase >cholestenone + H_2O_2

(4) H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene

diamine + H2O + H^+ peroxidase >qunoneimine dye + H_2O

Absorbance is measured at 600 nm.

LDL-cholesterol Most of the circulating cholesterol is found in three major

lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL.

[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and

HDL-cholesterol according to the relationship:

LDL-chol] = [total chol] - [HDL-chol] - [TG]/5

where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL

Maximal oxygen consumption test (VO_2max test)

Each subject was prepared for ECG monitoring prior to testing. Maximal oxygen uptake was determined on a motor driven treadmill (Nautilus™model: T 518). The expired gas was sampled continuously breath by breath for the measurement of oxygen consumption (Oxycon mobile, Cardinal Health, Germany). Oxycon mobile, was calibrated volume everyday.

After a few minutes of stretching, the subject was asked to perform a warm up on treadmill for 3-5 minutes. The VO_2max test was then started using a treadmill protocol consisting of walking or running at a speed roughly 70% age predicted maximal heart rate, with a 2% grade as stage 1. Throughout the test, the treadmill speed remained constant. The treadmill grade was increased to 6% at the second stage. While each stage was maintained for 2 minutes, the grade was increased 2% until VO_2max was achieved.

The test was terminated VO_2max was accepted when at least three of the following four factors were achieved:

a) A plateau in oxygen uptake with an increased work rate.

b) A respiratory exchanged ratio (RER) greater than 1.15

c) Achievement of age-predicted maximal heart rate±5%

[{220-age (in year)}±5%]

d) An RPE Borg Scale of 19 or 20

In addition, the test administrator subjectively evaluated and the characteristics of maximal performance if:

1. The exhaustion of the subject was expressed with body language.
2. The subject could not continue to run properly and safely because of exhaustion.

Intense aerobic exercise (20, 21)

An exercise bout was performed 1.5 hour before a standard breakfast. Volunteers performed aerobic exercise by switching between running and brick walking on the motorized treadmill which enable to adjust the speed and slope for setting to be the high level of aerobic exercise with intense intermittent aerobic exercise. The calculation of intensity was set on individual value based on the maximum heart rate from VO_2max test.

The exercise was divided into 3 stages: warm up, aerobic and cool down which took about 38 minutes as follows:

Warm up stage takes about 5 minutes by adjusting the speed and slope of treadmill until the heart rate reaches 70% of the maximum heart rate and stable for 3 minutes.

Aerobic stage was divided into 2 sub-stages: high intensity and active recovery. During the high intensity, the speed and slope of the treadmill were adjusted for the heart rate to reach 85-95% of the maximum heart rate for 4 minutes and during the active

recovery, the adjustment of speed and slope of the treadmill will let the heart rate to be at 70% of the maximum heart rate for 3 minutes, total 4 rounds.

Cool down stage took roughly 5 minutes

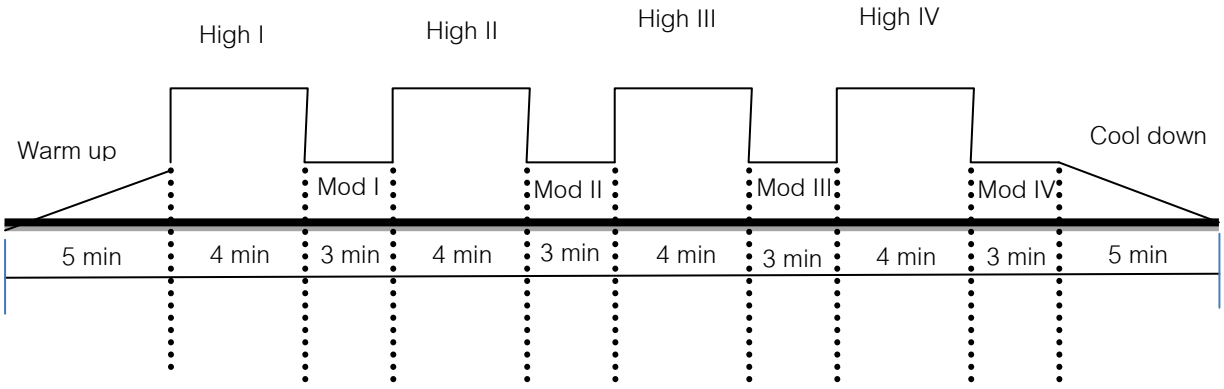


Figure 3.8 Intense interval exercise protocol (High : High intensity [85-95% of HRmax], Mod : Moderate intensity [70% of HRmax])

In the test date, Ex day, venipuncture and the measurements of forearm blood flow during fasting were done before exercise. All participants ate the prepared food after finishing exercise about 30 minutes.

Data processing and analysis

Flow measurements (FBF) were recorded for 5 sec every 15 sec. There 4 reading were obtained and average for each mean value. It takes a few seconds to obtain the first hyperemic flow, almost the first measurement for each protocol assumed as the

maximum hyperemic flow (peak blood flow), area under the curve of reactive hyperemic flow (AUC_{RH}). Reactive hyperemia blood flow was calculated as follow:

Area under the curve of reactive hyperemic flow (AUC_{RH}) using the trapezoidal rule, which was calculated the total of all data points of RH after occlusion

$$AUC_{RH} = \int_{t=0}^{t=\infty} RHt \text{ x dt}$$

When : RHt were sequential value of FBF after cuff occlusion beyond resting FBF

t was duration of 1 cycle of FBF record was 15 second

Resting forearm blood flow was estimated from 20 data points in 5 min of measurement.

$$\text{Resting FBF} = \frac{FBF_1+FBF_2+FBF_3+.....+FBF_{20}}{20}$$

Statistical analysis

All data were presented as mean ± standard deviation (S.D). Repeated- measure analysis of variance (ANOVA) was performed to examine the differences between each measurement in each of groups. The post-hoc test with Bonferroni correction was used when appropriated. An alpha level of 0.05 was used to determine statistical significant. All analyses were performed on the Statistical Package for the Social Sciences version 16.0 (SPSS, Chicago, IL, USA).

CHAPTER IV

RESULTS

In this study, 15 of normotensive offspring of hypertensive parents (OHT) and 15 of normotensive offspring of normotensive parents (ONT), who met inclusion/exclusion criteria volunteered for this study. All subjects completed the experimental protocols and measurements.

Characteristics of the subjects

The characteristics of the subjects in the OHT group and ONT group are summarized in Table 4.1. Age of 15 OHT subjects ranged from 20-28 years (mean 23±2 years) and the 15 ONT subjects aged from 22-28 years (mean 24±1 years). Both groups had similar height, weight and BMI. The VO_2max (P=0.21) and maximal heart rate (P=0.29) were not significantly different among all subjects. The blood level of cholesterol (P=0.025) and LDL-C (P<0.01) were significantly higher in OHT subjects. In contrast, HDL-C level was significantly lower in subjects from the OHT group (P=0.048). However, triglyceride (P=0.95), blood sugar (P=0.60), systolic blood pressure (P=0.17), diastolic blood pressure (P=0.19) and mean arterial pressure (P=0.17) were similar in among subjects. In the OHT group, the parental history of hypertension was dominately for one parent as opposed to both father and mother.

Table 4.1. Basic characteristics of 30 subjects

Variable	ONT(n=15)	OHT(n=15)	P value
Age (years)	24±1	23±2	P = .86
Height (cm)	172±5	172±5	P = .49
Body weight (kg)	65±8	66±8	P = .55
Body Mass Index (kg/m2)	21.8±2.1	21.9±1.8	P = .91
VO_2max (ml/kg/min)	39.2±3.5	37.1±1.8	P = .21
Maximum heart rate (BPM)	195±3	193±3	P = .29
Blood sugar (mg/dl)	79±9	78±7	P = .60
Triglyceride (mg/dl)	77±14	78±20	P = .95
Cholesterol (mg/dl)	172±14	184±14#	P < .05
LDL-C (mg/dl)	101±11	128±15#	P < .05
HDL-C (mg/dl)	54±9	48±6#	P < .05
Systolic blood pressure(mmHg)	117±5	120±6	P = .17
Diastolic blood pressure(mmHg)	74±5	77±6	P = .19
Mean arterial pressure(mmHg)	89.1±4.2	91.7±6.1	P = .17
Resting FBF(ml/100ml tissue/min)	3.55±0.2	4.02±0.9	P = .09
History of hypertensive parent(s)			
Father	-	3	
Mother	-	6	
Both	-	6	

#*P*<0.05 VS ONT group

Resting forearm blood flow (RFBF) and area under curve of reactive hyperaemia (AUC_{RH}) at baseline

At baseline, the OHT and ONT groups had similar RFBF (P= 0.09) measured before undergoing forearm ischemic procedure (Table 4.1). Also, the baseline AUC_{RH} not different between both groups (P=0.21) (Figure 4.1)

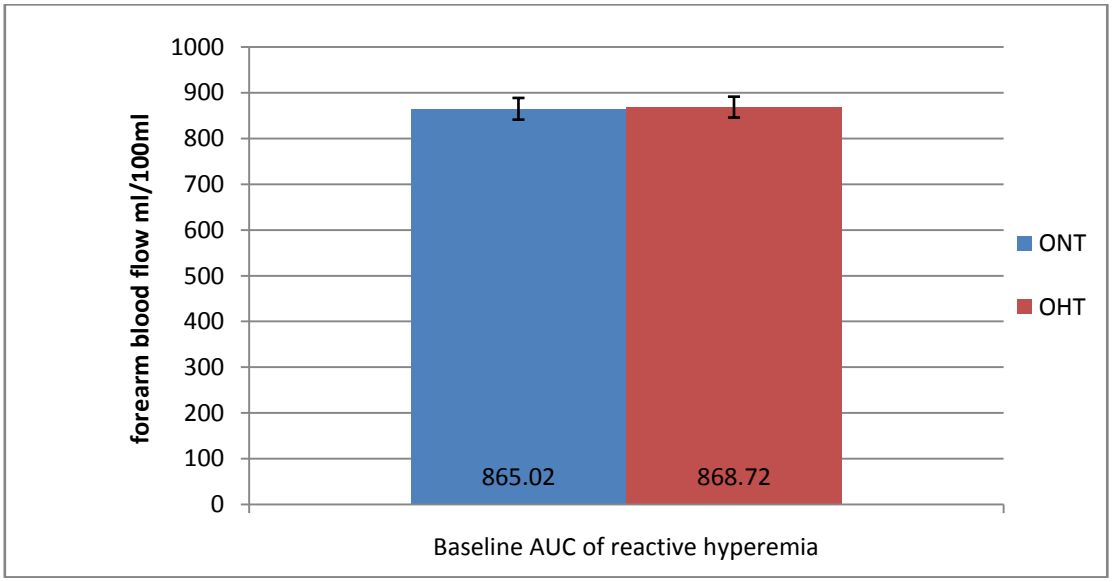


Fig 4.1 Average of baseline AUC of reactive hyperemia in both groups were similar (P=0.21).

Area under curve of reactive hyperemia during lipemia

After a high fat meal, the forearm blood flow in response to transient ischemia (reactive hyperemia) was measured and the AUC_{RH} was calculated. Both groups showed a reduction of AUC_{RH} after ingestion of high fat meal. The result showed a significant difference ($P_{ANOVA} < 0.05$) in AUC_{RH} between OHT and ONT groups at 2

hours (P = 0.048) and at 4 hours (P = 0.02) after meal. Interestingly, the reduction of AUC_{RH} in OHT was significantly greater (P<0.05) than ONT group at 4 hours.

Area under curve of reactive hyperemia during lipemia : in exercise VS no exercise

In exercise condition, an acute exercise bout of 38 min duration was performed prior to consumption of the standard meal. The result showed the magnitude of reduction of AUC_{RH} was attenuated in both OHT and ONT groups at 4 hours. Moreover, when comparing between OHT and ONT groups, AUC_{RH} in response to exercise was significantly different at 4 hours (800.1 vs 808.4 ml/100ml, P=0.032), but not at 2 hours (832.7 vs 829.2 ml/100ml, P=0.58), postprandially.

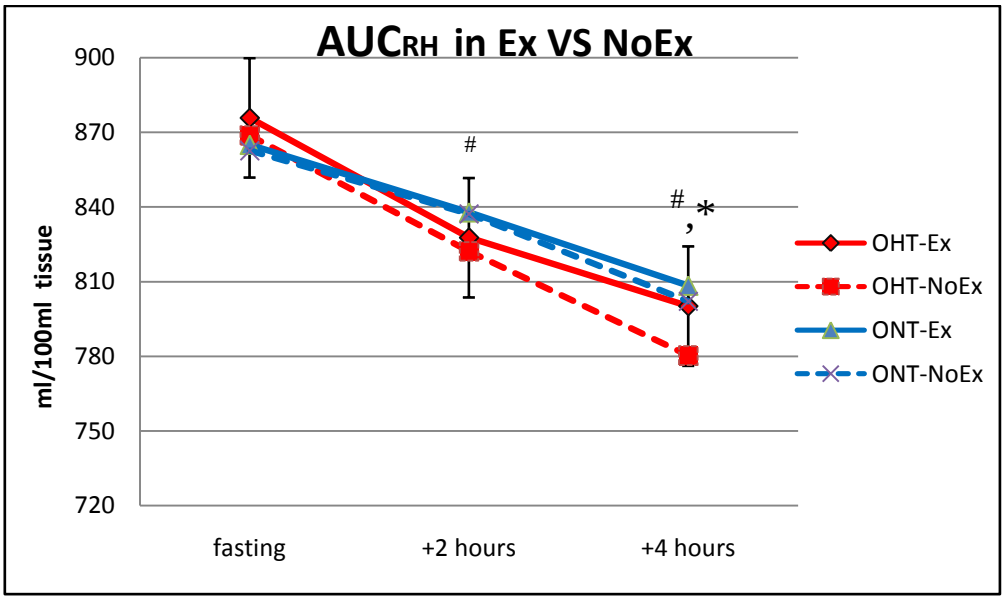


Figure 4.2 Area under curve (AUC_{RH}) in ONT and OHT : Ex VS NoEx (Fasting : AUC_{RH} at 0 hour. [before a meal], +2hours : AUC_{RH} at 2 hours [after a meal] , +4hours : AUC_{RH} at 4 hours [after a meal] * $p < 0.05$ vs NoEx, # $p< 0.05$ vs ONT group)

Triglyceride level (TG) in ONT and OHT in response to high fat meal: NoEx VS Ex

There was no significantly difference between ONT and OHT in baseline TG (P=0.375). The level of TG fasting, at 2 hours and 4 hours after meal were shown in figure 4.3, The high fat meal significantly increased TG in both OHT and ONT groups at 2 hours (P=0.014)and 4 hours (P= 0.003). Interestingly, an intense intermittent aerobic exercise 38 min. before high fat meal decreased an increment of TG in both OHT and ONT groups. The result showed a significantly greater decreasing of TG in OHT group at 4 hours (233 vs 176 mg/dl for OHT, p<0.01 and 179 vs 158 mg/dl for ONT, p=0 .21).

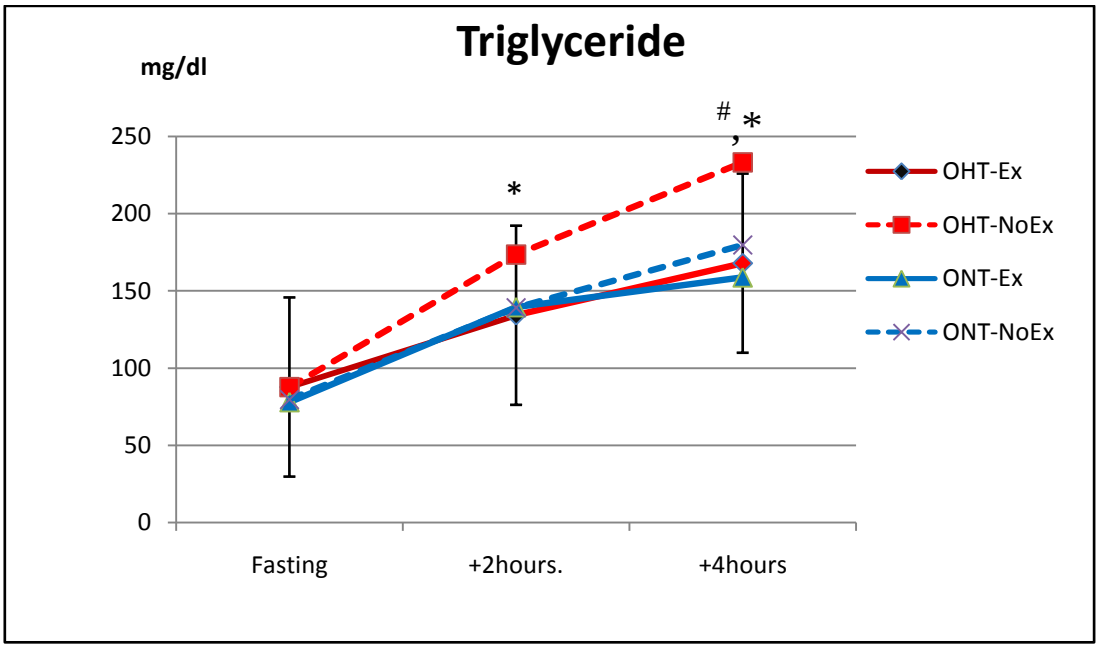


Figure 4.3 Triglyceride level in ONT and OHT : Ex VS NoEx (Fasting : TG at 0 hour. [before a meal], +2hours : TG at 2 hours [after a meal] , +4hours : TG at 4 hours [after a meal] * $p < 0.05$ vs NoEx, # $p < 0.05$ vs ONT group)

Lipid profile and blood sugar changes with meal in ONT and OHT : Ex VS NoEx

Serum levels of total cholesterol (table 4.2) and LDL-C (table 4.3) were significantly greater but HDL-C (table 4.4) was significantly lower in the OHT group at baseline, however, blood sugar (table 4.5) was not significantly different between the ONT and OHT group at baseline. The parameters did not significantly change from baseline of each group in response to the high fat meal during the control or exercise condition.

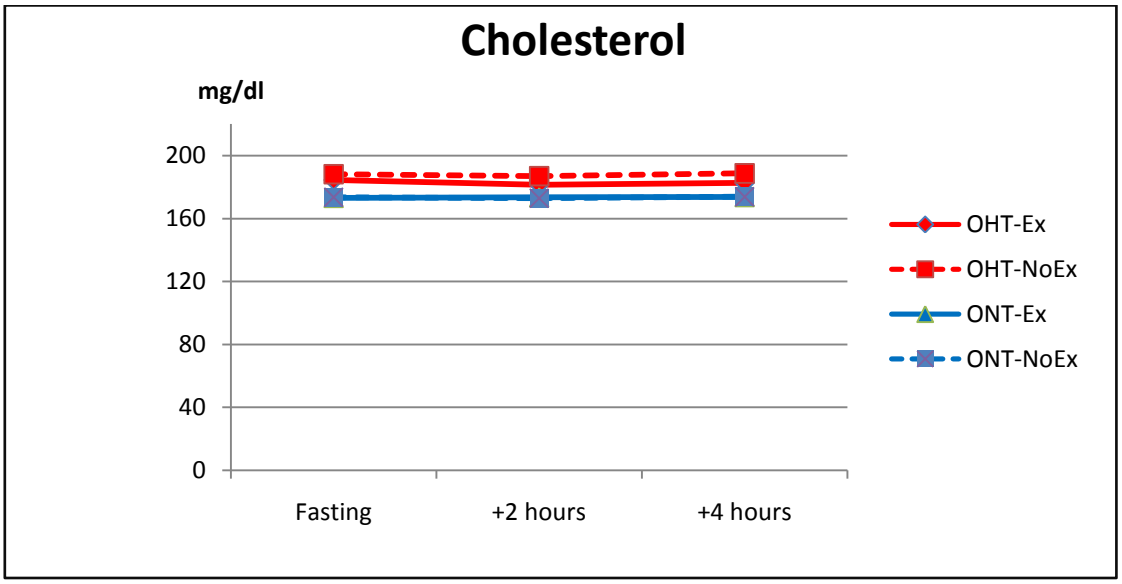


Figure 4.4 Cholesterol level in ONT and OHT : Ex VS NoEx [Fasting : cholesterol level at 0 hour.(before a meal), +2hours : cholesterol level at 2 hours (after a meal) , +4hours : cholesterol level at 4 hours (after a meal)]

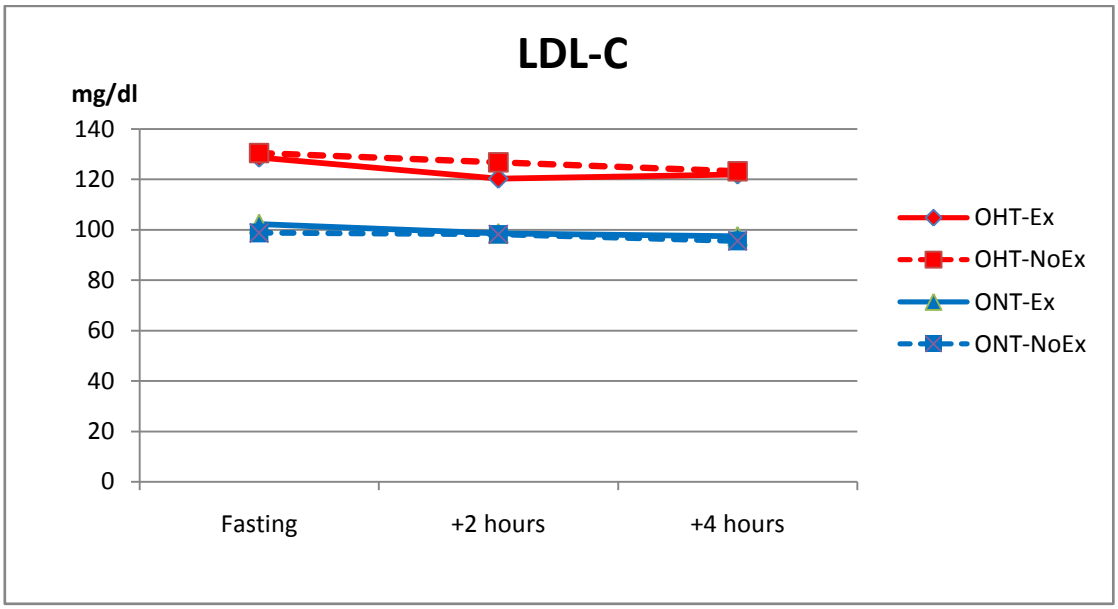


Figure 4.5 LDL-C level in ONT and OHT : Ex VS NoEx [Fasting : LDL-C level at 0 hour.(before a meal), +2hours : LDL-C level at 2 hours (after a meal) , +4hours : LDL-C level at 4 hours (after a meal)]

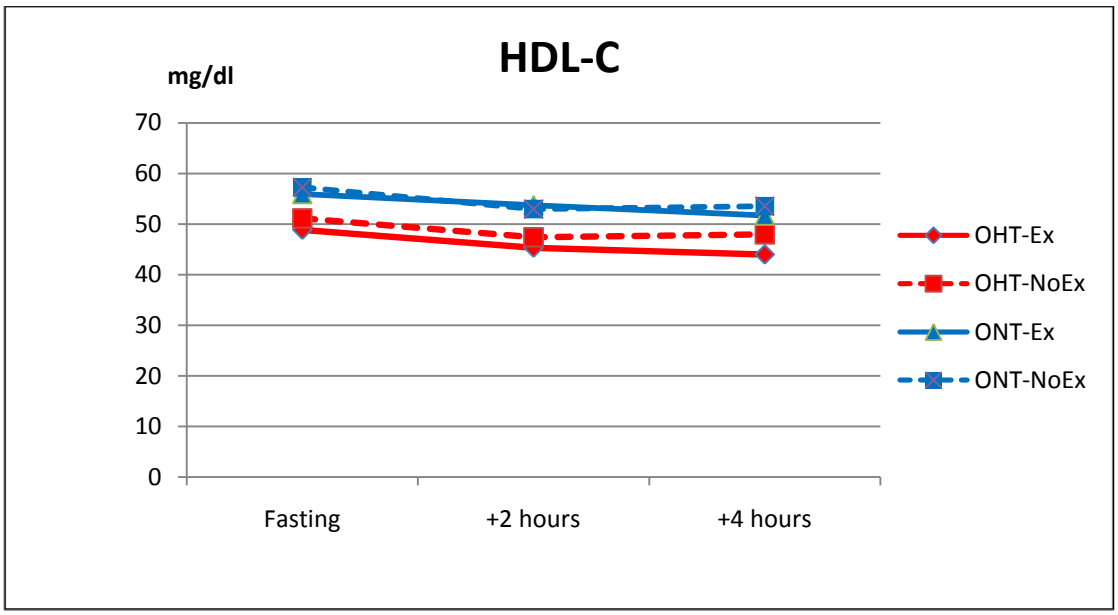


Figure 4.6 HDL-C level in ONT and OHT : Ex VS NoEx [Fasting : HDL-C level at 0 hour.(before a meal), +2hours : HDL-C level at 2 hours (after a meal) , +4hours : HDL-C level at 4 hours (after a meal)]

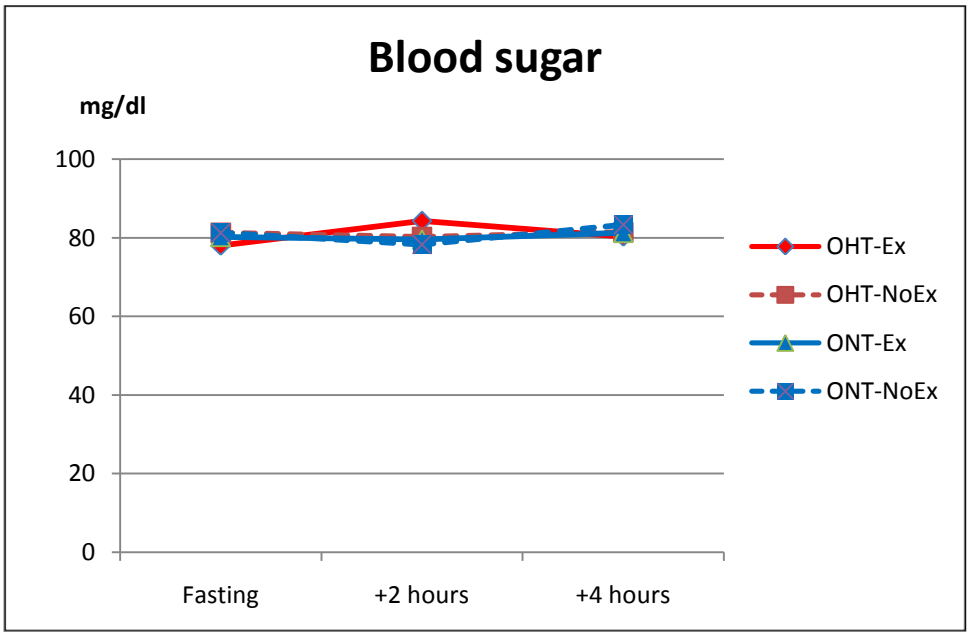


Figure 4.7 Blood sugar level in ONT and OHT : Ex VS NoEx [Fasting : Blood sugar level at 0 hour.(before a meal), +2hours : Blood sugar level at 2 hours (after a meal) , +4hours : Blood sugar level at 4 hours (after a meal)]

CHAPTER V

DISCUSSION AND CONCLUSION

In this study, we determined the effect of the prior intense interval aerobic exercise on the forearm blood flow using venous occlusion plethysmography in the offspring of hypertensive parents (OHT) and normotensive offspring of normotensive parents (ONT). It was hypothesized that endothelial function would be impaired, as indicated by a decrease in area under curve of reactive hyperemia (AUC_{RH}) following consumption of the high fat meal, and that a single bout of intense interval aerobic exercise before high fat meal would attenuate the meal effect**.** The result showed that there was a significantly greater reduction of AUC_{RH} in OHT group on postprandial lipemia state. In addition, prior intense aerobic exercise significantly decreased the magnitude of a reduction of AUC_{RH} in OHT compared to ONT group. Not only intense exercise induces high shear force which stimulates endothelial function , exercise also has an impact on postprandial lipemia which is likely to affect vascular function. The findings of the current study suggested that a reversible change in endothelial function appears to be attributed to multifactorial mediated mechanisms.

Characteristics of the study population

Baseline characteristics showed that all subjects had normal fasting triglyceride and blood sugar levels. However, at baseline, there was a significant difference of cholesterol and LDL-C levels between ONT and OHT. Interestingly, 6 of

OHT and 4 of ONT had dyslipidemia in their parental history.This was somewhat similar to previous study (75). It was suggested that the association of the offspring dyslipidemia with parental dyslipidemia probably due to genetic predisposition (75).This may be considered as the study limitation as history of dyslipidemia was not an exclusion. Despite some parameters on lipids, baseline (resting) forearm blood flow and reactive hyperemia were similar between ONT and OHT.

Effect of the high fat meal on postprandial lipid levels

One of the key events in the genesis of CVD is the transient postprandial hypertriglyceridemia observed after fat-rich food ingestion, which is equally relevant to younger populations given that the process of atherosclerosis typically begins in the first decade of life. The present study showed that a standard high fat meal incurred increasing of triglyceride level in both groups at 2 and 4 hours postprandially. However the OHT group was affected by high fat meal more than the ONT group. The Oslo study(76)reported that hypertensive persons have higher baseline blood lipid concentrations than normotensive individuals. Moreover Kalovou et al. (10) found that the untreated middle-aged men with uncomplicated essential hypertension have an abnormal response to a high fat meal increased triglyceride levels and delayed triglyceride clearance. It may be possible that a greater elevation of triglyceride levels induced by standard high fat meal in the OHT group is related to subclinically abnormal vascular function inherited from parents. Also a possibility of genetic predisposition on

abnormal lipid response in hypertensive offspring can not be excluded. It should be noted that other lipids including cholesterol, HDL-C, LDL-C did not change with meal in both groups. Similarly blood sugar level did not changed in 2 and 4 hours postprandially. This could be due to a limited carbohydrate content in the tested meal.

Effect of the high fat meal on forearm blood flow

Although forearm blood flow response to vascular occlusion (AUC_{RH}) was significantly attenuated in both groups after ingestion of a standard high fat meal, it was significantly lower in the OHT group. One possible mechanism of diminished forearm blood flow in response to transient ischemia after the ingestion of a standard high fat meal maybe related to increased free fatty acid (FFA) which affects nitric oxide (NO) production and reduces NO bioavailability (50). This was similar to the previous study(77) that showed a decreased forearm blood flow in response to vascular occlusion after ingestion of a high fat meal in healthy individuals (77). It was suggested to be due to the influence of thrifty genes (78). Meanwhile the high fat meal also affected endothelial function as showed by Vogel et al. (79), who investigated flow-mediated vasoactivity in 2 and 4 hours after a high fat meal. They found that impaired endothelial function had a significant correlation with elevation of triglyceride level (79).

Effect of intense interval aerobic exercise on postprandial lipemia

It has been known that exercise diminishes postprandial lipemia. For example Padilla et al. (80) examined the effect of prior aerobic exercise on postprandial values of triglyceride (TG) in healthy adults and found a decreased level of TG with exercise (80). In this study we found similar results, as report by previous studies (80, 81). TG was significantly decreased in OHT at 2 and 4 hours postprandially in Ex condition. Interestingly, TG was only slightly decreased in ONT. We suggested it is that postulated enhanced TG clearance and/or increased lipoprotein lipase (LPL) activity as a result of exercise may occur differently between ONT and OHT.

Effect of intense interval aerobic exercise on postprandial forearm blood flow

Exercisehas been shown to augment endothelium-dependent vasodilation through an increase in nitric oxide (NO) production (50). However, there has no report whether or not impaired vascular function induced by high fat meal consumption can be improved by exercise, particularly in individuals at high risk of hypertension.In this study we found that intense aerobic exercise prior to meal improved AUC_{RH} at 4 hour potprandially in both groups.This important finding suggested that exercise can improve vasodilating property of the vessels regardless of underlying status of endothelial function.As report by previous studies, the mechanisms may include improved plasma nitric oxide (82), increased antioxidant levels (20) or reduced synthesis and secretion of VLDL triglycerides in the liver (21). However, other studies have

reported discrepancies on the effect of exercise on oxidative stress and NO bioavailability. Goto et al. (83) demonstrated that changes in forearm blood flow, in response to exercise, depend on exercise intensities. Theysuggested that in humans, moderate-intensity exercise induces vasodilation through an increase in NO bioavailability and that high-intensity exercise increases oxidative stress (83). In contrast, the present study found a significantly improved AUC_{RH} with acute high intensity exercise in normotensive young men with or without family of hypertension. Interestingly, OHT demonstrated a greater improvement in postprandial AUC_{RH} in response to exercise. This supports the idea that there are reverse “hemodynamic, metabolic and hormonal alterations that are involved in the pathophysiology of hypertension” (29), which appear to response differently in subjects with and without genetic predisposition of vascular abnormality.

Conclusion

In summary, a single bout of intense aerobic exercise prior to a high fat meal effectively reduces postprandial TG and improve area under curve of reactive hyperemia in both ONT and OHT groups but greater in OHT group. Importantly, the finding is OHT group response to high fat meal and impair endothelial function more than ONT group that is confirmed OHT group is related to subclinically abnormal vascular function inherited from parents. However, our study manifest a single bout of intense aerobic exercise not only reduce TG level but improve vascular function in OHT group. To address the different effect of the high level of aerobic exercise on the blood lipid levels and the forearm blood flow after a meal of the offspring of hypertensive parents and those of normotensive parents. This ground information may provide important insight onto future research regarding lifestyle intervention for specific population.

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APPENDICES

APPENDIX A

Data of lipid and blood sugar parameters shown as Mean±SD

Parameters	Group/Condition	Fasting (mg/dl)	+2 hours (mg/dl)	+4 hours (mg/dl)
Cholesterol	ONT/Ex	173.1±13.9	173.5±12.5	173.6±11.9
	ONT/NoEx	173.5±15.9	172.8±15.9	173.6±13.1
	OHT/Ex	184.5±14.5	181.5±13.9	182.6±17.1
	OHT/NoEx	188.1±12.1	187±15.1	188.6±11.6
LDL-C	ONT/Ex	102.2±10.9	98.6±9.7	97.4±10.8
	ONT/NoEx	98.7±17.3	98.1±17.5	95.5±16.7
	OHT/Ex	128.6±15.1	120.2±14.9	121.9±16.9
	OHT/NoEx	130.4±15.7	126.7±19.9	123.2±21.8
HDL-C	ONT/Ex	55.9±9.6	53.7±9.3	51.6±8.7
	ONT/NoEx	57.2±11.2	52.9±10.2	53.3±10.3
	OHT/Ex	48.8±6.1	45.3±5.8	44.0±7.1
	OHT/NoEx	51.1±8.1	47.4±6.1	48.0±7.1
Blood Sugar	ONT/Ex	80.2±9.1	79.6±10.9	81.2±6.8
	ONT/NoEx	78.2±7.8	83.3±9.1	78.1±5.4
	OHT/Ex	78.1±7.9	82.6±9.9	80.3±8.7
	OHT/NoEx	81.2±8.1	80.2±9.1	81.4±7.8

APPENDIX B

Screening Questionnaire

(แบบสอบถามเพื่อการคัดกรอง)

ลำดับที่ ....

วันที่.....เดือน.....พ.ศ. .....

ตอนที่ 1 ข้อมูลเกี่ยวกับผู้ตอบแบบสอบถาม

1. วัน/เดือน/ปีเกิด..... อายุ.....ปี.....

เดือนเชื้อชาติ.....สัญชาติ.....อาชีพ.....

2. น้ำหนัก..... กิโลกรัม ส่วนสูง.....เมตร BMI…… kg/m^2

ตอนที่ 2 ข้อมูลเกี่ยวกับสุขภาพ

โปรดตอบคำถามต่อไปนี้ตามความเป็นจริง โดยทำเครื่องหมาย ✓ลงใน ❒ หรือเติมข้อความลงในช่องว่างที่เว้นไว้

1) ท่านมีโรคประจำตัวหรือไม่

❒ ไม่มี ❒ มี โปรดระบุ.....

2) ท่านตรวจร่างกายครั้งล่าสุด โดยมีผลของระดับไขมัน,น้ำตาลในเลือดและ คลื่นไฟฟ้าหัวใจ(EKG)เมื่อไร

❒น้อยกว่า 6 เดือน ❒มากกว่า 6 เดือน❒ไม่เคยได้รับการตรวจมาก่อน

3) บิดาของท่านเป็นความดันโลหิตสูงโดยมีความดันโลหิต ≥ 140/90 mmHg. และได้รับการวินิจฉัยโดยแพทย์หรือไม่

❒ ไม่มีตอบคำถามข้อ 6 ❒ มี ตอบคำถามข้อ 4

4) บิดาของท่านได้รับการวินิจฉัยว่าเป็นความดันโลหิตสูงก่อนอายุ 55 ปีหรือไม่

❒ใช่ ❒ ❒ไม่ใช่ ❒ไม่ทราบ

5) บิดาของท่านได้รับยาลดความดันโลหิต(antihypertensive) หรือไม่

❒ได้รับ ❒ ❒ไม่ได้รับ

6) มารดาของท่านเป็นความดันโลหิตสูงโดยมีความดันโลหิต ≥ 140/90 mmHg. และได้รับการวินิจฉัยโดยแพทย์หรือไม่

❒ไม่มีตอบคำถามข้อ 9 ❒ มี ตอบคำถามข้อ 7

7) มารดาของท่านได้รับการวินิจฉัยว่าเป็นความดันโลหิตสูงก่อนอายุ 65 ปีหรือไม่

❒ใช่ □ ❒ไม่ใช่ ❒ไม่ทราบ

8) มารดาของท่านได้รับยาลดความดันโลหิต(antihypertensive) หรือไม่

❒ได้รับ ❒ไม่ได้รับ

9) ท่านรับประทานยา/ วิตามิน หรืออาหารเสริมอยู่หรือไม่

❒ ไม่ ❒รับประทานโปรดระบุ.....

10) ท่านออกกำลังกายหรือไม่

❒ ไม่ ❒ ใช่

11) ท่านออกกำลังกายชนิดใด

❒ เดิน ❒ วิ่ง ❒ว่ายน้ำ ❒ ปั่นจักรยาน

❒ อื่นๆ.....

12) ท่านออกกำลังกายกี่ครั้งต่อสัปดาห์

❒<3ครั้งต่อสัปดาห์ ❒≥3ครั้งต่อสัปดาห์

13) ท่านออกกำลังกายเป็นระยะเวลาเท่าไรต่อครั้ง

❒<30 นาที ❒≥30 นาที

14) ขณะนี้ท่านมีปัญหาสุขภาพ และ/หรือมีภาวะเครียดหรือไม่

❒ ไม่มี ❒ มี

15) ท่านสูบบุหรี่หรือไม่

❒ ไม่เคยสูบ ❒ สูบ.....มวน/วัน เป็นระยะเวลา.....ปี

❒ เลิกสูบมาแล้ว ..... ปี ❒อื่นๆ โปรดระบุ.....

16) ท่านดื่มเครื่องดื่มที่ผสมแอลกอฮอล์หรือไม่

❒ ไม่เคยดื่ม ❒นานๆครั้งโปรดระบุ.....

❒ ดื่มเป็นประจำ

BIOGRAPHY

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