จุฬาลงกรณ์มหาวิทยาลัย

ทุนวิจัย
กองทุนรัชดาภิเษกสมโภช

รายงานผลการวิจัย

ผลกระทบของการออกกำลังกายต่อระดับไขมันในเลือดในคนไทยที่มี Apo E จีโนไทป์แบบต่างๆ

โดย

อ.ดร. นันทรี ชัยชนะวงศาโรจน์

เดือน กรกฎาคม ปีพ.ศ. 2547

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กิตติกรรมประกาศ

งานวิจัยนี้ได้รับการสนับสนุนจากกองทุนรัชดาภิเษกสมโภช จุฬาลงกรณ์มหาวิทยาลัย ขอขอบคุณอาสาสมัครทุกท่านที่ได้เสียสละในการเข้าร่วมการศึกษาวิจัยนี้ ขอขอบคุณภาควิชาเวชศาสตร์การธนาคารเลือด คณะสหเวชศาสตร์ จุฬาลงกรมณ์มหาวิทยาลัย ที่เอื้อเฟื้อสถานที่ อุปกรณ์ และ เครื่องมือในการวิจัย ขอขอบคุณ นางสาว วรารัตน์ เชียงจง ในฐานะผู้ช่วยวิจัย และลูกศิษย์ที่ปรึกษางานวิจัย ที่ได้ช่วยทำการวิจัยจนแล้วเสร็จด้วยดี

ชื่อโครงการวิจัย	ผลกระทบของการออกกำลังกายต่อระดับไขมันในเลือดในคนไทยที่มี Apo Eจีโนไทป์แบบต่างๆ
ชื่อผู้วิจัย	อ.ดร.นันทรี ชัยชนะวงศาโรจน์
เดือนและปีที่ทำวิจัยเสร็จ	กรกฎาคม พ.ศ. 2547

บทคัดย่อ

อะโปไลโปโปรตีน อี (อะโปอี) เป็นหนึ่งในหลายยีนที่มีความเกี่ยวข้องกับภาวะเสี่ยงของโรคหัวใจและหลอดเลือด อย่างไรก็ตามปฏิกิริยาระหว่างยีนและปัจจัยแวดล้อม เช่น การออกกำลังกาย อาหาร และยาลดระดับไขมันอาจมีผลต่ออัตราการเกิดโรคได้ วัตถุประสงค์ในการศึกษาคือเพื่อดูผลกระทบของระดับการออกกำลังกายกับความหลากหลายของยีนอะโปอีต่อระดับไขมันและระดับน้ำตาลกลูโคสในเลือดในคนไทยวัยผู้ใหญ่ที่สุขภาพแข็งแรงจำนวน 273 คน ความเข้มข้นของไขมันและน้ำตาลวัดโดยใช้ปฏิกิริยาเอมไซม์และวิธีเทียบสี ส่วนฟีโนไทป์ของอะโปอีใช้วิธีพีซีอาร์-อาร์เอฟแอลพี ดัชนีการออกกำลังกายคำนวณจากข้อมูลการออกกำลังกายซึ่งได้จากแบบสอบถาม ความถี่ของอะโปอียีนในประชากร 273 คน คือ E3/E3 78%, E2/E3 7%, E2/E4 5.9%, E3/E4 8.8%, E4/E4 0.7%. จีโนไทป์ของอะโปอีมีความสัมพันธ์กับโคเลสเตอรอลรวมและLDLที่ระดับนัยสำคัญ 0.05 พบว่าการมีอัลลีลE4จะทำให้มีระดับโคเลสเตอรอลรวมและLDLสูง (ที่ระดับนัยสำคัญ 0.05) และพบว่าระดับโคเลสเตอรอลรวมและLDLที่ตอบสนองต่อการออกกำลังกายนั้นเปลี่ยนแปลงตามชนิดของฟีโนไทป์ของอะโปอีด้วย การออกกำลังกายไม่มีผลต่อระดับโคเลสเตอรอลรวมและLDLในผู้ที่มีอัลลีล ε4 ของ ดังนั้นฟีโนไทป์ของอะโปอีมีอิทธิพลต่อผลของการออกกำลังกายต่อระดับโคเลสเตอรอลรวมและLDL ในประชากรไทยสุขภาพแข็งแรงที่มีการออกกำลังกายอยู่ในระดับปกติ

Project Title	The role of exercise on serum lipid concentrations varies with Apo E genotype : A study in Thais subjects.
Name of the Investigators	Dr. Nuntaree Chaichanawongsaroj
Year	July 2004

Abstract

Apolipoprotein E (apo E) is one of several candidate genes involved in cardiovascular risk. However, the interaction between genes and environmental factors, such as exercise, dietary and lipid lowering intervention, may be affected the incidence of the disease. Our aim was to investigate the effect of physical activity (PA) and apo E polymorphism on plasma lipids and plasma glucose levels in 273 healthy, adult Thais. Plasma lipid and glucose concentrations were determined by enzymatic–colorimetric methods and apo E phenotypes by PCR-RFLP. A PA index was calculated from exercise data which assessed by a questionnaire. Out of 273 subjects, apo E genotype frequencies were 78% for E3/E3, 7% for E2/E3, 5.9% for E2/E4, 8.8% for E3/E4, and 0.7% for E4/E4. Apo E genotypes were associated with total cholesterol (TC) and low-density lipoprotein (LDL), $P<0.05$. The presence of ε4 allele was related to higher TC and LDL ($P<0.05$). TC and LDL responses to exercise varied with apo E phenotypes. PA did not effect on TC and LDL in ε4 carrier. Thus, apo E phenotype partly determines the effect of PA on plasma TC and LDL in healthy, adult Thais with regular PA.

Introduction

Atherosclerosis is the cause of heart attacks, stroke, aortic aneurysms, and peripheral vascular disease, which together represent the most frequent causes of death in the industrialized world. Serum lipid concentrations have been linked to early arterial lesions in the aorta and coronary arteries. An atherogenic lipid profile is defined as a pattern of elevated serum cholesterol (TC) and triglyceride (TG) levels with an elevation of high-density lipoprotein cholesterol (HDL-C). Plasma lipid and lipoprotein concentrations are determined be genetic and environmental factors such as diet and physical activity (PA). Apolipoprotein E (apo E) determines serum TC and LDL cholecterol (LDL-C) concentrations and contributes to Coronary heart disease (CHD) risk. Apo E is a ligand for lipoprotein receptor. Physiologically, its most important function is to mediate specific uptake of plasma very-low-density lipoproteins (VLDL), chylomicron remnants, and intermediate-density lipoprotein (IDL) by the liver. Three major apo E isoforms, E2, E3, and E4 exist in plasma. These isoforms are coded by three codominant alleles, E2, E3, and E4, resulting in six major apo E phenotypes: E2/2, E3/2, E4/2, E3/3, E4/3, and E4/4. Apo E4 isoform is associated with high serum TC and LDL-C concentrations in most but not all populations. Previous intervention studies have shown that an increase in PA decreases body weight, percentage of body fat, serum TC, LDL-C, apo B, TG, and insulin concentrations and increases serum HDL-C and apo A-I concentrations. Responses to exercise may be mediated in large part by variation in genes. The aim of this study was to evaluate the effect of PA on plasma lipids in different apo E phenotype in Healthy adult Thais.

Survey of Related Literature

1. Lipoprotein

Lipoproteins are globular particles of varying size and composition. Their outer surface is hydrophilic and their inner core, which contains immiscible lipids, is hydrophobic. The surface of lipoprotein particles contains an amphipathic phospholipids bilayer, non-esterified cholesterol, and apolipoproteins. The core consists of cholesteryl esters and TG. Lipoproteins are synthesized in the liver, in the intestine, arise from metabolic changes of precursor lipoproteins, or are assembled at the cell membranes from cellular lipids or exogenous lipoproteins or apolipoproteins (1).

1.1 Lipoprotein classes

Lipoproteins are characterized by their size, density flotation constant and electrophoretic mobility (Table 1).

Table 1 Lipoproteins classes

Lipoprotein	Density (kg/l)	Particle diameters	Flotation rate (SI)	Electrophoretic mobility
Chylomicrons	<0.95	80-1200	>400	Origin
VLDL	0.95-1.006	30-80	60-400	Pre-beta
IDL	1.006-1.019	23-35	20-60	Broad beta
LDL	1.019-1.063	18-25	0-20	Beta
HDL	1.063-1.21	5-12	0-9	Alpha

Ultracentrifugation separates lipoproteins in plasma into five classes which are chylomicrons, very low-density lipoprotein (VLDL), intermediate-density

lipoprotein (IDL), low-density lipoproteins (LDL), and high density lipoproteins (HDL).

1.2 Apolipoproteins

The apolipoproteins found in plasma are classified into two broad types: the non-exchangeable and the exchangeable (or soluble) apolipoproteins. Apolipoprotein B-100 (apo B100) and apo B48, the principle protein components of LDL, VLDL, Lp(a) and chylomicron are non-exchangeable apolipoproteins. These non-exchangeable apolipoproteins circulate bound to the same lipoprotein particle through various metabolic transformations in plasma, until they are cleared, as lipoproteins, via specific receptor. In contrast, the exchangeable apolipoproteins (eg., apo A1, apo A2, apo Cs, apoE) have much smaller molecular masses than apo B100 or apo B48, are more or less soluble in water in their delipidated states, can transfer between lipoprotein particles, and can acquire lipids while in circulation. The common function of all apolipoproteins is to help solubilize neutral lipids in the circulation. Major human apolipoproteins are shown in Table 2.

The well-known functions of apolipoproteins involve in lipid binding and solubilization, modulation of enzymatic activities, and receptor recognition. Other functions have been described, for example, apo E has been implicated in in situ nerve repair and regeneration, as well as in plaque formation in Alzheimer,s disease. Apo(a) may have a function in the clotting process, while apo A4, produced in the intestine and hypothalamus, may have a role in signaling satiety in the fed state (2-4).

Table 2. Major human apolipoproteins

Apolipoprotein	Molecular weight	Lipoprotein class	Concentration in plasma (mg/dl)
Apo A1	28,100	HDL, CM	130
Apo A2	17,400	HDL	40
Apo A4	44,500	CM	15
Apo (a)	3-8 X 105	Lp(a)	0.1-40
Apo B100	512,000	LDL, VLDL	250
Apo B48	242,000	CM	
Apo C1	6,600	VLDL, CM	3
Apo C2	9,000	VLDL, CM	12
Apo C3	9,000	VLDL, CM	12
Apo D	22,000	HDL	12
Apo E	34,200	VLDL, CM, HDL	7

1. 3 Lipoprotein metabolism

A general schema of human lipoprotein metabolism is presented in Figure 1. There are three pathway of lipoprotein metabolism consisting of the exogenous pathway, the endogenous pathway, and the reverse cholesterol transport pathway. The exogeneous pathway involves transport of dietary lipid from the intestine to the liver. The endogenous pathway involves transport of lipids synthesized in the hepatocytes to peripheral tissues. The two pathways overlap at the stages of hydrolysis by lipoproteinlipase (LPL) in the periphery, and by hepatic triglyceride lipase (HTGL) in the liver. Reverse cholesterol transport is the pathway of cholesterol removal from the peripheral tissues to the liver.

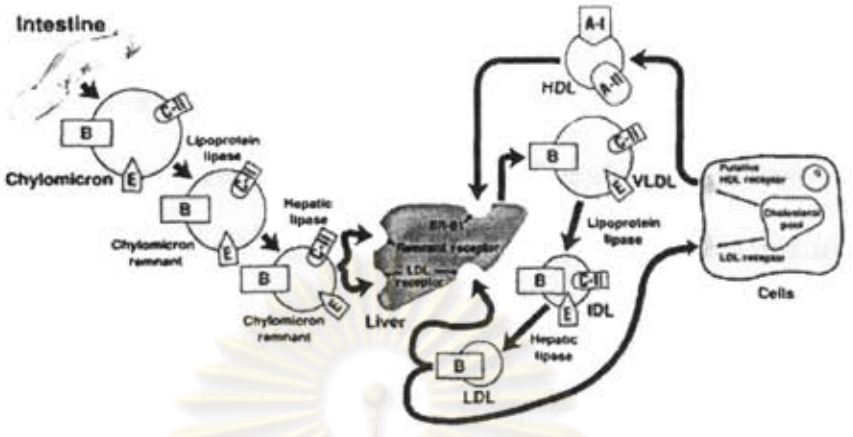

Figure 1. Human lipoprotein metabolism.

Dietary triacylglycerol, cholesteryl ester, and phospholipids are emulsified in the intestine by bile salts, their fatty acids are hydrolyzed by pancreatic lipases, and the resultant molecules (fatty acids, 2 monoacylglycerols, and cholesterol) are taken up by intestinal cells. Triacylglycerol and cholesteryl ester are reformed in the intestinal cells and packaged into chylomicrons for excretion into lymph and then blood. Apo B-48, A-I, and A-IV are on the surface of chylomicrons. Apo B-48 is essential for secretion of chylomicrons from the intestine. Once in the circulation, apo C-II, a cofactor for lipoprotein lipase (LPL) is transferred from HDLs to chylomicrons, a process facilitated by apo A-IV. Triacylglycerols in chylomicrons are hydrolyzed within minutes by LPL, which is located on the surface of endothelial cells lining the capillaries of adipose and other peripheral tissues, such as muscle. As the triacylglycerols are hydrolyzed, chylomicron remnants are produced, which are rapidly taken up by the liver through the interaction of apo E on the surface of the chylomicron remnant

with the chylomicron receptor. The released fatty acids are either taken up and stored in adipose tissue for future use or utilized by muscle for energy.

Once inside the liver, the cholesteryl esters in the chylomicron remnants are hydrolyzed and the liberated unesterified cholesterol down-regulates the genes for both the LDL receptor and the rate-limiting enzyme of cholesterol biosynthesis, hydroxymethylglutaryl-CoA (HMG-CoA) reductase. Thus, the potential effect of dietary cholesterol to increase LDL concentrations is offset by the inhibition of hepatic cholesterol synthesis. However, in 1 in 4 individuals, HMG-CoA reductase is not down-regulated as efficiently, leading to higher concentrations of LDL cholesterol. The saturated fatty acids from the chylomicron remnants, or those that are eventually mobilized from adipose tissue, appear to down-regulate the LDL receptor, leading to an increase in LDL concentrations.

In liver, the triacylglycerol-rich VLDLs are assembled and secreted as particles containing triacylglycerol and cholesteryl ester in the core, surrounded by apo B-100, E, C-I, C-II, and C-III. The fatty acids attached to the glycerol moiety of the triacylglycerol may be derived from either the endogenous synthesis of fatty acids from acetyl CoA or the mobilization of fatty acid from adipose tissue back to the liver. Acetyl CoA derived from amino acids, sugars, and fatty acid oxidation can be used to synthesize fatty acids. Apo B-100 is essential for the secretion of VLDL. Once in the circulation, the triacylglycerol in VLDL is hydrolyzed by LPL and apo C-II, producing fatty acids and the VLDL remnant. The triacylglycerol in the VLDL remnant can be further hydrolyzed to smaller particles called intermediate

density lipoproteins (IDLs), which are either taken up by the interaction of apo E with the LDL (B, E) receptor on the surface of the liver or converted by the action of hepatic lipase (hepatic triacylglycerol lipase) into the cholesteryl ester–rich LDL (1-5).

LDLs are then bound and internalized as the result of the interaction of apo B-100 with the LDL (B,E) receptor. The cholesteryl esters in the core of LDL are hydrolyzed in lysosomes, producing unesterified cholesterol, which can down-regulate the LDL receptor and HMG-CoA reductase genes through the sterol regulatory element. About two-thirds of LDL is removed by the liver and the rest by peripheral tissues. LDL also serves as the major carrier of vitamin E. Elevated concentrations of LDL, and its oxidized derivative, promote atherogenesis by causing endothelial dysfunction, proliferation of arterial smooth muscle cells, and conversion of monocytes into macrophages in the arterial wall, inducing foam cell formation (6). HDL is secreted from both intestine and liver as nascent particles. These nascent particles contain phospholipids and some unesterified cholesterol in the core, surrounded by a coat of apo A-I. Nascent HDL particles appear to have preß mobility on electrophoresis, and 9 subclasses of nascent HDL have been described (7). Once in the bloodstream, nascent HDLs are converted to a mature form of HDL through the interaction of the enzyme phosphatidylcholine–sterol *O*-acyltransferase (also called lecithin-cholesterol acyltransferase, or LCAT) and its cofactor, apo A-I. Unesterified cholesterol is removed from peripheral cells and esterified through the action of LCAT and apo A-I, producing cholesteryl ester in the core of more mature HDL particles.

During the process of lipolysis, apo C-I, C-II, and C-III can be transferred, along with phospholipids, from VLDL to the mature HDL particle. As more cholesteryl ester is formed, the subfraction of HDL called HDL_3 is converted to HDL_2. The structure and chemical composition of HDL can also be modified by cholesteryl ester transfer protein (CETP), in which a molecule of cholesteryl ester from HDL is exchanged for a molecule of triacylglycerol on VLDL or a VLDL remnant. The triacylglycerol content of the HDL_2 particles is often higher than that in HDL_3. HDL_2 can be converted back to HDL_3 through hydrolysis of triacylglycerol by hepatic lipase(5).

The cholesteryl ester in the core of HDL can be delivered to sterol-producing cells, such as liver, adrenal glands, ovary, and testis, through the interaction of apo A-I with a putative HDL receptor known as SRB-I (scavenger receptor, class B, type I). This process, often referred to as reverse cholesterol transport, promotes the delivery of cholesteryl ester to the cell. HDL is subsequently released from cells back into the bloodstream to gather more cholesteryl ester. At some point, the entire HDL particle is internalized for catabolism.

2. Atherosclerotic risk factors

Atherosclerosis is the leading cause of morbidity and mortality in the industrial world. CHD is now thought to be primary a problem of dysfunctional coronary endothelium rather than a simple accumulation of lipids as was thought previously. This dysfunctional endothelium then leads to inflammation, lipid accumulation, and fibromuscular hyperplasia, the foundation for a coronary

atherosclerotic plaque formation. The risk factors for artherosclerotic cardiovascular disease are as follows (8).

2.1 Hypercholesterolemia

Lipoproteins are high-molecularweight complexs that contain both lipid and protein. The role of lipoproteins in normal physiology includes transport of lipids to cells for energy, assistance in normal growth, and storage of energy. LDL plays a particularly important role in atherogenesis because of its effect on the influx and efflux of lipids into the vessel wall. Inaddition, increased LDL-C concentrations may promote thrombus formation, which is the final step in most acute coronary events, Another lipoprotein, HDL is beginning to seen as an important factor in protection against atherosclerosis progression. HDL promotes cholesterol efflux from atherosclerotic lesions. In addition, HDL inhibits the oxidation and subsequent accumulation of LDL.

2.2 Hypertension

Increased systematic blood pressure has been established clinically as a risk factor for atherosclerotic heart disease. Hypertension increases the progression of coronary vascular disease by promoting endothelial dysfunction. High blood pressures attenuate the coronary vessels' response to endothelium-derived vasodilators and increase vascular permeability to macromolecules including LDL. In addition, high blood pressures increase the production of endothelin, which plays an important role in artherogenesis.

Diabetes Mellitus

Insulin resistance in patients with Type 2 diabetes or in patients with poorly controlled Type 1 diabetes leads to hyperinsulinemia. This pathology

elevates certain growth factors such as insulin growth factor 1. These growth factors, in the presence of hyperglycemia, promote proliferation of the fibromuscular components of the growing atherosclerotic lesion. Although absolute levels of LDL-C may be normal in patients with diabetes mellitus, LDL function is often abnormal due to glycosylation in the blood. The typical lipid profile in patients with diabetes consists of elevated total triglycerides with low levels of HDL-C. This profile often causes abnormal triglyceride-rich lipoprotein metabolism, which in turn modifies LDL structure. This modification results in smaller, denser LDLs, which are known to be markedly atherogenic. In addition, increased levels of Lp(a) are common in patients who have poorly controlled diabetes mellitus.

2.3 Obesity and physical inactivity

Obesity is a significant risk factor for cardiovascular disease. It is a primary risk factor in young patients, and is associated with established risk factors such as hypertension and hyperlipidemia in other patients. Physical activity favorably alters lipid profiles, lowers adiposity and blood pressure, and increases cardiovascular and pulmonary functional capacities. In addition, physical fitness, a condition that is amenable to being tested in an exercise laboratory, independently reduces the risk of premature CHD.

2.4 Family history

It is clear that single gene mutations influence lipid metabolism. Complex polygenic disorders including hypertension, diabetes, and homocysteinemia also portend significant risk for CHD. Interestingly,

currently mapped genetic abnormalities only partially account for the risk predicted by a positive family history for premature CHD.

2.5 Smoking

Several observational studies have demonstrated that smoking increases atherosclerotic risk by increasing fibrinogen levels in the blood, enhancing platelet reactivity, and increasing whole blood viscosity by inducing secondary polycythemia vera. In addition, the chemical irritants found in tobacco injure the endothelium. Smoking also lowers HDL-C and promotes oxidation of LDL-C. The mechanism of these effects on lipid profile is thought to be the exposure of LDL to the free radicals that are present in cigarette smoke.

3. ApoE polymorphism and cardiovascular disease.

3.1.1 apoE gene

The APO E gene, located on human chromosome 19, is 3.7 kb in length and contains four exons. The genomic organization of APO E is similar to that of the APO A and APO C gene families, suggesting that these genes arose from a common ancestor by gene duplication. The primary product of the APO E gene is a 317-amino acid protein that gives rise to the 299-amino acid mature protein by cleavage of an 18-amino acid signal peptide. Apo E is a constituent of TG-rich chylomicrons, VLDL particles and their remnants, and a subclass of HDL. The primary role of apo E in plasma lipid metabolism is to mediate the interaction of chylomicron remnants and intermediate density lipoprotein particles with lipoprotein receptors, including the LDL

receptor and the chylomicron remnant or apo E receptor. The remnant receptor appears to be the LDL receptor-related protein. Three major apo E isoforms are coded by three alleles at the APO E locus, designated E2, E3, and E4, giving rise to six common phenotypes. The most common isoform, E3, is characterized by a cysteine at amino acid residue 112 and an arginine at residue 158. The E2 isoform has a cysteine at residues 112 and 158, whereas the E4 allele product has an arginine at residues 112 and 158. In populations of European origin, the E3 allele ranges in frequency between 0.7 and 0.8, the E4 between 0.10 and 0.15, and E2 between 0.05 and 0.10 (9).

3.1.2 Associations of apo E with lipid parameters

In the general population, the E2 allele is consistently associated with lower levels of total plasma cholesterol, LDL-C, and apo B and elevated levels of TG and apo E compared with the ε3 allele. Elevated levels of TG and apo E are consistent with impaired clearance of remnant particles containing apo E2, presumably due to defective receptor recognition of apo E2 containing particles (10). The basis for the reduced apo B and LD-C levels in APO E2/3 and 2/2 individuals is less clear. Ehnholm et al. (11) suggested that the presence of apo E2 in intestinal VLDL particles impairs their conversion to LDL by interfering with normal lipolytic processing. Conversely, the ε4 allele is associated with higher levels of total and LDL cholesterol and apo B and lower levels of apo E. These observations are consistent with the faster rate of catabolism of particles containing apo E4 compared with those

containing apo E3 (12). In general, the lower plasma total cholesterol levels observed in subjects carrying the ε2 allele correlate with reduced coronary and peripheral artery atherosclerosis, and the higher cholesterol levels seen in ε4 carriers are associated with a higher prevalence of cardiovascular disease (CV) disease. However, the effect of APO E variation on clinical atherosclerosis is not completely explained by its impact on risk factor levels, as recent studies have demonstrated associations between carotid atherosclerosis (13) and coronary artery calcification (14) in asymptomatic adults. There is convincing evidence that the relationship between APO E genotype and plasma lipoprotein-lipid levels is context dependent, being significantly influenced by age (15), sex (16, 17, 18) and body weight distribution (17). Recent evidence also indicates that the responses of plasma lipoprotein-lipid levels to different lipid-lowering interventions may be affected by an individual's APO E genotype, indicating the significance of gene-environment interactions.

3.1.3 Associations of apo E with CHD

Epidemiologic studies have investigated the direct impact of apo E on CHD, as well as its impact on cholesterol levels. One study, addressing the contribution of apo E to CHD, reported that about ~6% of the variation in risk for CHD in North America can be attributed to this locus. Another study of middle-aged men from nine populations estimated a ~40% increased risk for CHD mortality for ε4 carriers compared with E3/3 genotype or ε2 carriers (19). Some studies have also suggested that ε4 carriers are particularly prone

to developing disseminated coronary lesions or to have an increased risk of death from CHD (20, 21, 22). It has been proposed that the biochemical mechanism is related to dys-function of the E4 isoform in lipoprotein metabolism and an increased concentration of serum cholesterol and TG (23). Studies from Finland, Scotland, and Northern Ireland have shown that populations with higher cholesterol levels and higher CHD mortality rates also have a higher frequency of the ε4 allele (19, 24). Other studies have also associated the ε2 allele with increased CHD risk (20).

An association between apo E2/2 and type III hyperlipoproteinemia has been known for decades. This disorder is characterized by increased cholesterol and triglyceride levels, the presence of β-VLDL (cholesterol-enriched remnants of intestinal chylomicrons and hepatic VLDL), xanthomas, and premature vascular disease, both CHD and peripheral artery disease. Overt hyperlipoproteinemia III occurs with a frequency of 1-5 per 5,000, whereas homozygosity for E2/2 occurs with a frequency of 0.5-1 per 100 in Caucasian populations (23). Thus, this genotype contributes to the hyperlipoproteinemia III phenotype without being its sole cause.

Strains of apo E deficient and apo E-overexpressing transgenic mice have been developed to increase our understranding of apo E in disease processes. Apo E-deficient mice accumulate VLDL and remnant particles in plasma and develop atherosclerosis, even on low-fat diets (25). Increased expression of human apo E3 in transgenic mice results in hypertriglyceridemia (26).

In addition to being studied in association with cardiovascular disease outcomes and intermediate phenotypes, the apo E polymorphism has been investigated as a risk factor for other chronic diseases, such as diabetes mellitus, β- thalassemia, rheumatoid arthritis, Alzeimer's disease, Parkinson's disease, schizophrenia, and psychosis (27).

4. Genes and environmental interaction: the role of exercise

Regular exercise has been shown to improve control of lipid abnormalities, diabetes mellitus, hypertension, and obesity, with the greatest benefits realized by sedentary individuals who begin to exercise. Response to exercise may be mediated in large part by variation in genes. To understand how genes and exercise can interact to modify a phenotypic trait or health outcome, it is necessary to consider multiple levels of interaction. Figure 2 illustrates the many complex ways in which genes and exercise, both together and separately, can influence the health status of an individual. In this model, exercise can produce direct and immediate effects on health status, without necessarily altering gene expression or function. Such is the case in exercise-induced asthma, in which the increased air flow and temperature resulting from rapid breathing during exercise can very quickly alter the short-term health status in genetically susceptible individuals. Another example of an acute exercise effect is that of exercise-related sudden cardiac death occurring in individuals with genetic defects leading to hypertrophic cardiomyopathy or coronary artery anomalies. These examples are illustrative of gene-environment interactions in which the same environment (i.e., exercise) produces differential effects in genetically different individuals. Exercise can

also affect health status indirectly by altering the expression or action of one or more genes that influence intermediate phenotypes (e.g., cholesterol level) that ultimately produce disease outcomes. This is an example of biological interaction, in which two or more factors influence a phenotype interdependently. Biological interaction, in which multiple genetic and environmental factors are interconnected in complicated ways, is inherent in complex chronic diseases, such as cardiovascular disease. Because of the complex nature of many diseases and intermediate phenotypes, and of health status in general, the model in Fig. 2 necessarily includes other genes and other environments, in addition to exercise, that may or may not interact with one another in determining overall health (28).

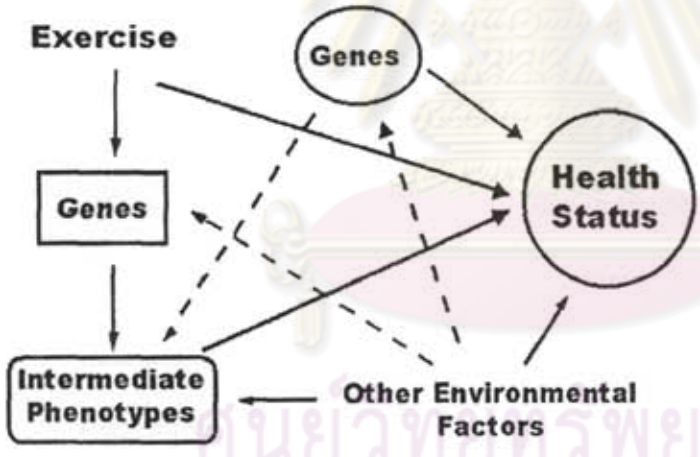

Figure 2. Model of gene-exercise interaction illustrates the complex interaction among exercise, genes, and other environmental factors in overall determination of health status.

Several strategies have been used to detect genes whose effects may be altered by exercise. Many studies have investigated the effect of exercise on intermediate traits related to chronic diseases such as hypertension, obesity, and cardiovascular

disease. Although the positive effects of exercise on reducing hyperlipidemia, high blood pressure, obesity, and related factors are well documented, there is typically a great amount of heterogeneity in the responsiveness to exercise intervention. A limited number of studies have investigated the role of genetic variation in the control of traits such as blood pressure and lipid processing within the context of exercise, and several gene-exercise interactions have been identified (28).

4.1 Apo E genotype and plasma lipoprotein-lipid with exercise training

The first evidence that plasma lipoprotein-lipid responses to exercise training might be influenced by Apo E genotype was provided by Taimela et al. (29). They assessed the plasma lipoprotein-lipid profiles of ~1,500 Finnish children and young adults aged 9–24 yr in whom leisure-time physical activity was assessed by questionnaire. Leisure-time physical activity levels did not affect plasma lipoprotein-lipid profiles in the females. However, in the males, in addition to physical activity levels affecting plasma lipoprotein-lipid profiles, the interaction between apo E phenotype and physical activity also affected plasma lipoprotein-lipid levels. In Apo E4/4 men physical activity levels did not affect plasma lipoprotein-lipid levels, whereas in Apo E3/4 and 3/3 men, there was an inverse effect of physical activity level on plasma TC and LDL-C and a positive effect on HDL-C /TC ratio. In Apo E2/3 men there were even stronger relationships between physical activity levels and these same components of the plasma lipoprotein-lipid profile. However, there are a number of inconsistencies between the text and figures in Taimela et al. (29) with respect to these apo E phenotype-physical activity interaction effects on plasma lipoprotein-lipid

levels. Thus, bearing in mind these inconsistencies, these data, though cross-sectional, may be consistent with the possibility that exercise training does not affect plasma lipoprotein-lipid levels in Apo E4 individuals, has a moderate effect in Apo E3 individuals, and has an even greater effect in Apo E2 individuals. More recently, in another cross-sectional study St.-Amand et al. (30) concluded that plasma lipoprotein-lipid profiles of Apo E2 individuals appear to be especially affected by increased CV fitness. However, while this does appear to be the case for plasma TG levels, the data are more consistent with the possibility that the overall plasma lipoprotein-lipid profiles of APO E3 men and women appear to affected more by increased CV fitness than those of Apo E2 and E4 men and women. In a third cross-sectional study, we recently reported that Apo E genotype was not associated with plasma lipoprotein-lipid levels in sedentary postmenopausal women or postmenopausal women who had undergone 5–6 h/wk of low- to moderate-intensity aerobic activity for the previous 12 yr. Furthermore, postmenopausal women athletes with only Apo E3 or E4 alleles who ran an average of 30 miles/wk for the preceding 15 yr had plasma lipoprotein-lipid profiles only slightly better, in terms of total, LDL, HDL, and HDL_2 cholesterol and TG levels, than the sedentary or physically active women. Only women distance runners with at least one Apo E2 allele had better plasma lipoprotein-lipid profiles than the sedentary and physically active women. Women athletes with at least one Apo E2 allele also had better overall plasma lipoprotein-lipid profiles than women distance runners with only APO E3 or E4 alleles, despite the fact that they were otherwise similar in terms of training mileage and history, body composition, diet, and hormone replacement

therapy status. The middle-aged and older Apo E2 genotype men had larger overall plasma lipoprotein-lipid profile improvements with prolonged endurance exercise training than otherwise comparable Apo E3 and E4 genotype men (31). Men in all Apo E genotype groups generally reduced body weight and percent body fat to the same degree with exercise training. The Apo E2 and E3 men tended to reduce plasma total and LDL cholesterol more with exercise training than E4 men, but the differences were not significant. However, Apo E2 genotype men increased plasma HDL-C three to four times more than E3 and E4 genotype men. Apo E2 genotype men also increased plasma HDL_2 cholesterol dramatically more with exercise training than Apo E3 and E4 genotype men. Apo E2 and E3 genotype men both decreased plasma TG more with exercise training than E4 men. The differences in plasma HDL and HDL_2 cholesterol increases with exercise training among Apo E genotype groups remained significant after controlling for body weight changes, whereas the reduction in plasma TG in the Apo E2 genotype men tended to still be greater than in the Apo E4 men after controlling for changes in body weight ($P = 0.09$). Thus these longitudinal intervention data provide stronger evidence supporting the conclusion that the plasma lipoprotein-lipid profiles of Apo E2 individuals may be affected most by prolonged endurance exercise training. Plasma lipoprotein-lipid profiles of Apo E4 individuals do not appear to change as a result of exercise training, whereas Apo E3 individuals appear to have responses intermediate between those of E2 and E4 individuals (9).

Procedure

Study population

Two-hundred seventy three adult Thais residing in the Bangkok Metropolitan area were interviewed about their personal information (sex, age, body weight) and physical activity (PA) with a questionnaire. The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Chulalongkorn University.

Physical activity (PA)

Participants were asked about the frequency and intensity of participation in PA. An index of leisure-time PA was calculated from the product of intensity times estimated duration times monthly frequency (32). For intensity, we used coefficient values of 4 (never sweating and becoming breathless) corresponding to light aerobic activity, 6 (some sweating and becoming breathless) corresponding to moderate aerobic activity, and 10 (heavy sweating and becoming breathless) corresponding to intense aerobic activity. The coefficient values 4, 6, and 10 for different levels of intensity were chosen to estimate the metabolic cost of each intensity level. A mean value of 30minutes for duration (coefficient 0.5) was used in the case of PA other than supervised exercise. In the case of supervised exercise (participation in a sports club session), a mean value of 45 minutes for duration (coefficient 0.75) was used. A coefficient value of 0 for duration was used in case of no leisure-time PA. Coefficient values for monthly frequency of activity were 0.5 (< once per month), 1 (once per month), 2.5 (two to three times per month), 4.3 (once per week), 17 (two to six times per week), and 30 (once per day).

Blood sampling and lipid analysis

Blood samples were obtained in EDTA tubes from individuals after fasting for 12-16 h. Concentrations of plasmaTC, TG and HDL were measured by enzymatic colorimetry methods (bioMerieux, France) and a VITALAB FLEXOR automated chemistry analyzer. LDL levels were calculated using Friedewald formula (33). Two milliliter of plasma samples was stored at -20^{O}C for DNA extraction.

Determination of Apo E genotypes

Total genomic DNA was extracted from blood using FlexiGene DNA kit (Qiagen, USA). 200-1000 ng of DNA was amplified in a thermocycler (PTC-200, MJ research Inc., USA). The sequence of apo E primers are GCACGGCTGTCCAAGGAGCTGCAGGC and GCCGCTCGCGATGGCGCTGAG , respectively. PCR reaction was performed in 25 µl containing 7 pmole of forward and reverse primer, 200 µM dNTP, 1.5 mM $MgCl_2$, 4% dimetyl sulfoxide (DMSO), 1 units of Taq DNA polymerase (Qiagen, USA) and 1Xbuffer supplied by the manufacturer. The PCR conditions of *apo E* gene were as follows: denaturation for 5 min at 95^{O}C, 35 cycles of denaturation for 1 min at 95 ^{O}C, annealing for 1 min at 66 ^{O}C, and extension for 2 min at 72 ^{O}C. The PCR product was digested at 37 ^{O}C for 3 h with 10 units of *Hha*I (New England Biolabs Inc., USA). The digested PCR product was resolved on 15% native polyacrylamide gel with ethidium bromide staining and visualized on a UV transluminator. The fragment sizes from polymorphic HhaI were as follows; E2/E2: 91 bp and 83 bp, E2/E3: 91 bp, 83 bp and 48 bp, E2/E4: 91 bp, 83 bp,72 bp and 48 bp E3/E3: 91 bp, 48 bp and 35 bp, E3/E4: 91 bp, 72 bp and 48 bp,

E4/E4: 72 bp, 48 bp and 35 bp. From the PCR results, the genotypes and allele frequencies were calculated.

Statistical analyses

Statistical analysis was carried out using SPSS software. Allelic frequencies were estimated by gene counting method. The biochemical characteristics of the individuals, age, body mass index and physical activity index were expressed in term of mean±SD. Comparisons between plasma lipids in each gender were tested by using independent sample t-test. The one way analysis of varience (ANOVA) test was performed to compare the means of lipid parameters among different genotypes except LDL and total cholesterol in females and LDL in total subjects were compared by using Kruskal-Wallis test. Statistical significance was accepted at the P = 0.05 level. Association between serum lipids and apo E alleles was analyzed using Pearson' correlation. Stepwise multiple linear regressions were used to determine the concurrent effects of multiple variables on plasma lipids and plasma glucose. The PA X apoE phenotype interaction was tested by linear regression analyses. Dependent variables were total cholesterol, triglyceride, HDL, LDL, and glucose. The independent variables included main effects for PA (log PA index) and apo E phenotypes (indicator variables), and a PA X apo E4 phenotype interaction term (interaction between presence of ε4 allele).

Results

Clinical characteristics and plasma lipid concentration in 273 healthy Thais subjects (age between 20-45 years) are as followed: BMI , 21.9±3.9 Kg/m^2; Total cholesterol, 188.3±35.6 mg/dl; Triglyceride, 75.5±56.6 mg/dl; HDL, 58.5±12.5 mg/dl; LDL, 117.3±35.5 mg/dl; Glucose, 77.7±12.2 mg/dl and Log PA index, 2.2±1.5 (Table 3). As demonstrated in Table 4, advancing age was associated with higher total cholesterol, triglyceride, LDL and lower HDL and PA. The distribution of *apo E* genotypes were 78% for E3/E3, 7% for E2/E3, 5.9% for E2/E4, 8.8% for E3/E4, and 0.7% for E4/E4 (Table 5). Allele frequencies were 0.859 for ε3, 0.1085 for ε4 and 0.0645 for ε2 (Table 6). The ε2 allele was rare in our population. *ApoE* genotypes were significantly associated with total cholesterol and LDL in study population, $p < 0.05$ (Table 7). While, PA was significantly associated with total cholesterol, LDL and glucose, $p < 0.05$ (Table 8). Comparison of lipid and glucose concentrations between apo E polymorphisms (Table 9.) was shown that the E4 genotypes (E2/4, E3/4 and E4/4) had significantly higher total cholesterol and LDL than the E3 genotypes (E3/3 and E2/3) ($p < 0.05$). The stepwise increases in total cholesterol and LDL from E2/3, E3/3, E2/4, E3/4 and E4/4 were demonstrated. The highest total cholesterol and LDL were observed in E4/4 genotype (260.5±84.15, 183.5±95.46) and lowest in E2/3 genotypes (180.84±39.64, 110.11±37.04). The present of ε2 and ε3 allele did not affect any lipid and glucose concentrations (Table 10 and 11.) while the presence of ε4 allele resulted in increased total cholesterol and LDL in study population (Table 12.). Results of regression analyses with apo E entered separately as predictors of total cholesterol and LDL are presented in Table 13. Using stepwise regression analyses to assess the simultaneous effects of age, exercise and apo E polymorphism on plasma lipid and

plasma glucose, it was found that exercise and apo E polymorphism was positively associated with total cholesterol and LDL (Table 14.). Exercise also had a positive role on plasma glucose. For age, it was the predictor of triglyceride and HDL only. Due to no association of plasma lipid and plasma glucose was found in ε2 and ε3 allele, therefore we presented results for ε4 allele only. No effect of exercise on total cholesterol and LDL in ε4 carrier was found (Table 15.).

Table 3. Clinical characteristics of plasma lipid and plasma glucose concentrations in 273 healthy Thais subjects.

parameters	Males (n=73)	Females (n = 200)	Total (n = 273)
Age (Year)	28.2±7.3	30.8±8.1	30.13±7.9
BMI (Kg/m^2)	22.5±4.2	21.75±3.8	21.9±3.9
Total cholesterol (mg/dl)	193.0±39.9	186.5±33.9	188.3±35.6
Triglyceride (mg/dl)	96.2±84.5	68.0±39.7	75.5±56.6
HDL (mg/dl)	52.2±10.8	60.8±12.3	58.5±12.5
LDL (mg/dl)	123.9±40.8	114.9±33.2	117.3±35.5
Glucose (mg/dl)	80.3±11.6	76.8±12.3	77.7±12.2
Log PA index	1.5±0.7	1.4±0.7	2.2±1.5

Table 4. Comparison of plasma lipid and plasma glucose concentrations in different age groups.

Age (years)	20-25 (N =102)	26-35 (N=91)	36-45 N =80	P-value
BMI (Kg/m^2)	20.77±4.13	22.0±3.47	23.38±3.68	0.000
Total cholesterol (mg/dl)	182.83±38.17	189.37±38.12	193.88±27.93	0.015
Triglyceride (mg/dl)	64.0±31.86	71.78±37.37	94.54±87.04	0.006
HDL (mg/dl)	61.3±12.3	57.58±11.92	55.96±12.74	0.008
LDL (mg/dl)	109.72±34.89	121.63±36.14	122.15±34.38	0.004
Glucose (mg/dl)	78.39±9.0	75.46±8.44	79.45±17.89	0.067
Log PA index	1.89±1.6	2.56±1.35	2.2±1.52	0.043

P values calculated using Kruskal-Wallis test.

Table 5. Distribution of apo E genotypes in males and females.

	Apo E genotype					
	E3/E3	E2/E3	E2/E4	E3/E4	E4/E4	E2/E2
Males (n =73)	57 (78.0%)	9 (12.3%)	1 (1.4%)	4 (5.5%)	2 (2.7%)	0 (0%)
Females (n=200)	156 (78%)	10 (5.0%)	14 (7.5%)	20 (10%)	0 (0%)	0 (0%)
Total (n=273)	213 (78.0%)	19 (7.0%)	15 (5.9%)	24 (8.8%)	2 (0.7%)	0 (0%)

Table 6. Allele frequencies of apo E in study population.

	Allele frequency
ε2	0.0645
ε3	0.859
ε4	0.1085

Table 7. Correlations between BMI, plasma lipid and plasma glucose concentrations and apoE genotype in study population.

	Total (n = 273)	
	r	p
BMI (Kg/m^2)	-0.068	0.264
Total cholesterol (mg/dl)	0.151	0.012
Triglyceride (mg/dl)	0.042	0.494
HDL (mg/dl)	0.043	0.482
LDL (mg/dl)	0.152	0.012
Glucose (mg/dl)	-0.059	0.333

Table 8. Correlations between BMI, plasma lipid and plasma glucose concentrations and PA in study population.

	Total (n = 273)	
	r	p
BMI (Kg/m^2)	0.054	0.377
Total cholesterol (mg/dl)	0.134	0.026
Triglyceride (mg/dl)	-0.088	0.147
HDL (mg/dl)	0.024	0.697
LDL (mg/dl)	0.184	0.002
Glucose (mg/dl)	0.194	0.001

Table 9. Comparison of plasma lipid and plasma glucose concentrations among apoE genotypes in study populations.

		N	Mean±SD	p
Total cholesterol (mg/dl)	E3/3	213	185.8967±32.4617	0.002
	E2/3	19	180.8421±39.6376	
	E2/4	15	192.5333±48.0296	
	E3/4	24	206.3333±38.1218	
	E4/4	2	260.5000±84.1457	
	Total	273	188.2527±35.6281	
Triglyceride (mg/dl)	E3/3	213	73.9718±55.2195	0.635
	E2/3	19	85.6842±66.0505	
	E2/4	15	64.1333±30.6358	
	E3/4	24	87.6667±73.4448	
	E4/4	2	87.0000±2.8284	
	Total	273	75.5458±56.5683	
HDL (mg/dl)	E3/3	213	58.4977±12.3076	0.915
	E2/3	19	56.5263±14.7097	
	E2/4	15	59.8000±12.3531	
	E3/4	24	59.1250±12.9457	
	E4/4	2	59.5000±12.0208	
	Total	273	58.4945±12.4638	
LDL (mg/dl)	E3/3	213	114.8310±32.5121	0.025*
	E2/3	19	110.1053±37.0374	
	E2/4	15	120.4667±44.2765	
	E3/4	24	137.7500±39.9513	
	E4/4	2	183.5000±95.4594	
	Total	273	117.3297±35.5258	
Glucose (mg/dl)	E3/3	213	77.9765±13.0045	0.515
	E2/3	19	76.5789±8.9027	
	E2/4	15	81.2000±7.7201	
	E3/4	24	74.5000±9.1366	
	E4/4	2	74.5000±10.6066	
	Total	273	77.7253±12.2229	

* = P values calculated using Kruskal-Wallis test.

Table 10. Comparison of plasma lipid and plasma glucose concentrations in study population with and without ε2 allele.

	With E2 (n = 19)	Without E2 (n = 254)	p
BMI (Kg/m^2)	22.4438±4.7330	21.9117±3.8644	0.569
Total cholesterol (mg/dl)	180.8421±39.6376	188.8071±35.3337	0.348
Triglyceride (mg/dl)	85.6842±66.0505	74.7874±55.8713	0.419
HDL (mg/dl)	56.5263±14.7097	58.6417±12.3006	0.476
LDL (mg/dl)	110.1053±37.0374	117.8701±35.4267	0.359
Glucose (mg/dl)	76.5789±8.9027	77.8110±12.4448	0.673

Table 11. Comparison of plasma lipid and plasma glucose concentrations in study population with and without ε3 allele.

	With E3 (n = 213)	Without E3 (n = 60)	p
BMI (Kg/m^2)	21.9581±3.9991	21.9156±3.6726	0.941
Total cholesterol (mg/dl)	185.8967±32.4617	196.6167±44.4340	0.085
Triglyceride (mg/dl)	73.9718±55.2195	81.1333±61.2805	0.387
HDL (mg/dl)	58.4977±12.3076	58.4833±13.1103	0.994
LDL (mg/dl)	114.8310±32.5121	126.2000±43.7914	0.065
Glucose (mg/dl)	77.9765±13.0045	76.8333±8.9465	0.523

Table12. Comparison of plasma lipid and plasma glucose concentrations in study population with and without ε4 allele.

	With E4 (n = 41)	Without E4 (n = 232)	p
BMI (Kg/m^2)	21.6708±3.1017	21.9979±4.0547	0.623
Total cholesterol (mg/dl)	203.9268±45.0801	185.4828±33.0370	0.016
Triglyceride (mg/dl)	79.0244±59.6781	74.9310±56.1132	0.670
HDL (mg/dl)	59.3902±12.3893	58.3362±12.4969	0.619
LDL (mg/dl)	133.6585±45.0692	114.4440±32.8431	0.012
Glucose (mg/dl)	76.9512±9.0746	77.8621±12.7095	0.661

Table 13. Regression coefficients of the relation between apo E polymorphism, plasma lipids and plasma glucose in study population.

	Total	
	β	p
Total cholesterol (mg/dl)	0.151	0.012
Triglyceride (mg/dl)	0.042	0.494
HDL (mg/dl)	0.043	0.482
LDL (mg/dl)	0.152	0.012
Glucose (mg/dl)	-0.059	0.333

Table 14. Regression coefficients from stepwise multiple linear regression analyses of the relation between age, log PA, apoE polymorphism and plasma lipids, plasma glucose in study population.

	Age		Log PA		apoE genotype	
	β	P	β	P	β	P
Total cholesterol (mg/dl)	-	NS	0.128	0.031	0.191	0.001
Triglyceride (mg/dl)	0.244	0.000	NS	NS	-	NS
HDL (mg/dl)	-0.179	0.003	NS	NS	-	NS
LDL (mg/dl)	-	NS	0.177	0.003	0.199	0.001
Glucose (mg/dl)	-	NS	-0.194	0.001	-	NS

Table 15. Regression coefficients of plasma lipids, plasma glucose and PA and their interaction terms with ε4 allele on plasma lipids and plasma glucose.

	Interaction (ε4 X PA)	
	β	p
Total cholesterol (mg/dl)	0.169	0.292
Triglyceride (mg/dl)	-0.137	0.392
HDL (mg/dl)	0.161	0.315
LDL (mg/dl)	0.147	0.359
Glucose (mg/dl)	-0.281	0.075

Discussion

Human *apo E* alleles are polymorphic with significant different frequencies among different ethnic groups and have been associated with increased risk of coronary heart disease. The allele frequencies of our study population were similar to previously report in Asian populations including Chinese, Mongolian, Taiwan, Japanese, and Indian. The frequencies of apoE phenotypes and allele in different race of Chinese population were: E3/3 69.8-72.93%, E2/3 5.0-12.98%, E3/4 11.33-13.2%, E2/4 1.38-2%, E4/4 0-1.38%, E2/2 0-2%, ε2 3.2-8.6%, ε3 80.4-87.5% and ε4 4.9-16.4%) (34, 35).The allele frequencies in the three ethnic groups (Chinese, Malays and Asian Indians) in the Singapore population were: ε2 3.9-10.5%, ε3 71.5-85.3% and ε4 8.5-18% (36). However, the distribution of apo E phenotypes in previous healthy Thais study were E3/3 81.1%, E2/3 12.4%, E3/4 0%, E2/4 0%, E4/4 0.9%, E2/2 5.5% which slightly different from our study and others (37).The inconsistent data, partly due to the common pitfalls in these type of association studies, such as inadequate sample size and population stratification (38). Northern Europeans (Finns, Germans) tend to have higher frequencies of the ε4 allele than southern Europeans (French, Italians). Nigerians, Japanease, Mexican Americans and American Indians have relatively low frequencies of ε2 (27).

Apo E genotypes E2/3, E3/3, E2/4, E3/4 and E4/4 (in this order) were found to correlate with stepwise increases in TC and LDL. In Caucasian population, TC and LDL increased in the order E2/2<E2/3< E2/4<E3/3<E3/4 in both genders, and HDL and apo A-I levels decreased in that order in women, but not in men (39). ε4 carriers have higher TC and LDL while E3/3 has lower TC and LDL. High levels of LDL have been associated with increased risk of CHD. Apo E contributes more to normal

cholesterol variability than any other gene identified thus far in cholesterol metabolism (40). In general, ε2 lowers TC levels and ε4 raise them. However, no effect of ε2 was found in our study. Ilveskoski *el al* concluded that ε4 is a significant genetic risk factor for coronary artherosclerosis in early middle age but that it loses its importance with age (41). Myocardial infarction survivors carrying the ε4 allele have an 80% increased risk of dying compared with other patients (42).

The association between PA and plasma lipids is influenced by apo E phenotype. PA was positively associated with total cholesterol and LDL in our study. The effect of PA on plasma lipids was not found in ε4 carriers. Cholesterol adsorption efficiency from the intestine increases in the order of E2<E3/3<E4 and the LDL catabolic rate from plasma decreases in the order of E2>E3/3>E4 (43). Moreover, apo E4 binds to VLDL with higher affinity compared with apo E2 and apoE3 (44). The mechanism in ε4 carriers is effectively enough to override the possible metabolic effect of PA on TC and LDL (29). The sedentary men with ε2 allele had significantly greater increases in HDL than those with ε3 or ε4 allele subsequent to exercise training. Conversely, another study reported that hypertensive individuals with a ε2 allele exhibited a lesser response in both systolic and diastolic blood pressure after exercise training compared with hypertensive individuals with either a ε3 or ε4 allele. It was suggested that apo E genotype can interact both positively and negatively with exercise (45).

Conclusion

Apo E was one of the first polymorphisms associated with cardiovascular disease to be studied thoroughly in both health and disease. It influences lipoprotein metabolism and the plasma concentration of TC, LDL and confers a risk for CHD. Asian populations including Thais have relatively lower frequencies of ε2 allele than Caucasian populations. TC and LDL varied with apo E phenotypes which increased in the order E2/3<E3/3<E2/4<E3/4<E4/4. The presence of ε4 allele is associated with higher TC and LDL. The differences in plasma TC and LDL between subjects with different apo E phenotypes are influenced by the level of PA in healthy, adult Thais. Exercise did not affect the total cholesterol and LDL in ε4 carriers.

Suggestion for further work

Due to gene-exercise interactions is essential to improving human health and performance both in healthy subjects and patients. The effect of PA on plasma lipid and apolipoprotein should be further studied in subjects with various types of supervised exercise training such as Yoda, exercise in fitness center, aerobic dance, etc. Whether exercise associated with lipid levels in CHD patients of different apo E phenotypes is also interesting. Moreover, interaction of exercise with other candidate genes involved in lipid metabolic pathway such as *apolipoprotein AII* (*apo AII*), *apolipoprotein AI-CIII-AIV* gene cluster (*apo AI-CIII-AIV*), *apolipoprotein E* (*apoE*), *cholesteryl ester transfer protein* (*CETP*), *cholesterol 7 α-hydroxylase* (*CYP7α*), *hepatic lipase* (*HL*), and *microsomal triglyceride* (*MTP*) should also be studied in our Thai population.

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Appendix
ข้อมูลสำหรับอาสาสมัคร

ชื่อโครงการวิจัย (ไทย) Apo E จีโนไทป์มีผลกระทบกับผลของการออกกำลังกายต่อระดับไขมันในเลือดในคนไทยที่มีสุขภาพแข็งแรง

(อังกฤษ) The role of exercise on serum lipid concentrations influenced by Apo E genotype in healthy Thais

เรียน อาสาสมัครทุกท่าน

ท่านเป็นผู้ได้รับเชิญจากคณะผู้วิจัยให้เข้าร่วมโครงการวิจัยเกี่ยวกับการศึกษา ยีน Apolipoprotein E และ ผลของการออกกำลังกายต่อระดับไขมันในเลือด ก่อนที่ท่านจะตกลงเข้าร่วมการวิจัยดังกล่าว ขอเรียนให้ท่านทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยในครั้งนี้

ข้อมูลเบื้องต้น

ยีน Apolipoprotein E เป็นยีนที่ความสำคัญและเกี่ยวข้องในขบวนการเมตาบอลิสมของไขมันในเลือด ยีน Apo E มี 3 แบบ คือ E2, E3 และ E4 ซึ่งในแต่บุคคลจะถูกกำหนดลักษณะการแสดงออกของยีนแตกต่างกันไปได้ 6 แบบ จากการศึกษาวิจัยในต่างประเทศพบว่ายีนนี้มีความสัมพันธ์ต่อระดับไขมันในเลือด และยังพบว่ายีนนี้มีความเกี่ยวข้องกับความเสี่ยงในการเกิดโรคหัวใจและหลอดเลือด โดยพบว่าผู้ที่มียีนแบบ E2 มักจะมีระดับโคเลสเตอรอลในเลือดต่ำ และระดับไตรกลีเซอไรด์ในเลือดต่ำกว่าผู้ที่มียีนแบบ E3 และ E4 นอกจากนั้นยังพบว่าผู้ที่มียีนแบบ E2 มีความเสี่ยงต่อการเป็นโรคหัวใจและหลอดเลือดต่ำ ขณะที่ผู้ที่มียีนแบบ E4 มีความเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือดสูง

เป็นที่ยอมรับว่าการออกกำลังกายมีผลในเชิงบวกต่อระดับไขมันในเลือด พบว่าการออกกำลังกายสามารถช่วยลดและควบคุมระดับไขมันในเลือดได้ อย่างไรก็ตามมีการศึกษาในต่างประเทศบางอันพบว่าผลของการออกกำลังกายต่อระดับไขมันในเลือดนั้นขึ้นอยู่กับลักษณะยีน Apo E ในบุคคลนั้นๆด้วย โดยพบว่าผู้ที่มียีน Apo E2 การออกกำลังจะมีผลในเชิงบวกต่อระดับไขมันในเลือดมากกว่าผู้ที่มียีนแบบ E3 และ E4 ซึ่งช่วยลดความเสี่ยงของการเกิดโรคหัวใจและหลอดเลือดได้ ในผู้ที่มียีนแบบ E4 พบว่าการออกกำลังกายไม่สามารถเปลี่ยนแปลงระดับไขมันในเลือดได้ ดังนั้นผู้ที่มียีนแบบ E4 จึงค่อนข้างจะมีความเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือดสูง ในการศึกษาครั้งนี้จึงต้องการดูความสัมพันธ์ของยีน Apo E ในการออกกำลังกายต่อระดับไขมันในเลือดในกลุ่มคนไทยที่มีสุขภาพแข็งแรง

วัตถุประสงค์ในการวิจัย

เพื่อศึกษาความสัมพันธ์ของยีน Apo E ในการออกกำลังกายต่อระดับไขมันในเลือดในกลุ่มคนไทยที่มีสุขภาพแข็งแรง

วิธีการวิจัย

ทำการสัมภาษณ์อาสาสมัครจำนวน 200 คน ที่มีช่วงอายุระหว่าง 17-50 ปี ในด้านข้อมูลพื้นฐาน เช่น เพศ อายุ น้ำหนัก และข้อมูลด้านการออกกำลังกาย และความถี่ในการออกกำลังกาย ทำการเจาะเลือดอาสาสมัคร

จำนวน 5 มิลลิลิตร โดยอาสาสมัครต้องอดอาหารก่อนเจาะเลือดประมาณ 8-12 ชั่วโมง เพื่อนำไปวิเคราะห์ระดับไขมันในเลือด และ ลักษณะยีน Apo E และ นำข้อมูลที่ได้มาวิเคราะห์สถิติ

ความเสี่ยงที่เกี่ยวข้องกับการวิจัยนี้

การเจาะเลือดจากท่านเป็นจำนวน 5 มิลลิลิตรนั้นเป็นจำนวนที่น้อยมาก ซึ่งจะไม่ก่อให้เกิดอันตรายใดๆ เว้นเพียงแต่อาจจะเกิดรอยช้ำบริเวณที่เจาะเลือดซึ่งไม่มีความเจ็บปวดและจะหายเองได้ภายใน 7 วัน

ประโยชน์จากการเข้าร่วมโครงการวิจัย

อาสาสมัครในโครงการวิจัยทุกท่านจะได้รับการตรวจวิเคราะห์ระดับไขมันในเลือดโดยไม่เสียค่าใช้จ่ายใดๆ ซึ่งอาสาสมัครสามารถทราบสภาวะของระดับไขมันในเลือดของตนเอง

ข้อมูลส่วนตัวและข้อมูลอื่นๆของอาสาสมัครจะถูกเก็บไว้เป็นความลับเฉพาะคณะผู้วิจัย และ รายงานผลเฉพาะข้อมูลที่ผ่านการวิเคราะห์แล้ว

การเข้าร่วมการศึกษาครั้งนี้เป็นไปด้วยความสมัครใจ ท่านอาจจะปฏิเสธที่จะเข้าร่วม หรือถอนตัวจากการศึกษาได้ทุกเวลา

ในการร่วมเป็นอาสาสมัครในการศึกษาครั้งนี้ ทางผู้ทำการศึกษายินดีที่จะรับผิดชอบเป็นค่าชดเชยให้ท่านเป็นจำนวนเงิน 200 บาท

หากท่านมีปัญหา หรือข้อสงสัยประการใด กรุณาติดต่อ ดร. นันทรี ชัยชนะวงศาโรจน์ ภาควิชาเวชศาสตร์การธนาคารเลือด คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทร 02-2189931

ขอขอบคุณในความร่วมมือของท่านมา ณ ที่นี้

ข้อมูลพื้นฐานของอาสามัคร

โครงการวิจัยเรื่อง Apo E จีโนไทป์มีผลกระทบกับผลของการออกกำลังกายต่อระดับไขมันในเลือดในคนไทยที่มีสุขภาพแข็งแรง

ส่วนที่1 ประวัติส่วนตัว

อายุ.....ปี น้ำหนัก.....ส่วนสูง.....

ท่านมีโรคประจำตัวหรือไม่.....ถ้ามีโปรดระบุ.....

.....

ท่านเคยมีประวัติการเจ็บป่วยด้วยโรค

โรคหัวใจ โรคความดันโลหิตสูง

โรคเบาหวาน อื่นๆ

ส่วนที่ 2 ข้อมูลการออกกำลังกาย

1. ท่านออกกำลังกายหรือไม่.....
2. ท่านออกกำลังกายอะไร.....
3. ท่านออกกำลังกายครั้งละเฉลี่ยนานเท่าใด.....
4. ท่านออกกำลังกายบ่อยเพียงไร

☐ ไม่เคยเลย ☐ บางครั้ง (อย่างมากเดือนละ1-2 ครั้ง)

☐ บ่อยครั้ง (อย่างน้อยสัปดาห์ละ 1-2 ครั้ง) ☐ บ่อยมาก (ออกกำลังกายทุกวัน)

5. ท่านมีเหงื่อหรือรู้สึกเหนื่อยหอบหลังจากการออกกำลังกายหรือไม่.....

ใบยินยอมด้วยความสมัครใจให้ทำการวิจัยในมนุษย์

Subject No.....

การวิจัยเรื่อง Apo E จีโนไทป์มีผลกระทบกับผลของการออกกำลังกายต่อระดับไขมันในเลือดในคนไทยที่มีสุขภาพแข็งแรง

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

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียดและมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบัง ซ่อนเร้น

ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้โดยความสมัครใจ และมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการในการวิจัยครั้งนี้เมื่อใดก็ได้

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวข้องกับข้าพเจ้าเป็นความลับและจะเปิดเผยเฉพาะในรูปที่เป็นผลสรุปการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆที่เกี่ยวข้องกระทำได้ในเฉพาะกรณีที่จำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ลงนาม.....ผู้ยินยอม

(.....)

ลงนาม.....พยาน

(.....)

ลงนาม.....ผู้ทำวิจัย

(.....)