ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร

นางสาวเอมอร ชัยประทีป

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาอาหารเคมีและโภชนศาสตร์ทางการแพทย์ ภาควิชาอาหารและเภสัชเคมี
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553

EFFECTS OF SOY PROTEIN ISOLATE SUPPLEMENTATION ON BIOMARKERS OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS AT PUBLIC HEALTH CENTER 66, HEALTH DEPARTMENT, BANGKOK METROPOLITAN ADMINISTRATION

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Food Chemistry and Medical Nutrition
Department of Food and Pharmaceutical Chemistry
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Thesis Title	EFFECTS OF SOY PROTEIN ISOLATE SUPPLEMENTATION ON BIOMARKERS OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS AT PUBLIC HEALTH CENTER 66, HEALTH DEPARTMENT, BANGKOK METROPOLITAN ADMINISTRATION
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เอมอร ชัยประทีป : ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร (EFFECTS OF SOY PROTEIN ISOLATE SUPPLEMENTATION ON BIOMARKERS OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS AT PUBLIC HEALTH CENTER 66, HEALTH DEPARTMENT, BANGKOK METROPOLITAN ADMINISTRATION) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. อรอนงค์ กังสดาลอำไพ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.ดร. กุลวรา เมฆสวรรค์, 138 หน้า.

โรคหัวใจและหลอดเลือดเป็นสาเหตุหลักของการเสียชีวิตในผู้ป่วยโรคเบาหวานชนิดที่ 2 การวิจัยนี้ศึกษาผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 โดยผู้ป่วยเบาหวานชนิดที่ 2 ที่สมัครเข้าร่วมการวิจัย จำนวน 36 คน ได้รับคำแนะนำด้านโภชนบำบัด และถูกแบ่งแบบสุ่มออกเป็น 2 กลุ่ม คือ กลุ่มทดลอง (ได้รับโปรตีนสกัดจากถั่วเหลืองวันละ 30 กรัม ซึ่งมีปริมาณไอโซฟลาโวนส์ 32 มิลลิกรัม เป็นเวลา 6 สัปดาห์) และกลุ่มควบคุม (ไม่ได้รับโปรตีนสกัดจากถั่วเหลือง) ทำการวัดความดันโลหิต และตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ได้แก่ ระดับน้ำตาล ไกลโคซิเลทฮีโมโกลบิน (HbA1c) ไขมัน โฮโมซิสเทอีน โฟเลต วิตามินบี 12 และ C-reactive protein (hs-CRP) เมื่อเริ่มต้นและสัปดาห์ที่ 6 ของการวิจัย

ผลการศึกษาพบว่าความดันโลหิต และตัวบ่งชี้ทางชีวภาพต่างๆ ไม่มีความแตกต่างกันอย่างมีนัยสำคัญระหว่างกลุ่มทดลองและกลุ่มควบคุม แต่หลังจากได้รับโปรตีนสกัดจากถั่วเหลืองกลุ่มทดลองมีระดับ HbA1c และโฮโมซิสเทอีนลดลง และระดับโฟเลตเพิ่มขึ้น เมื่อเปรียบเทียบกับค่าเริ่มต้นอย่างมีนัยสำคัญทางสถิติ ($p < 0.01$) สำหรับระดับไขมันในเลือดพบว่าระดับคอเลสเตอรอลรวม แอลดีแอล-คอเลสเตอรอล และไตรกลีเซอไรด์ ลดลงในกลุ่มทดลองมากกว่ากลุ่มควบคุม แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับค่าเริ่มต้น นอกจากนี้ยังพบความสัมพันธ์ในทางลบระหว่างระดับโฮโมซิสเทอีนกับระดับโฟเลต ($r = -0.47$) วิตามินบี 12 ($r = -0.39$) และการทำงานของไต (GFR) ($r = -0.47$) แต่มีความสัมพันธ์ในทางบวกกับอายุ ($r = 0.59$) อย่างมีนัยสำคัญทางสถิติ สำหรับระดับ hs-CRP พบว่ามีความสัมพันธ์ในทางลบกับการทำงานของไต (GFR) ($r = -0.36$) แต่มีความสัมพันธ์ในทางบวกกับอายุ ($r = 0.40$) และ HbA1c ($r = 0.35$) อย่างมีนัยสำคัญทางสถิติ

ผลการวิจัยแสดงให้เห็นว่า การเสริมโปรตีนสกัดจากถั่วเหลืองในผู้ป่วยโรคเบาหวานชนิดที่ 2 มีผลดีต่อตัวบ่งชี้ทางชีวภาพบางตัว ซึ่งอาจเป็นประโยชน์ในการลดความเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือดในผู้ป่วยโรคเบาหวานชนิดที่ 2

ภาควิชา……อาหารและเภสัชเคมี.....ลายมือชื่อนิสิต……
สาขาวิชา..อาหารเคมีและโภชนศาสตร์ทางการแพทย์...ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก….....
ปีการศึกษา.....2553……..ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

##5176611533 : MAJOR FOOD CHEMISTRY AND MEDICAL NUTRITION
KEY WORDS: SOY PROTEIN ISOLATE/ CARDIOVASCULAR BIOMARKERS/ LIPID PROFILE/ HOMOCYSTEINE/C-REACTIVE PROTEINE/ TYPE 2 DIABETES

EM-ON CHAIPRATEEP: EFFECTS OF SOY PROTEIN ISOLATE SUPPLEMENTATION ON BIOMARKERS OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS AT PUBLIC HEALTH CENTER 66, HEALTH DEPARTMENT BANGKOK METROPOLITAN ADMINISTRATION. THESIS ADVISOR: ASSOC. PROF. ORANONG KANGSADALAMPAI, Ph.D., THESIS CO-ADVISOR: ASST. PROF. KULWARA MEKSAWAN, Ph.D., 138 pp.

Cardiovascular disease (CVD) accounts for the majority of morbidity and mortality in type 2 diabetic patients. This study evaluated the effects of soy protein isolate (SPI) supplementation on biomarkers of CVD in type 2 diabetic patients at Public Health Center 66. Thirty-six type 2 diabetic patients were recruited. They received nutrition counseling and were randomized into 2 groups: SPI group (supplemented with 30 g/day of SPI containing 32 mg of isoflavones for 6 weeks) and control group (no SPI supplementation). Blood pressure and biomarkers of CVD, which included blood glucose, glycosylated hemoglobin (HbA1c), lipid profile, total homocysteine (tHcy), folate, vitamin B_{12} and C-reactive protein (hs-CRP) were examined at baseline and at the end of week 6 of the study.

The results showed that there was no significant difference in blood pressure and biomarkers of CVD between the SPI and control groups. However, after SPI supplementation, HbA1c and tHcy levels were significantly decreased and folate level was significantly increased from baseline ($p < 0.01$). The concentrations of TC, LDL-C and TG were decreased greater in SPI group than those in control group, but there were no significant differences from baseline. Furthermore, plasma tHcy level was significantly negatively correlated with plasma folate (r = -0.47), vitamin B_{12} (r = -0.39) and renal function (GFR) (r = -0.47), but it was significantly positively correlated with age (r = 0.59). The serum level of hs-CRP was significantly negatively correlated with GFR (r = -0.36) and significantly positively correlated with age (r = 0.40) and HbA1c (r = 0.35).

This study indicated that supplementation of SPI with isoflavones improves some biomarkers of CVD and may be beneficial on cardiovascular events in type 2 diabetic patients.

Department:….Food and Pharmaceutical Chemistry…..Student’s Signature.…...….....
Field of Study:.Food Chemistry and Medical Nutrition..Advisor’s Signature..……
Academic Year:…….2010...…..…….Co-Advisor’s Signature.…...…….

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AHA	American Heart Association
BMI	body mass index
BP	blood pressure
BUN	blood urea nitrogen
CAD	coronary artery disease
CHD	coronary heart disease
cm	centimeter
CRP	C-reactive protein
CVD	cardiovascular disease
CVE	cardiovascular events
d (s)	day (s)
DM	diabetes mellitus
ECLIA	electro-chemiluminescence immunoassay
etc.	et cetara
et al.	et alia (and others)
FBS	fasting blood sugar
FMD	flow mediated dilatation
FPG	fasting plasma glucose
g (s)	gram (s)
GDM	gestational diabetes mellitus
GFR	glomerular filtration rate
HbA1c	glycosylated hemoglobin
HDL-C	high density lipoprotein cholesterol
hr (s)	hour (s)
hs-CRP	high sensitivity C-reactive protein
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
kcal	kilocalorie
kg	kilogram

l	litre
LDL-C	low density lipoprotein cholesterol
m^2	square in metres
MAC	mid arm circumference
MAMC	mid-arm muscle circumference
mg	milligram
mg/dl	milligram per deciliter
MI	myocardial infarction
min (s)	minute (s)
ml	milliliter
mmol/l	millimole per litre
mm	millimeter
mmHg	millimeter-mercury
MS	methionine synthase
MTHFR	methylenetetrahydrofolate redutase
n	number
ng	nanogram
NICE	National Institute for Health and Clinical Excellence
OGTT	oral glucose tolerance test
PAD	peripheral artery disease
PAI	physical activity index
pg	picrogram
pmol	picromole
pH	potential of hydrogen ion
PVD	peripheral vascular disease
RCT	randomized controlled trials study
RDA	Recommended Dietary Allowance
Scr	serum creatinine
SD	standard deviation
SPC	soy protein concentrate
SPI	soy protein isolate
TC	total cholesterol
TG	triglyceride

tHcy	total homocysteine
TSF	triceps skinfold thickness
US.FDA.	The United States Food and Drug Administration
UKPDS	United Kingdom Prospective Diabetes Study
vs.	versus
WC	waist circumference
WHO	World Health Organization
WHR	waist-hip circumference ratio
wk (s)	week (s)
β	beta
0.0259	conversion factor from mg/dl to mmol/l for TC, LDL-C, HDL-C (mg/dl x conversion factor = mmol/l)
0.0113	conversion factor from mg/dl to mmol/l for TG (mg/dl x conversion factor = mmol/l)
0.0555	conversion factor from mg/dl to mmol/l for FPG (mg/dl x conversion factor = mmol/l)

CHAPTER I

INTRODUCTION

1.1 Background and Significance of the Study

The incidence and prevalence of diabetes mellitus (DM) worldwide is increasing, due almost exclusively to an increase in non-insulin-dependent (Type 2 DM) (Rao and McGuire, 2004). Diabetes mellitus is a group of metabolic disorder characterized by high blood glucose (hyperglycemia) resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (American Diabetes Association [ADA], 2010).

Diabetes patients, particularly those with type 2 diabetes, are at increased risk of developing atherosclerotic cardiovascular disease (CVD) including coronary heart disease (CHD), stroke, and peripheral vascular disease (PVD) (Toth et al., 2007). Cardiovascular disease accounts for the majority of morbidity and mortality associated with type 2 diabetes (Rao and McGuire, 2004). A person with type 2 diabetes is 2 – 6 times more likely to get CVD, and up to 80% of patients with diabetes will die from this disease (International Diabetes Federation [IDF], 2007).

Numerous factors for heart disease appear promising as independent risk factors in predicting progression to CVD. The new risk factors are increased levels of total-homocysteine (tHcy) and C-reactive protein (CRP). These risk factors, in combination with conventional high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) analysis, offer better diagnostic value for CVD (McNamara, 2000). Both tHcy and CRP levels were significantly elevated in

type 2 diabetic patients with atherosclerosis vascular disease when compared with patients without vascular disease. It is also found that low folate levels were responsible for the majority of elevation of tHcy levels (Akalin, Alatas, and Colak, 2008). Commings et al. (2005) showed that diabetic patients with atherosclerosis had significant increase in CRP and tHcy concentrations.

Homocysteine (Hcy) is an intermediate sulfur-containing amino acid formed during the conversion of methionine through remethylation pathway. In remethylation cycle Hcy is remethylated to methionine and the methyl donor is 5-methyltetrahydrofolate (5-Methyl THF). This reaction is catalyzed by methionine synthase (MS), which requires folic acid and vitamin B_{12} (Ciccarone et al., 2003). Troen et al. (2003) demonstrated that folate and vitamin B_{12} deficiency and excess dietary methionine resulted in increase plasma tHcy. The association of hyperhomocysteinemia with an increased risk for the premature development of atherosclerotic CVD and increased risk of death in diabetic patients has been identified by several studies. Refsum et al. (2004) showed the combined effects of elevated tHcy on increasing the risk of mortality in diabetic patients who had been diagnostically confirmed for coronary artery disease (CAD). Hyperhomocysteinemia appeared to be a stronger (~ 2-fold) risk factor for mortality in type 2 diabetic patients than in non-diabetic patients. It has been estimated that lowering tHcy by 5 µmol/l may reduce the risk of CVD death by ~10% (Hoogeveen et al., 2000). The mean plasma tHcy levels were significantly higher in type 2 diabetic patients who died from CHD than in those who did not (Soinio et al., 2004). Therefore, folic acid and vitamin B_{12} may be useful as replacement therapy in diabetic patients to prevent future atherogenic processes and diabetes complications due to hyperhomocysteinemia (Ismail, Fahmy, and Farrag, 2008). Observational data suggested that increases

dietary folate is beneficial for prevention of some cancers and CVD (Bailey et al., 2010).

Soybean is an excellent source of protein, folate and minerals. The U.S. Food and Drug Administration approved the health claim that "25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" (U.S.FDA., 1999). Nagata et al. (2003) reported that soy products intake was inversely associated with plasma tHcy (r = -0.15, P = 0.04) and positively correlated with serum folate (r = 0.15, P = 0.04). In diabetic patients, supplementation with soy protein (50 g/day) containing isoflavones for 6 weeks resulted in reduction in plasma tHcy, total cholesterol (TC), LDL-C and triglycerides (TG) (Hermansen et al., 2001). Supplementing 30 g soy protein/day in hyperlipidemic subjects significantly reduced LDL-C and tHcy concentrations compared with the control group who consumed casein. Moreover, plasma tHcy at baseline was inversely associated with serum folate and vitamin B_{12} (Tonstad, Smerud, and Hoie, 2002).

The study in postmenopausal women found that after 6 months of treatment with isoflavones resulted in a significant decrease in TC, LDL-C, TG and plasma tHcy concentrations (Turhan et al., 2009). Hanson et al. (2006) reported that supplementation of soy protein isolate (SPI 40 g/day) in postmenopausal women for 6 weeks significantly reduced plasma tHcy. In postmenopausal women with metabolic syndrome, consumption of soy protein (30 g/day) for 8 weeks significantly reduced fasting insulin, LDL-C, TG and hs-CRP (Azadbakht et al., 2007). In type 2 diabetic patients with nephropathy, soy protein consumption significantly decreased fasting plasma glucose (FPG), hs-CRP and improved lipid profiles (Azadbakht, Atabak, and Esmaillzadeh, 2008).

Epidemiological studies have demonstrated that type 2 diabetic patients had a higher incidence of CVD , which is a major cause of death in diabetic patients (Rao and McGuire, 2004). Many studies have shown that soy protein reduced the risk of heart disease by improving the lipid profiles and blood glucose control (Anderson, Johnstone, and Cook-Newell, 1995; Gardner et al., 2001); however, little evidence exists regarding the effects of SPI consumption on other biomarkers of CVD including plasma tHcy, hs-CRP, folate and vitamin B_{12} levels. In addition, the effects of SPI focusing specially on diabetic patients are rarely investigated in Thai population. Hence, the purpose of the present study was to evaluate the effect of SPI supplementation on biomarkers of CVD in type 2 diabetic patients.

1.2 Objectives of the Study

The specific objectives of the present study were to determine the effects of SPI supplementation on biomarkers of CVD including glycemic control, lipid profiles, tHcy, hs-CRP, folate and vitamin B_{12} in type 2 diabetic. The correlations between biomarkers of CVD were also investigated.

1.3 Benefits of the Study

This study provides the information about the effects of SPI supplementation on biomarkers of CVD in type 2 diabetic patients, thus the study results can apply for using in therapeutic planning for decreasing vascular complications in type 2 diabetic patients.

1.4 **Operational Definition of Terms**

Soy protein isolates (SPI)**:** a product that prepared from defatted soybeans and both soluble and insoluble carbohydrates are removed. The resulting product contains protein at least 90% on a moisture-free basis (Egbert, 2004).

Total Homocysteine (tHcy): an intermediate metabolite of the essential amino acid, methionine, which can be degraded through two enzymatic pathways namely remethylation and transulfuration. Through the remethylation pathway, Hcy receives a methyl group from 5-Methyl THF to form methionine and vitamin B_{12} is an essential cofactor for the MS (Jacobsen, 2001).

High-sensitivity C-reactive protein (hs-CRP): an acute-phase response protein that is considered a marker of inflammation and a predictor of cardiovascular events (CVE), including myocardial infarction (MI), stroke and peripheral arterial disease (PAD) (Bruno et al., 2009)

Glomerular filtration rate (GFR): a good index of renal fuction. GFR is difficult to measure in clinical practice, most clinicians estimate the GFR from the serum creatinine concentration by Cockroft-Gault formula: GFR (ml/min) = [(140-age) × weight (kg)]/[(72 × serum creatinine (mg/dl))] for men, multiplied by 0.85 for women, if GFR value ≥ 90 ml/min was considered as indication of normal renal function (Levey et al., 1999; Ndrepepa et al., 2008).

CHAPTER II

LITERATURE REVIEW

2.1 Prevalence and Costs of Diabetes Mellitus

The prevalence of type 2 diabetes is increasing and quickly becoming a global concern. The World Health Organization (WHO) estimates that there will be 300 million people with diabetes worldwide by the year 2025, which is more than twice the estimated prevalence reported in 1995 (Chapman-Novakofski, 2008; Rao and McGuire, 2004). Diabetes is strongly associated with cardiovascular disease (CVD) risk, which is the primary cause of morbidity and mortality among patients with diabetes, accounting for more than 80% of death in this population (Rao and McGuire, 2004).

The economic burden of diabetes mellitus was approximately $98 billion in 1997. More than half of medical expenditures related to DM result from hospitalizations for CVD (Isley, 2004). The costs of diabetes are staggering, in 2002, direct medical costs of diabetes were estimated to be $92 billion, with an additional $40 billion in indirect costs due to disability, work loss and premature mortality. Overall health care costs for people with diabetes are more than double the costs for people who do not have diabetes and a significant contributor for rising health care cost (Buse, 2006).

2.2 Type of Diabetes Mellitus (Chapman-Novakofski, 2008; Franz, 2008)

2.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (type 1 DM) accounts for 5-10% of all diagnosed cases of diabetes. Most cases are diagnosed in people younger than 30 years of age,

with a peak incidence at around ages 10 to 12 years in girls and ages 12 to 14 years in boy. The primary defect is pancreatic β-cell destruction, usually leading to absolute insulin deficiency and resulting in hyperglycemia, polyuria (excessive urination), polydipsia (excessive thirst), dehydration, electrolyte disturbance, weight loss and ketoacidosis. Person with type 1 DM are dependent on exogenous insulin to prevent ketoacidosis and death.

2.2.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (type 2 DM) accounts for 90-95% of all diagnosed cases of diabetes. Hyperglycemia develops gradually and is often not severe enough in the early states for the patient to notice any of the classic symptoms of diabetes. Although undiagnosed, these individuals are at increased risk of developing macro- and micro-vascular diabetes complications. In most cases type 2 DM results from combination of insulin resistance and β-cell failure. Endogenous insulin levels are inadequate to overcome concomitant “insulin resistance” (decreased tissue sensitivity or responsiveness to insulin); as a result, hyperglycemia ensures. Insulin resistance is first demonstrated in target tissue, mainly muscle, liver and adipose tissue. Initially there is a compensatory increase in insulin secretion, which maintains normal glucose concentrations, but as the disease progresses, insulin production gradually decreases.

2.2.3 Gestational Diabetes Mellitus (GDM)

GDM is defined as any degree of glucose intolerance with onset of first recognition during pregnancy. It occurs in about 7% of all pregnancies, usually diagnosed during the second or third trimester of pregnancy. During pregnancy gestational diabetes requires treatment to normalize maternal blood glucose levels to avoid complications in the infant.

2.2.4 Other Type of Diabetes

This type of diabetes may account for 1-5% of all diagnosed cases of diabetes. This includes diabetes associated with specific genetic syndromes, drugs, malnutrition, infections and other illnesses.

2.3 Diagnostic and Screening Criteria

Diagnostic criteria for diabetes are summarized in Table 1. There are three ways to diagnose diabetes and each, in the absence of unequivocal hyperglycemia, must be confirmed on a subsequent day by any one of the three methods. Testing or screening for diabetes should be considered in all individuals at age 45 years and above, particularly in those with a body mass index (BMI) of 25 kg/m^2 or more, and if the result is normal, the test should be repeated at 3-year intervals (ADA, 2010). Testing should be considered at a younger age or be carried out more frequently in individuals who are overweight (BMI > 25 kg/m^2) and have additional risk factors (a family history of diabetes, older age, obesity and physical inactivity).

2.4 Management

The management of diabetes is aimed at three goals: (1) elimination symptoms of hyperglycemia, (2) prevention of microvascular diabetes complications and (3) prevention of macrovascular diabetes complications, all without producing excess hypoglycemia or other untoward effects (Isley, 2004). The management of type 2 diabetes hinges on non-pharmacological measures (diabetes education, diet, exercise and weight loss) and drug therapy (Dagogo-Jack, 2006).

TABLE 1 Criteria for the diagnosis of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes mellitus

Impaired fasting glucose (IFG)

- Fasting plasma glucose (FPG) [a] 100-125 mg/dl (5.6-6.9 mmol/l)

Impaired glucose tolerance (IGT)

- 2-hour plasma glucose 140-199 mg/dl (7.8-11.0 mmol/l)

Diabetes mellitus

- FPG ≥ 126 mg/dl (7.0 mmol/l) or
- Symptoms of hyperglycemia and a casual plasma glucose ≥ 200 mg/dl (11.1 mmol/l) [b] or
- 2-hour plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an OGTT [c]

[a] fasting is defined as no caloric intake for at least 8 hr.

[b] casual is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss.

[c] the test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water

Source: adapted from ADA (2009*a*)

2.4.1 Pharmacological Treatment

The maintenance of long-term glycemic control (necessary for prevention of complications) in person with type 2 DM often requires the use of multiple agents in combination. Medication for combination therapy should be selected from drug classes that lower blood glucose by different mechanism, to ensure additive or synergistic effects and to maximize nonglycemic benefits related to weight, lipid profiles and biomarkers of CVD. The available agents that are currently approved for drug therapy of type 2 DM present in Table 2.

TABLE 2 Oral hypoglycemic agents

Classes	Mechanism of action	Example
Insulin secretagogues		
Oral sulfonylureas (1^{st} generation)	stimulate insulin release from β-cells	Chlorpropamide Tolazamide Tolbutamide
Oral sulfonylureas (2^{nd} generation)	stimulate insulin release from β-cells	Glimepiride Glipizide Glyburide Gliclazide
Non-sulfonylurea secretagogues	stimulate insulin release from β-cells	Nateglinide Repaglinide
Insulin sensitizers		
Biguanides	increase insulin sensitivity increase cellular glucose uptake and utilization decrease hepatic glucose production decrease morbidity and mortality in obese patients	Metformin
Thiazolidinediones	insulin sensitizers decrease hepatic output of glucose increase peripheral insulin uptake	Pioglitazone Rosiglitazone
α-Glucosidase Inhibitors		
	inhibit α-glucosidase in brush border of small intestine prevent hydrolysis and delay carbohydrate digestion	Acarbose

Source: adapted from Jain and Saraf (2008)

Functionally, these agents can be classified into insulin secretagogues, insulin sensitizers and alpha-glucosidase inhibitors. All of these agents have tissue-specific actions to improve blood glucose control. Thus, the initial choice of medication for control of hyperglycemia in type 2 diabetic patients is a matter of

clinical judgment. In most patients of type 2 diabetes, β-cell function had decreased by ~50% at the time of diagnosis and continued to deteriorate over time. The progressive decline in β-cell function predicts a future need for exogenous insulin. Immediate insulin therapy is indicated for initial stabilization of type 2 diabetic patients with ketoacidosis or severe glycemia. In stable patients, exogenous insulin can be considered as an adjunct to oral agents if glycemic control is suboptimal. Patients with type 2 DM that has been managed with oral agents for many years are understandable reticent about the prospects of starting insulin.

2.4.2 Prevention of Macro- and Micro-vascular Complications

A comprehensive approach to modification of cardiovascular risk factors is recommended, because most diabetic patients die from heart disease or stroke. Therapeutic interventions include smoking cessation, control of dyslipidemia, blood pressure control and maintenance of HbA1c of < 7% (Table 3).

Intensive glucose control in patients with acute myocardial infarction has been demonstrated to reduce short-term and long-term mortality in diabetic patients. Thus, excellent glycemic control not only is necessary to prevent the development of complications, but also helps improve survival in persons with diabetes who have suffered CVD. The microvascular complications including retinopathy, nephropathy and neuropathy develop after several years of uncontrolled diabetes. The best prophylaxis against microvascular complications is tight glycemic control.

TABLE 3 Recommendations for lipid and blood pressure for diabetic patients

Classes	
LDL cholesterol	<2.6 mmol/l (<100 mg/dl)
HDL cholesterol	
Men	>1.1 mmol/l (>40 mg/dl)
Women	>1.4 mmol/l (>50 mg/dl)
Triglyceride	<1.7 mmol/l (<150 mg/dl)
Blood pressure	<130/80 mmHg

LDL =low density lipoprotein, HDL = high density lipoprotein, mmol/l = millimole per litre, mg/dl = milligram per decilitre, mmHg = millimetre of mercury

Source: adapted from ADA (2007)

2.5 Diabetes and Cardiovascular Disease

Diabetes is strongly associated with CVD risk, which is the primary cause of morbidity and mortality among patients with diabetes, CVD accounts for more than 80% of mortality in this population. The cardiovascular complications of type 2 diabetic patients include coronary heart disease (CHD), cerebrovascular disease and peripheral artery disease (PAD). Uncontrolled diabetic patients are more likely to experience rapid progression of CVD and eventually the clinical implications (Lahham, 2009). The most effective interventions to improve the CVD risk of patients with diabetes involve aggressive biomarkers of CVD control: glycemic and blood pressure control and improve lipid profiles (Rao and McGuire, 2004).

2.6 Biomarkers of Cardiovascular Disease

The development of atherosclerosis is multifactorial and generally starts long before the development of clinically evident diabetes, a global approach to biomarkers of CVD reduction is mandated in all patients with diabetes (Al-Ozairi, Middelbeek, and Horton, 2006). In recent years, the study by Lahham (2009) confirmed that patients with type 2 diabetes had many associated traditional risk factors for CVD. The frequencies of traditional risk factors in these diabetic patients were hypercholesterolemia 26.8%, hypertriglyceridemia 31.9%, obesity 32.8%, smoking 68.5% and hypertension 59.1%.Traditional risk factors do not explain all of the risk for incident CVE in type 2 diabetic patients. Various new or nontraditional risk factors have the potential to improve global risk assessment for CVD (Helfand et al., 2009). So early detection of traditional and nontraditional risk factors (Table 4) and intervention in diabetic patients can reduce mortality and improve health outcomes in this high-risk population (Lahham, 2009).

2.6.1 Hyperglycemia

Hyperglycemic control in type 2 diabetic patients is mandatory for the prevention of development of both macro- and micro-vascular complications. The relationship of glycosylated hemoglobin (HbA1c) to CVD risk has been clarified in type 2 diabetes with the demonstration that HbA1c levels are an independent predictor of CVD (Al-Ozairi et al., 2006).

Current recommendation for glycemic control includes reducing FPG and HbA1c to near normal (FPG < 126 mg/dl and HbA1c < 7%). Uncontrolled blood glucose in diabetic patients was more likely to experience rapid progression of CVD. In addition, plasma tHcy levels decreased even with modest improvement of glycemic control in type 2 DM (Get-ngern et al., 2005)

TABLE 4 Risk factors for cardiovascular disease

Traditional risk factors	Nontraditional (emerging) risk factors
Hyperglycemia	C-reactive protein
Hypertension	Homocysteine
Dyslipidemia	Fibrinogen
Smoking	Lipoprotein
Obesity	Oxidized LDL-cholesterol

LDL = low density lipoprotein

Source: adapted from Hofimann, Tucker, and Parker (2009)

2.6.2 Hypertension

Hypertension has been identified as one of the most potent antecedents of CVD. Because hypertension is usually as asymptomatic, healthcare providers have a responsibility to identify individuals at risk by measuring blood pressure. Elevated blood pressure accelerates the development of coronary artery disease (CAD) and contributes significantly to the pathogenesis of cerebrovascular accidents, heart failure and renal failure, especially in type 2 DM. Blood pressure (BP) should be checked annually or more frequently as indicated. BP > 160/100 mmHg should always be treated in those with and without diabetes aiming for a level of < 140/80 mmHg. BP of 140-159/90-99 mmHg should be treated in all diabetic patients, and it aims for < 140/80 mmHg. Other factors may also help reduce BP including salt restriction, weight reduction or exercise and reduction of excessive alcohol intake (Watkins, 2003; Williams et al., 2002).

2.6.3 Dyslipidemia

Dyslipidemia is common in patients with diabetes and further increase the risk of ischemic heart disease, especially in type 2 DM. Indeed, even when low density lipoprotein cholesterol (LDL-C) concentration is normal or slightly raised in type 2 diabetes, the LDL particles may be qualitatively different and more atherogenic than those in non-diabetic patients (Watkins, 2003). Several epidemiological studies have established that every 1% decrease in LDL-C was associated with a 2% decrease in CHD risk whereas every 100 mg/dl increase in triglyceride (TG) was associated with an increase in cardiovascular events (CVE) of 16% in men and 42% in women. For high density lipoprotein cholesterol (HDL-C), each 1 mg/dl decrease in HDL-C is associated with a 2-3% increase in CVD risk (Babu and Fogelfeld, 2006; Greene and Fernandez, 2007). Diabetic patients had borderline to high risk levels of TC, TG, LDL-C and HDL-C. These high frequencies of dyslipidemia increase their risk of CVD. The results indicated the need of control not only glycemic levels in diabetic patients but also to their lipid profiles (Lahham, 2009).

2.6.4 Smoking and Obesity

Smoking double the risk of CVD in normal subjects, stopping smoking is one of the most effective ways to decrease cardiovascular risk. In diabetic subjects, smoking is associated with increased formation of subclinical atherosclerosis. Obesity, commonly seen in patients with type 2 DM, is associated with increased risk of CVD (Al-Ozairi et al., 2006). Obesity classified by Body mass index (BMI), which calculated as weight/height^2 (kg/m^2). BMI cut-off points in Asian populations for overweight and obesity are 23-24.9 kg/m^2 and 25-29.9 kg/m^2, respectively (Inoue and Zimmet, 2000).

2.6.5 C-Reactive Protein (CRP)

C-reactive protein, a marker of inflammation, is recently recognized as an independent predictor of future CVE. Furthermore, hs-CRP is associated with DM (Ma et al., 2006). Almost 50% of CVE occur in patients with normal LDL-C levels. The measurement of hs-CRP in these patients helps to identify those patients at greater risk. American Heart Association (AHA) guidelines have designated hs-CRP levels greater than 3 mg/l as high and associated with increased cardiovascular risk, 1-3 mg/l as moderate risk and <1.0 mg/l as low risk. It is necessary to perform 2 separate measurements to accurately classify a patient's risk. If the initial hs-CRP is >10 mg/l, it should be discarded and the test should be repeated in 2 weeks to allow for the resolution of acute inflammation (Hofimann et al., 2009).

C-reactive protein assays have recently been developed that provide very sensitivity, called "**high sensitivity CRP (hs-CRP)**". The levels of hs-CRP increased in patients with acute inflammatory caused by infection, autoimmune disorders and chronic inflammation caused by atherosclerotic plaque formation (Hofimann, et al., 2009). Patients with type 2 DM are at an increased risk for atherosclerosis (Toth et al., 2007). Evidence is accumulating in the literature supporting a role for inflammation in the pathogenesis of DM, thereby possibly linking the manifestation or progression of diabetes with a number of "inflammatory disorders" (Ridker and Silvertown, 2008). Systemic inflammation, measured by increased serum acute-phase reactants such as hs-CRP, has been recognized to occur in type 2 DM. Significantly higher serum concentrations of hs-CRP have been found in patients with type 2 DM. The levels of hs-CRP decreased significantly after improvement of metabolic control in type 2 diabetic patients, indicating that the inflammatory pathway are improved (Holly, 2007).

2.6.6 Homocysteine (Hcy)

Homocysteine is a sulphur-containing amino acid. An increase in the plasma level of Hcy, an intermediate in the catabolism of methionine, has been identified as a risk factor for CVD (Wijiekoon, Brosnan, and Brosnan, 2007). Diabetic patients with CHD had significantly higher plasma tHcy concentrations in comparison to the diabetes without history of CHD (Rudy et al., 2005). Homocysteine is present in plasma in four forms. About 70-80% of plasma Hcy is in the form of disulfide bound to plasma proteins, chiefly albumin. About 1% circulates as the free thiol, and the remaining 20-30% of plasma Hcy combine with itself to form the dimer Hcy or with other thiols, including cysteine, which forms the Hcy-cysteine mixed disulfide. The term "**total plasma homocysteine**" **(tHcy)** refers to all of the four forms of Hcy (Figure 1) (Govindaraju et al., 2003; Tewari, Zhang, and Bluestein, 2004). Plasma tHcy levels are measured during fasting conditions. Fasting values of tHcy between 5 - 12 µmol/l are considered to be normal. However, for subjects at increase risk the AHA has recommended a basal level <10 µmol/l (Genser et al., 2006). The results from meta-analysis suggested that tHcy was an independent graded risk factor for atherosclerotic disease. An increment of 5 µmol/l had an effect on the risk of heart disease comparable to the effect of 0.5 mmol/l serum cholesterol (Ashfield-Watt, Burr, and McDowell, 2004). Finally, unlike cholesterol, which the body needs for the production of certain cell parts and hormones, tHcy provides no health benefit. The higher levels of tHcy, greater the risk of CVD. Therefore, tHcy levels must be as low as possible (Strand and Wallace, 2002).

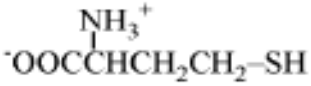
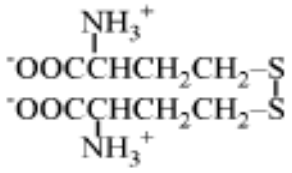
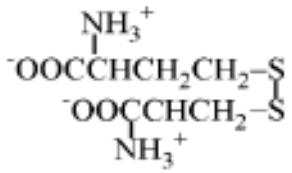
Reduced:		
Homocysteine		**1%**
Oxidized:		
Homocystine		**5-10%**
Mixed-disulfides:		
Protein-bound Homocysteine		**80-90%**
Cysteine-Homocysteine		**5-10%**

FIGURE 1 Structural all forms of homocysteine present in plasma

- **Homocysteine Metabolism**

Homocysteine is a naturally occurring sulfur containing amino acid. Homocysteine derived from the metabolism of dietary methionine. Methionine is found in large quantities in meats, eggs, milk, cheese, white flour, canned foods, and highly processes foods. Homocysteine has two main metabolic phases, remethylation (reconstituting methionine) and transulfuration (Figure 2).

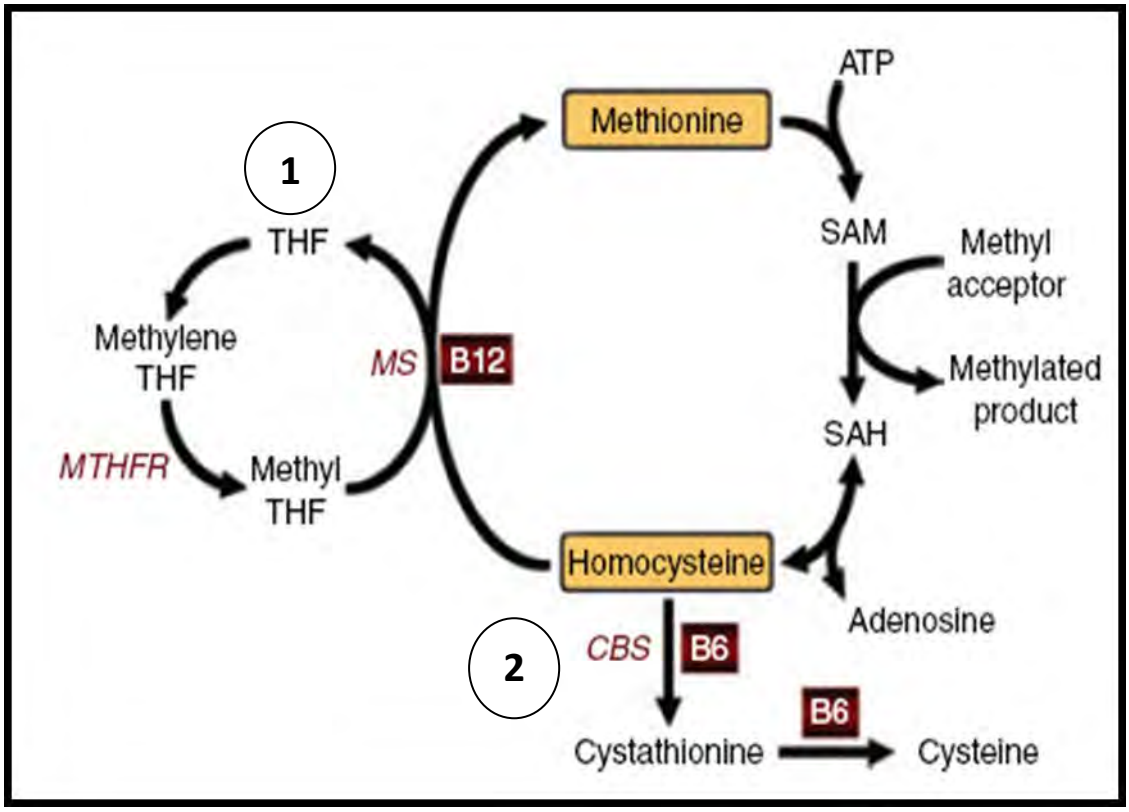


FIGURE 2 Homocysteine metabolism

In most tissues, remethylation involves a derivative of dietary folate (5-methyltetrahydrofoate: 5-Methyl THF) as the methyl donor. The process is catalyzed by methionine synthase (MS) with vitamin B_{12} as an essential cofactor. Methionine synthase and another enzyme (methylenetetrahydrofoate reductase; MTHFR) also participate in successive stages of the conversion of dietary folate into methyl donor. Transulfuration pathway involves conversion of tHcy into cysteine, which required vitamin B_6 as a cofactor (Ashfield-Watt et al., 2004; Govindaraju, et al., 2003; Strand and Wallace, 2002). If one or more of the tHcy metabolic pathways are inhibited due to enzymatic defects or vitamin deficiencies, tHcy accumulates causing an increased tHcy levels in plasma (Tewari et al., 2004).

TABLE 5 Causes of hyperhomocysteinemia

Primary: Inherited enzyme defects
- Cystathione beta synthase
- Methylene tetrahydrofolate reductase
- Methionine synthase

Secondary:

a) **Physiological:**
- Increasing age
- Male gender
- Menopause

b) **Life style factors:**
- Tobacco use
- Coffee consumption
- Animal protein consumption

c) **Vitamin deficiencies:**
- Folic acid
- Vitamin B_{12}
- Vitamin B_6

d) **Systemic disorders:**
- Chronic renal failure
- Pernicious anemia
- Hypothyroidism
- Systemic lupus erythematous
- Psoriasis

e) **Malignancies:**
- Breast
- Ovary
- Pancrease

f) **Drugs:**
- Dihydrofolate reductase inhibitors (Methotrexate)
- Folic acid antagonist (Phenytoin)
- Vitamin B_{12} antagonist (Nitrous oxide)
- Vitamin B_6 antagonist (Theophylline)

Source: adapted from Govindaraju et al. (2003)

- **Cause of Hyperhomocysteinemia**

The causes of hyperhomocysteinemia can be either primary or secondary (Table 5). The primary causes of hyperhomocysteinemia include the genetic defects in the enzymes involved in Hcy metabolism, whereas the secondary causes include life style factors, chronic diseases and drugs. In general, tHcy levels tend to increase with age. Men have higher tHcy levels than age matched women.

Cigarette smoking also interferes with the synthesis of pyridoxal phosphate. Nutritional deficiencies in the vitamin cofactors that are required for metabolism of tHcy through the remethylation or transsulfuration pathways: folate, vitamin B_{12} and vitamin B_6. In developed countries, these vitamins are partially removed from foods during processing and typical diets are rich in the precursor amino acid methionine (which is derived from animal proteins). In addition, metabolic disorders such as hypothyroidism, pernicious anemia, psoriasis and chronic renal failure may also give rise to hyperhomocysteinemia. Plasma tHcy increase in chronic renal failure patients, not because of impaired urinary excretion but because of impaired metabolism of tHcy by the kidney, the major route by which tHcy is cleared from plasma. Several drugs increase tHcy concentrations. Methrotrexate depletes folate and phenytoin also interfere folate metabolism. As folate is a co-substrate for MS, altering folate levels can lead to increase in plasma tHcy concentrations (Govindaraju, et al., 2003; Loscalzo, 2006).

- **Homocysteine and Pathogenesis of Atherosclerosis**

A unifying hypothesis for the mechanism of Hcy-mediated vascular injury has not yet been established. One frequently described mechanism involves oxidative damage due to the auto-oxidation of tHcy in the plasma or intracellular to form various reactive oxygen species including superoxide and hydrogen peroxide which initiate lipid peroxidation and support the oxidation of LDL-C. Plasma tHcy has been shown to decrease the activity as well as the expression of the antioxidant enzyme glutathione peroxidase. Excess tHcy can form Hcy thiolactone, a highly reactive intermediate, which combines with LDL-C to form aggregates that are taken up intimal macrophages and be incorporated into atheromatous plaques. Plasma tHcy is also a potent mitogen for vascular smooth

muscle cells. Figure 3 shows the adverse vascular effects of tHcy in promoting atherothrombosis (Govindaraju, et al., 2003; Wijiekoon et al., 2007).

- **Homocysteine-Lowering Therapy**

Lowering tHcy levels by 25% (a reduction of ~3 μmol/l) from current levels would reduce the risk of ischemic heart disease by 11% and 19% lower risk of stroke (Loscalzo, 2006). Evidence from several studies confirmed the importance of tHcy as a powerful predictor of future risk of CVD and other complications of atherosclerosis. An inexpensive vitamin therapy with folic acid, vitamin B_{12} and vitamin B_6 is generally effective in reducing tHcy (Govindaraju, et al., 2003). Heart Outcome Prevention Evaluation (HOPE-2) reported reduction in tHcy levels but not with a significant reduction in death from cardiovascular causes, in patients with vascular disease or diabetes who were treated daily with a combination of folic acid, vitamin B_{12} and vitamin B_6 for 5 years. In addition, this trial showed a marginally significant reduction in stroke among the patients receiving vitamins than those receiving placebo (Loscalzo, 2006).

Folic acid in dose equal to the RDA (400 μg/day) is associated with a 25-30% reduction of tHcy levels. An additional 7% of tHcy reduction can be achieved by vitamin B_{12} 0.02-1 mg/day co-administration. However, current data do not support pharmacological treatment with folic acid and B vitamins in general population. In contrast, tHcy lowering treatment should be considered when homocysteinemia is present (Antoniades et al., 2009). Therefore, in general population should avoid excessive meat intake and increase consumption of leafy green vegetables, fresh fruits and beans, which are good sources of dietary folate to protects against CVD from elevated plasma tHcy (Refsum et al., 1998).

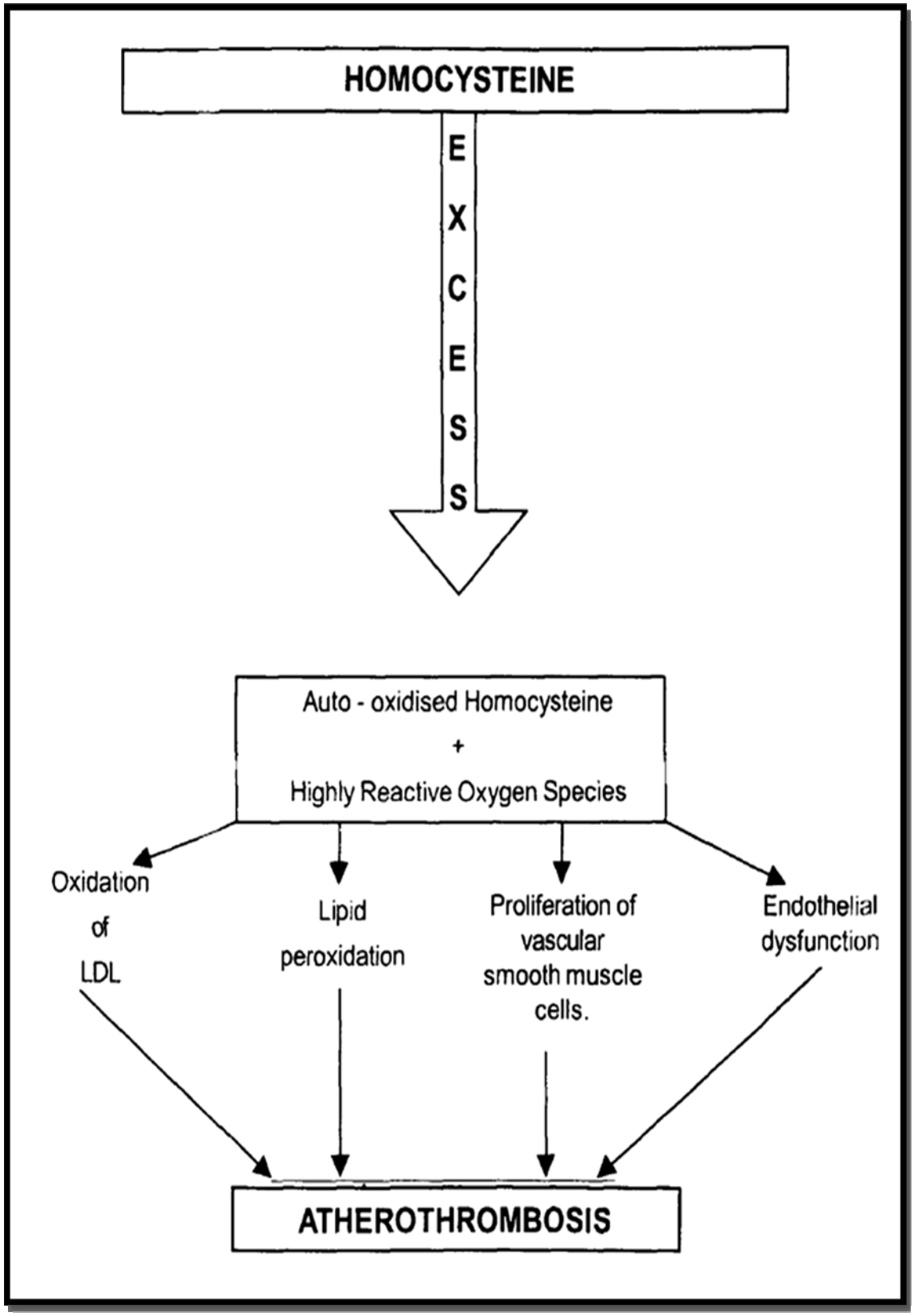


FIGURE 3 Adverse vascular effects of homocysteine

2.6.7 Folic acid

Total folate is an umbrella term used to represent the different forms of the B vitamin. Folate is the form that occurs naturally in food sources. Folic acid is the form of the vitamin found in fortified foods and dietary supplements. The term “dietary folate” is used to represent food folate and folic acid in fortified foods together. The governments of both the United States and Canada instituted national fortification programs with folic acid to enhance the diets of reproductive-aged women, and neural tube defect rates decreased in both country. However, the fortification program increased folic intake among virtually all segments of the population. Moreover, these data suggested that food folate is beneficial for prevention of CVD (Bailey et al., 2010).

Elevation of tHcy levels in folate deficiency is clear from the impairment of remethylation cycle. In general, folate seems to be a stronger determinant of tHcy levels than dose vitamin B_{12}. A rise in plasma tHcy levels was seen in the decline of folate levels, with folate levels < 4.8 mmol/l. Folate levels correlate inversely with tHcy levels in virtually all surveys, whether national surveys of presumably health persons or surveys of patients with CVD. Foods contain folate are shown in Table 6. Rich sources include beans, nut, dairy products, fruits, grains and cereals. Yeast is the single richest source of folate available. Folate is the predominant nutritional factor determining plasma levels of tHcy. Supplementation of folic acid >500 µg/day has been shown to reduce elevated tHcy levels (Genser et al., 2002). The RDA of folate, based on an intake from all sources that is sufficient to maintain red cell folate levels, is shown in Table 7. The red cell folate levels are slightly less sensitive indicator of early developing deficiency than serum folate because the red cell’s life span in the circulation is 120 days (Carmel, 2001b)

TABLE 6 Folate content in foods (µg per 100 g)

Foods	Folate content
Beans	
Soy protein isolate (current study)	211
Soybean	305
Black beans	149
Kidney beans	144
Green beans	37
Vegetables	
Spinach	160
Asparagus	95
Cabbage	80
Brown rice	41
Meat	
Pork	156
Chicken	61
Steamed mackerel	24

Sources: กระทรวงสาธารณสุข (2548)

TABLE 7 Recommended Daily Allowance (RDA) of folate

Subgroup	US RDA (µg/day)	Thai RDA (µg/day)
1-3 years old	150	40
4-8 years old	200	50-65
9-13 years old	300	90-130
14-19 years old	400	135-145
Adults (over 20 years old)		
Male	400	175
Female	400	150
Lactating women	500	250
Pregnancy women	600	500

Sources: adapted from Carmel (2001b) and กระทรวงสาธารณสุข (2532)

2.6.8 Vitamin B_{12}

Vitamin B_{12} deficiency may contribute to the risk of vascular disease through its association with elevated levels of tHcy in the blood. Elevation of tHcy is a very sensitive indicator of clinical vitamin B_{12} deficiency and do not recover until vitamin B_{12} therapy is given. Plasma vitamin B_{12} levels criteria for deficiency is <148 pmol/l (<200 pg/ml) (Carmel et al., 2003). Vitamin B_{12} in the human diet derives from animals that ingest vitamin B_{12}. Poultry and seafood are the major food sources, but eggs and dairy products are also important. The Institute of Medicine recently proposed the increased daily intake of vitamin B_{12} to 2.4 µg from the RDA of 2 µg. Vitamin B_{12} deficiency is a significant public health problem, particularly among the elderly. Therefore, the use of oral supplements has become increasingly common in recent years (Carmel, 2001a).

2.7 Soybeans *(Glycine max)*

Soybean belongs to the subgenus *Soja*, a member of the genus *Glycine*, in the family Leguminosae. Soybean is an extremely rich source of protein, fat, dietary fiber, vitamins, minerals and phytochemicals. Interest in the composition of soy products has grown because potential CVD prevention and other therapeutic agents have been reported in soybeans and related products. The U.S. Food and Drug Administration (U.S. FDA) has allowed the following food claim for soy protein: Diets low in saturated fat and cholesterol that include 25 g soy protein a day may decrease the risk of heart disease (Isanga and Zhang, 2008).

2.7.1 **Soybean and Soy Protein Products** (Endres, 2001)

The proximate composition of soybean and soy protein products is shown in Table 8. The protein isolate which is prepared by acid precipitation of soybean protein is very high in protein content as a result of its low fat and carbohydrate content and its almost total absence of fiber. Soybean oil is composed of approximately 16% saturated fatty acids, 24% monounsaturated fatty acids, and 60% polyunsaturated fatty acids. The major fatty acid is linoleic acid which by itself comprises 53% of total fatty acids in soybean oil. Another importance fatty acid component of soybean oil is linolenic acid, representing 7% of total fatty acids in soybean oil. Soybean is a good source of several vitamins (thiamine, riboflavin, niacin and folate) and minerals (phosphorus and calcium) but not zinc, manganese and copper. Soybeans are a major source of phytochemicals. The major bioactive isoflavones are genistein, daidzein and glycetein. The isoflavones content of different soy protein products depends on crop year and on the type of processing used to produce the ingredients (Hendrich and Murphy, 2001) (Table 9)

The amino acid profiles of soybean and various soy products are shown in Table 10. Soybean is consisted of high quantity essential amino acids, with the exception of the sulphur containing amino acids (cystine, methionine) in which soybean is deficient. However, there are sufficient other essential amino acids. Very high lysine content is noteworthy which places soybean protein in a distinct class of vegetable proteins. Since lysine is limiting in most cereal proteins, it is possible that soy protein may improve total protein status in populations dependent on cereal grains for stable food.

TABLE 8 Proximate composition of soybean and soy protein products

Constituents (g/100g)	Raw soybean	Defatted flour	Protein concentrate	Protein isolate
Water	8.54	7.25	5.80	4.98
Food energy (kcal)	416	329	332	338
Protein	36.49	47.01	58.13	80.69
Lipids	19.94	1.22	0.46	3.39
Carbohydrate	30.16	38.37	31.21	0.36
Crude fiber	4.96	4.27	3.77	0.26
Fatty acids (g/100g)				
Saturated	2.88	0.13	0.05	0.42
Monounsaturated	4.40	0.21	0.08	0.64
Polyunsaturated	11.25	0.53	0.20	1.65
Vitamins (mg/g)				
Thiamin	0.87	0.70	0.31	0.17
Riboflavin	0.87	0.25	0.14	0.10
Niacin	1.62	2.61	0.71	1.43
Pyridoxine	0.37	0.57	0.13	-
Folate	375.1	305.4	340	176.1
Vitamin B_{12}	0	0	0	0
Minerals (mg/g)				
Calcium	277	241	363	178
Magnesium	280	290	315	39
Potassium	1797	2384	2202	81
Phosphorus	704	674	839	776
Zinc	4.89	2.46	4.4	4.03
Copper	1.65	4.06	0.97	1.60
Manganese	2.51	3.01	4.19	1.49

Sources: adapted from Nwokolo (1996)

TABLE 9 Isoflavones contents of soy products (mg/100 g)

Soy products	Genistein	Daidzein	Glycitein	Total
Soybeans, raw (Japan)	74.33	45.95	9.01	130.65
Soybeans, raw (Europe)	39.78	45.44	22.37	103.56
Soybeans, raw (United States)	86.33	61.33	13.33	159.98
Soybeans, roasted	75.78	62.14	13.33	148.50
Soy flour, full-fat, raw	98.77	72.92	16.12	178.10
Soy flour, full-fat, roasted	85.12	89.46	16.40	165.04
Soy flour, defatted, raw	87.31	64.55	15.08	150.94
Soy protein concentrate, aqueous washed	52.81	38.25	4.94	94.65
Soy protein concentrate, alcohol extraction	5.26	5.78	1.57	11.49
Soy-protein isolate	57.28	30.81	8.54	91.05
Soybean, curd, fermented	21.12	12.18	2.30	34.68
Miso	23.24	16.43	3.00	41.45
Natto	37.66	33.22	10.55	82.29
Soymilk, unfortified	6.07	4.84	0.93	10.73
Tempeh, raw	36.15	22.66	3.82	60.61
Tempeh, cooked	21.14	13.12	1.39	35.64
Tofu, pressed, raw	16.01	15.59	2.77	33.91
Tofu, firm, cooked	10.83	10.26	1.35	22.05

Source: adapted from US Department of Agriculture (2008)

TABLE 10 Amino acid contents of soybean and soy protein products (g/100g)

Amino acids	Raw soybean	Defatted Flour	Protein concentrate	Protein isolate
Tryptophan	0.53	0.68	0.83	1.11
Threonine	1.58	2.04	2.47	3.13
Isoleucine	1.77	2.28	2.94	4.25
Leucine	2.97	3.82	4.91	6.78
Lysine	2.42	3.12	3.92	5.32
Methionine	0.49	0.63	0.81	1.13
Cystine	0.58	0.75	0.88	1.04
Phenylalanine	1.90	2.45	3.27	4.59
Tyrosine	1.38	1.77	2.30	3.22
Valine	1.82	2.36	3.06	4.10
Arginine	2.83	3.64	4.64	6.67
Histidine	0.98	1.26	1.57	2.30
Alanine	1.71	2.21	2.68	3.59
Aspartic acid	4.58	5.91	7.24	10.20
Glutamic acid	7.06	9.10	12.01	17.45
Glycine	2.13	2.75	3.29	4.96
Proline	2.13	2.75	3.29	4.96
Serine	2.11	2.72	3.36	4.59

Sources: adapted from Nwokolo (1996)

- **Soy flour**

Soy flour is made by grinding soybeans into a fine powder. Three type of flour are produced from soybeans for use in foods, natural or full-fat (contains natural oils), defatted (oils removed) with 50% protein content and lecithinated (lecithin added). The functionality, nutritional value and low cost of soy flour products account for their growing use in foods and as a base material for manufacture of further processed food ingredients.

- **Soy protein concentrate (SPC)**

SPC is basically defatted soy flour without the water soluble carbohydrates. It is made by removing part of the carbohydrates (soluble sugars) from defatted soybeans. They contain at least 65% protein on a moisture free basis, with the remaining portion being mainly insoluble carbohydrates.

- **Soy protein isolate (SPI)**

SPI is prepared from defatted soybeans by alkaline extraction, followed by precipitation at acidic pH. As a result, both soluble and insoluble carbohydrates are removed and the product is nearly fat free. Because of this, it has a neutral flavor and will cause less gas due to bacterial flatulence. SPI is used throughout the food industry for both nutritional and functional reasons, including meat analogs, soup and dairy type products (beverage powders and infant formulas) and it is added to food products like shakes, energy bar and cereals.

2.7.2 Soy Isoflavones (Isanga and Zhang, 2008)

Isoflavones are found mainly in soybean. They are present in soybean at the level of 0.1 to 5 mg/g. Isoflavones are also known as phytoestrogen because they are found in plant food (primarily soy products) and appear to have estrogen-like activity. They are structurally similar to estrogen and bind to estrogen receptors,

therefore, they share the physiological features and behavior of endogeneous estrogens. Many potential health benefits of isoflavones in soy products have been investigated, including effects on vascular disease, cognitive function and cancer (Hendrich and Murphy, 2001).

Genisteine and daidzein in soybean are mainly presented the form of their glycoside. It is generally believed that isoflavone glycosides are hydrolyzed to their corresponding aglycones prior to gastrointestinal absorption. The metabolism of isoflavones is shown in Figure 4. Isoflavones when ingested are metabolized extensively in the intestinal tract, absorbed, transported to the liver and undergo hepatic recycling. Intestinal bacterial glucosidases cleave the sugar moieties and release the biologically active isoflavones, daidzein and genistein. All of these phytoestrogen are eliminated mainly by the kidney.

2.7.3 Soy and Cardiovascular Disease

Epidemiological studies have shown that the consumption of soy-containing food may prevent or slow-down the development of CVD. In 1998, Soy protein consumption in Japan was reported to be as high as 55 g/day, compared with the United States which less than 5 g/day. Deaths from CVD per 100,000 people were 300 for Japanese population and 598 for US population. Hence, a higher consumption of soy containing foods was suggested to be an important factor in preventing atherosclerosis in Asian populations. However, the active principle of this protective effect is still controversial. Several studies have shown favorable effects of soy protein, isoflavones, or both in CVD risk reduction (Erdman, 2000; Wenzel, Fuchs, and Daniel, 2008).

Glycoside forms
Biologically inactive

Aglycone forms
Biologically active

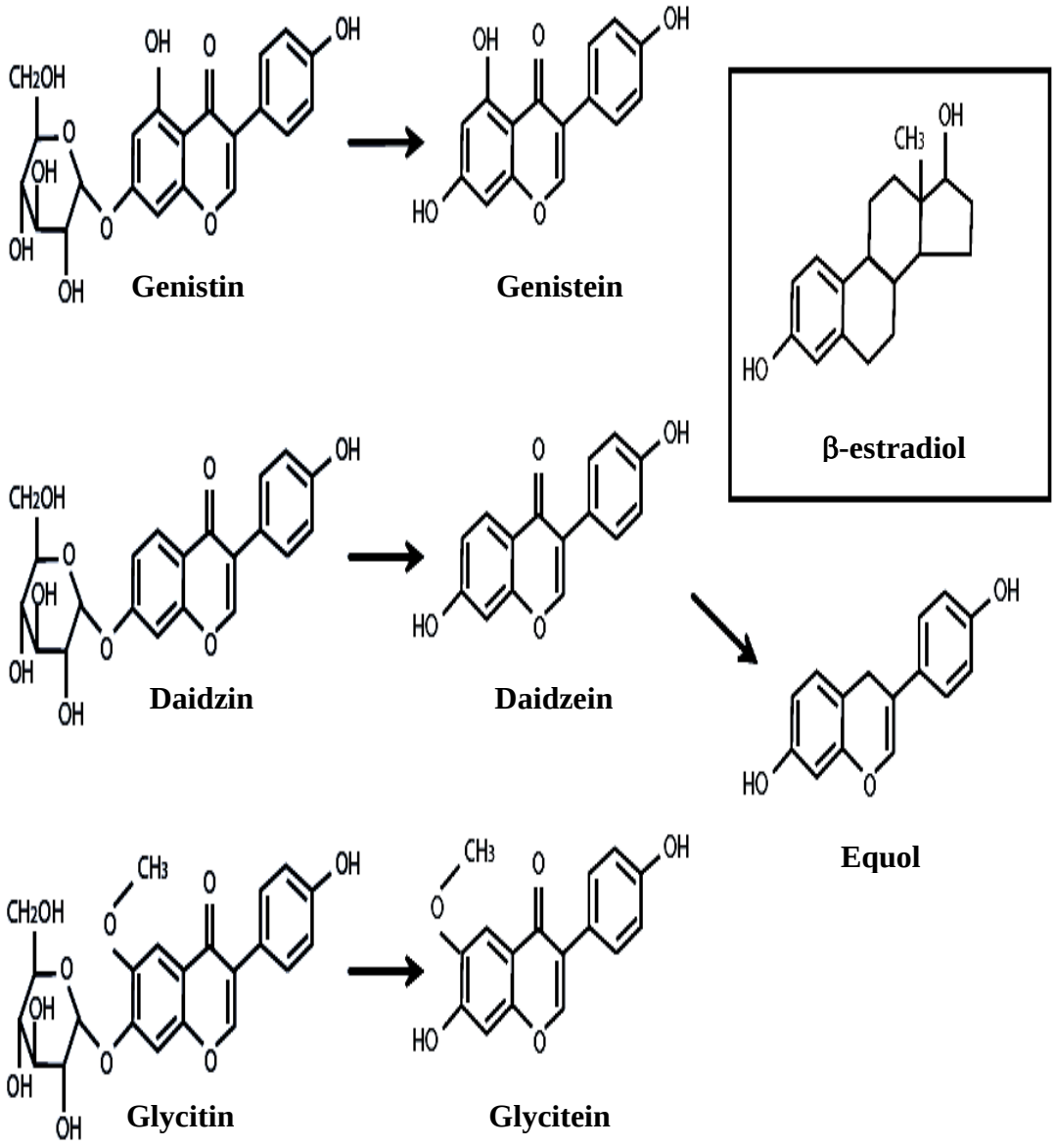


FIGURE 4 Metabolism of isoflavones

2.7.4 Effects of Soy on Glycemic Control

Eating legumes regularly, particularly soybeans may reduce the risk of developing type 2 DM and benefit people with existing diabetes who are also at increased risk of other chronic disease such as CVD and kidney disease (Jayagopal, Albertazzi, and Atkin, 2004). A large study from Shanghai found that among postmenopausal women, a regular intake of tofu and other soy products was strongly protective against type 2 DM (Yang, Shu, and Jin, 2004). Several randomized controlled trials (RCT) study reported the effects of soy protein or isoflavones in type 2 diabetic patients. Hermansen et al. (2001) found that daily supplementation with isoflavones (165 mg/day) in combination with soy protein (50 g/day), compared with placebo, for 6 weeks showed no difference in plasma glucose, insulin and HbA1c.

The most recent trial investigated at daily supplementation with 132 mg/day purified isoflavones over 12 weeks and found no effect on plasma glucose, HbA1c and insulin resistance (Gonzalez et al., 2007). However, Jayagopal et al. (2002a) found that soy protein (30 g/d) with isoflavones (132 mg/d) for 12 weeks significantly reduces mean values for fasting insulin, insulin resistance and HbA1c. Similarly, in longitudinal RCT soy protein consumption for 4 years significant reduced FPG compared with animal protein (Azadbakht et al., 2008). Azadbakht (2007) found that red meat replaced by soy protein diets for 8 weeks in postmenopausal women with metabolic syndrome significantly reduced fasting insulin and insulin resistance compared with control diet. Hence, purified isoflavones were found to have a positive effect on insulin resistance and other cardiovascular outcome measures, only when combine with soy proteins (Bartlett and Eperjesi, 2008).

2.7.5 Effects of Soy on Lipid Profile

The efficacy of soy and soy derivatives in lowering TC and LDL-C was recently supported by the US Food and Drug Administration (US.FDA, 1999) which approving a health claim that "**25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease**". Soy intake has been linked to the improvement of blood lipid levels in humans, therefore reducing the risk of developing atherosclerosis, especially in people with type 2 DM (Brynes, 2003). Consumption of soy protein has been shown to improve the blood lipid levels in diabetic subjects. The randomized double-blind supplementation for 6 weeks with soy protein (50 g/day) with high levels of isoflavones (165 mg/day), compared with placebo (casein 50 g/day) in type 2 DM demonstrated significantly lower mean values for LDL-C and TG but no significant change in HDL-C (Hermansen et al., 2001). The meta-analyses in 23 RCT revealed that intake of soy protein containing isoflavones significant decreased TC (3.8%), LDL-C (5.3%), TG (7.3%) and increased HDL-C (3.0%). The lowering effects of soy protein containing isoflavones on TC, LDL-C and TG occurred within short initial period of intervention (6-12 weeks), whereas improvements in HDL-C were only observed in studies of longer than 12 weeks duration.

Jayagopal et al. (2002a) reported that soy protein with phytoestrogen supplementation (soy protein 30 g/day, isoflavones 132 mg/day) in postmenopausal women with type 2 diabetes for 12 weeks significantly lower mean values for TC, LDL-C and TC/HDL-C ratio when compared with placebo (cellulose 30 g/day). In type 2 diabetic patients with nephropathy, consumption of soy protein for 7 weeks, compared with animal protein, showed significant reductions in TC, TG, LDL-C,

urinary urea nitrogen and proteinuria. Therefore, soy in the diet can modify the risk factors of heart disease and improve kidney function in these patients (Azadbakht et al., 2003). A recent RCT of study that explore the effects of soy proteins (30 g/d) with isoflavones (60 mg/day) versus purified soy isoflavones on lipid profiles for 3 months in postmenopausal women demonstrated a highly significant improvement in serum lipid profiles (TC, TG, LDL-C, Apolipoprotein B and HDL-C) in soy protein with isoflavones group whereas purified soy isoflavones group demonstrated significant improvement in only serum TG (Jassi et al., 2010)

Potential mechanisms by which isoflavones might prevent atherosclerosis include a beneficial effects on thrombus formation, antioxidant effects, anti-proliferative effects on smooth muscle cells, anti-inflammatory effects and maintenance of normal vascular reactivity (Anthony, Clackson, and Williams, 1998). However, tablets containing only soy isoflavones extracted did not have a significant effect on TC reduction (Zhan and Ho, 2005). Jassi et al. (2010) observed that soy protein as a whole appears to be required for the hypocholesterolemic effect as compared to its isoflavone alone.

2.7.6 Effects of Soy on Inflammatory Biomarker (hs-CRP)

Soy-based diets could block the inflammatory processes associated with atherogenesis thereby reducing risk of CVD (Nagarajan, 2010). Isoflavones supplementation (80 mg/day) in patients with clinically manifest atherosclerosis and impaired endothelial function for 12 weeks reduced serum hs-CRP and improved brachial flow mediated dilatation (FMD). These findings suggested that isoflavones alleviated vascular inflammation which was important implication for the use of isoflavones as secondary prevention in patients with CVD (Chan et al., 2008). Two

short-term trials studied on the effect of soy bean and soy protein diet in the period for 8 weeks in postmenopausal women. The results demonstrated that both soy bean and soy protein diet significantly reduced hs-CRP concentrations (Azadbakht, et al., 2007; Nasca, Zhou, and Welty, 2008). Azadbakht et al. (2008) found that soy protein consumption (35% soy protein, 35% animal protein, 30% vegetable protein) for 4 years significantly decreased hs-CRP compared with the control group (70% animal protein, 30% vegetable protein).

The levels of hs-CRP was not changed after isoflavones supplementation at 114 mg/day for 3 months in postmenopausal women (Nikander et al., 2003). Supplementation of soy foods regimen (soy protein 50 mg/day with sioflavones 73 mg/day) for 1-month in hyperlipidemic subjects caused no changes in the levels of hs-CRP (Jenkins et al., 2002a). Fanti et al. (2006) studied in end-stage renal disease patients who received isoflavone-containing soy-based nutritional supplements (soy group) compared with isoflavones free milk-based supplements (control group) for 8 weeks. The levels of hs-CRP were not statistically different after intervention although a trend towards lower levels was noted in the soy group and blood isoflavones concentration correlated with hs-CRP. Supplementation of soy food containing 50 mg isoflavones in premenopausal women for 2 years, did not modify serum level of hs-CRP (Maskarinec et al., 2009). D'Anna et al (2005) suggested that isoflavones neutral effect on hs-CRP. This finding agrees with several studies that there were no effects of soy foods or soy isoflavones on hs-CRP, but a trend toward reduced hs-CRP concentrations was seen.

2.7.7 Effects of Soy on Plasma tHcy, Folate and Vitamin B_{12}

Plasma tHcy act as a risk factor for atherosclerosis, whereas folate and vitamin B_{12} are essential co-factor in tHcy metabolism. Higher tHcy levels were correlated with lower plasma folate and vitamin B_{12} levels (Antoniades et al., 2009). Numerous studies have shown the effects of soy isoflavones on plasma tHcy levels. Studies in postmenopausal women indicated that genisteine aglycone (54 mg/day) supplementation for 3 years significantly decreased plasma tHcy compared to placebo group (Marini et al., 2010). Turhan et al. (2009) showed that postmenopausal women received isoflavones 80 mg/day for 6 months resulted in a significant decrease in plasma tHcy and increase in serum nitrite/nitrates. In contrast, Reimann et al. (2006) found that consumption of fruit cereal bars containing isoflavones (50 mg/day) in postmenopausal women for 8 weeks did not affect plasma tHcy. D'Anna et al. (2005) evaluated the effect of purified genisteine 54 mg/day for 6 months in postmenopausal women and found no significant difference in plasma tHcy compared with placebo.

Jenkins et al. (2002b) reported no significant differences in plasma tHcy between high- (50 g soy protein and 73 mg isoflavones daily) and low- (52 g soy protein and 10 mg isoflavones daily) isoflavone soy diets for 1 month in hyperlipidemic subjects. Both high and low isoflavones soy diets resulted in significantly lower plasma tHcy from baseline, however, no significant differences in plasma tHcy was seen between groups. SPI with low isoflvones (soy protein 40 g/day, 1.2 mg isoflavones and phytate 0.64 mg) supplement for 6 weeks significantly reduced plasma tHcy in postmenopausal women (Hanson et al., 2006). Two intervention studies examined the effects of soy protein on plasma tHcy in diabetic subjects (Hermansen et al., 2001) and hypercholesterolemic subjects (Tonstad et al., 2002). Both studies reported that plasma tHcy concentrations were decreased with SPI

compared with casein, suggesting a novel, possibly anti-atherosclerotic effect of dietary soy. These findings may be ascribed to the higher methionine content in casein compared with SPI. Soy product intake (soy protein and/or soy isoflavoes) inversely correlated with plasma tHcy (r=-0.15, P=0.04) after controlling for covariates and significantly positively with folate (r=-0.15, p=0.04). Moreover, plasma tHcy concentration was significantly inversely correlated with serum folate (r=-0.46, p=0.0001) and marginal significance with serum vitamin B_{12} (r=-0.14, p=0.07). The strong correlation between plasma tHcy and serum folate levels indicates that serum folate is an important determinant of plasma tHcy. Although the extent to which each component of soy, such as protein, B-vitamins and isoflavones in association with the lowering plasma tHcy concentrations is unclear, this biochemical complex appears to have a favorable effect on tHcy metabolism (Nagata et al., 2003).

CHAPTER III

MATERIALS AND METHODS

This study was an extension of a previous study (Daoroong Komwong, 2009) of the effects of SPI supplementation on insulin resistance, fasting blood glucose and lipid profile in type 2 diabetic patients. In the present study, the biomarkers of CVD were investigated. The experimental design and procedures were described again in the following sections.

3.1 Subjects

The subjects in this study were selected from type 2 diabetic patients at Public Health Center 66, Bangkok Metropolitan Administration. Males or females aged 35 years old and over who had fasting blood sugar (FBS) between 5.56-13.89 mmol/l (100-250 mg/dl) and used only sulfonylureas and/or biguanide as oral hypoglycemic agent were recruited to the study. All subjects had BP less than 160/100 mmHg and had body mass index (BMI) between 18.5-29.9 kg/m^2. Their total cholesterol (TC) and triglyceride (TG) levels were less than 6.21 mmol/l (240 mg/dl) and 2.26 mmol/l (200 mg/dl), respectively. They were not taking antibiotics or did not stop using antibiotics less than 7 days before participating in the study. In addition they were not currently on medicines and any nutrition supplements or herbal products, which affected the immune system or inflammation. The subjects were not vegetarians, and they did not eat soy or soy products regularly. They were free from chronic diseases or conditions including liver disease, renal disease, cancer, immunodeficiency, infection, history of soy or soy products allergy, malnutrition, and surgery within 1 month before and during the study. None of them smoked and drank

alcohol regularly. Every subject could read and write Thai.

The experimental protocol was approved by the Ethics Committee for Researches Involving Human Subjects, Faculty of Pharmaceutical Sciences Chulalongkorn University (Appendix A). The investigator fully explained the purpose and the protocol of the study to the subjects. The written informed consent was obtained from each subject prior to entry into the study.

3.2 Experimental Design

The subjects participated in a 10-week study starting with a 4-week pre-experimental period (phase I), followed by a 6-week of experimental period (phase II). In phase II, subjects were randomly assigned into 2 groups: a SPI group (supplemented with 30 g/day of SPI containing 32 mg of isoflavones for 6 weeks) and a control group (no supplementation). For both groups, they were advised to consume diets that were suitable for diabetic patients and informed about diabetic self-care. They were also informed to maintain their diets and level of physical activity throughout the study. The biomarkers of cardiovascular disease (CVD) were measured at the beginning and the end of experimental period.

3.3 Experimental Protocol

3.3.1 Phase I: Pre-Experimental Period (4 weeks)

In phase I, the subjects received nutrition counseling to control blood glucose levels and choose a diet suitable for diabetes management. Dietary advice involved a consideration of energy intake and the proportion of protein, fat, carbohydrate and other nutrients. At the first time, all subjects were interviewed about personal information, health history on diabetes, dietary and physical activity

behaviors and nutritional status (Appendix B). Each subject was asked to maintain amount of energy intake, level of physical activity, avoid taking vitamins, soybean, soy products and legumes and take their regularly medications throughout the study.

3.3.2 Phase II: Experimental Period (6 weeks)

At the beginning of phase II, 8-hour fasting venous blood was obtained from each subject for determining baseline levels of biomarkers of CVD including glycemic control, lipid profile, hs-CRP, tHcy, folate and vitamin B_{12}. Blood pressure, body weight and height measurements were also taken. A 3-day food record was done by the subjects prior to and during phase II of the study.

The subjects were randomly assigned into a SPI group and a control group. Each subject in SPI group received 42 sachet of 30-g SPI/sachet (containing 32 mg isoflavones) for 6 week supplementation. They were advised to take 1 sachet daily by mixing with water, milk, fruit juice, soup and continued consuming the SPI until the end of week 6. At the end of the study, venous blood was obtained from each subject for determining biomarkers of CVD. Blood pressure was also taken. Habitual diets, medication and level of physical activity were maintained constantly throughout the period of study. Each subject received a fixed number of SPI sachet (42 sachets) and instructions to return all the sachet of SPI at the end of the study for ascertainment of compliance. The eaten sachets of SPI were counted and calculated for % of compliance. In addition, any adverse effects that occurred during SPI supplementation were also asked over the telephone. The study protocol is presented in Figure 5.

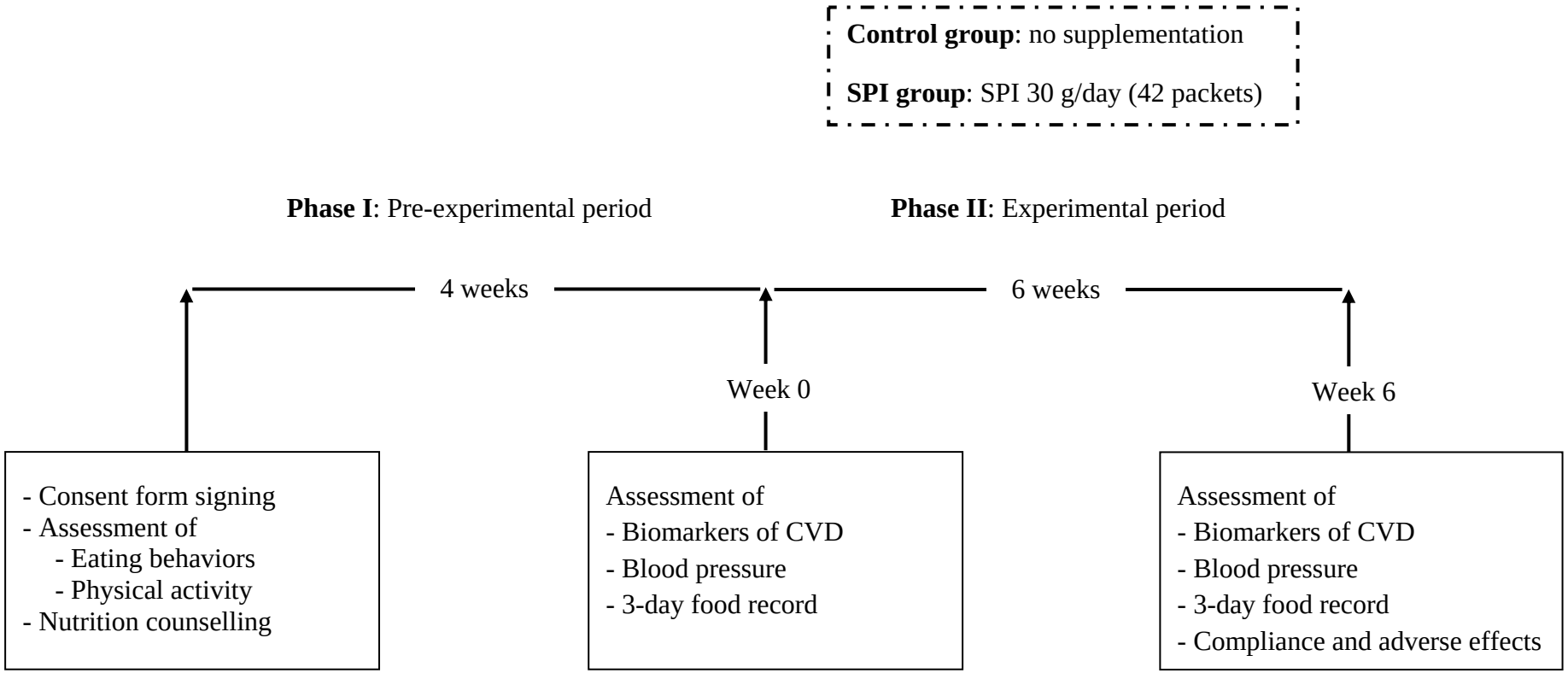


FIGURE 5 Study protocol

3.4 Research Instruments

3.4.1 Soy Protein Isolate Supplement

Soy protein isolate (SPI) (ISOPRO 612B; Shandong Sinoglory Soya Protein, Qingdao, China) containing 90.4% protein in powder form was packed in sealed aluminum foil packaging to protect from moisture and light (30 g/sachet). One sachet of the SPI contained 27.12 g of protein and 32 mg of total isoflavones (30.51% genistein, 27.14% daidzein and 2.07% glycitein) (Appendix C).

3.4.2 3-Day Food Record

During the phase I of the study, all subjects were instructed how to record their food intake. The record was used for assessment of food consumption and nutrient intake. They were asked to perform a 3-day food record (2 weekdays and 1 weekend day) before starting the phase II and again at the end of the phase II. Subjects were provided with dietary record forms (Appendix D). All items and portions of food consumed including name and method of preparation and cooking were recorded. The subjects estimated food portion size using standard household measuring cups and spoons then investigator converted portion size into gram of foods. Individual's total energy, protein, carbohydrate, fat and cholesterol were calculated using software THAI NUTRISURVEY version 2 (2008) software developed for Thai food by Department of Health, Ministry of Public Health and Faculty of Tropical Medicine, Mahidol University, Thailand.

3.4.3 Physical Activity Assessment

The physical activity was assessed from physical activity, which was modified from National Institute for Health and Clinical Excellence (NICE) guideline (NICE, 2006). The data were then classified into four levels of physical activity (inactive, moderately inactive, moderately active and active).

3.5 Blood Pressure and Body Mass Index Measurements

Blood pressure was measured in upper arm while the subject was seated comfortably using sphygmomanometer (Hico Medical Co.Ltd., Tokyo, Japan) and stethoscope (Spirit Medical, UK). The body weight and height were measured at baseline and week 6 of the phase II by weight with height meter (402 KL Health meter® Professional, USA). The body weight was measured as the subjects were wearing light clothing without shoes. The height of the subjects was measured when they were not wearing shoes and stood with their heels against the wall by height meter. Body mass index (BMI) was calculated from body weight in kilogram divided by the square of height in meters (kg/m^2). Then BMI was interpreted and categorized using standard categories of BMI for Asian adults (Table 11).

3.6 Blood Sample Collection

At the beginning (baseline) and the end of the phase II, 15 ml of venous blood was obtained from each subject after 8-hour fast. All blood samples were appropriately prepared for determination of CVD biomarkers. Blood in the amount of 8 ml was kept in clotted blood tube to determine fasting plasma glucose (FPG), TC, LDL-C, HDL-C, TG, blood urea nitrogen (BUN), serum creatinine (Scr), uric acid and hs-CRP. Additionally, 5 ml of blood was kept in EDTA containing tube for HbA1c determination then centrifuged at 5,000xg for 5 minutes by using centrifugal machine (S-8 Boeco, Germany). Plasma was collected in microtubes and kept at -80 ^{o}C until plasma tHcy, folate and vitamin B_{12} were assayed.

TABLE 11 Standard categories of Body Mass Index for Asian adults

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal weight	18.5 - 22.9
Overweight	23.0 - 24.9
Obesity	
Obese I	25.0 -29.9
Obese II	≥ 30.0

BMI= body mass index; kg/m^2= kilogram/square meter

Source: Inoue and Zimmet, 2000.

3.7 Biomarkers of Cardiovascular Disease Determination

The levels of FPG, HbA1c, TG, total-C, LDL-C, HDL-C, albumin, BUN, SCr and uric acid were performed by colorimetry and turbidimetry using clinical chemistry analyzer (ABX-Pentra 400, Horiba, France) at laboratory unit, Public Health Laboratory Division, Bangkok Metropolitan Administration. The hs-CRP was determined by immunoturbidimetry method (Cobas Intrigra 400 plus, Roche diagnostics, Canada) at Bangkok Pathology-Laboratory Co. Ltd., Bangkok. Plasma tHcy was carried out by a fully automated immunoassay using the IMx analyzer (Abbott Diagnostics, Chicago, U.S.A.) at Faculty of Medicine, Chulalongkorn University. Folate analysis in plasma and SPI were determined by microbiological assay using *Lactobacillus casei* as the test organism at Faculty of Tropical Medicine, Mahidol University (Appendix E). Vitamin B_{12} was determined by electro-chemiluminescence immunoassay (ECLIA) technique (Elecsys immunoassay analyzers, Roch Diagnostics, Switzerland) at Faculty of Tropical Medicine, Mahidol University.

3.8 Statistical Analysis

Categorical data were expressed as number and percentage of subjects. Continuous data were expressed as mean ± standard deviation (SD). For comparison of the proportion of demographic data, Chi-square was used to test for significant difference between groups. Normal distribution of the data was checked by Shapiro-Wilk's statistics, Skewness, and Kurtosis (Appendix F). When the distribution of variables was normal, paired *t*-test and independent *t*-test were used to compare within group and between groups differences, respectively. The associations between continuous variables were assessed by Pearson's correlations. A p value of < 0.05 was accepted as significant.

CHAPTER IV

RESULTS

4.1 Demographic Data of Subjects

Fifty-eight type 2 diabetic patients enrolled in this study, but only 41 subjects (15 males and 26 females) met the inclusion criteria. The subjects were randomly divided into: control and SPI groups. The control group comprised of 19 patients and the SPI group comprised of 22 patients. Five subjects dropped out from the study: 1 subject in the control group (loss follow-up) and 4 subjects in the SPI group (1-loss follow up, 3- had low compliance as they consumed SPI less than 80%). All of dropped out patients were female. Finally, 36 subjects (14 males and 22 females) completed the study and were included in final analysis.

The characteristics data of the subjects are presented in Table 13. The control group included 18 subjects (8 males and 10 females), and the SPI group included 18 subjects (6 males and 12 females). Most of the participants in both groups were in the age group of $\geq$ 65 years old. Obesity (BMI: 25.0-29.9 kg/m^2) and overweight (BMI: 23.0-24.9 kg/m^2) in the control group were equally found (38.9%) , and 22.2% of the subjects were normal weight (BMI: 18.5-22.9 kg/m^2). In the SPI group, 66.7% of the subjects were obese, 22.2% were overweight, and 11.1% were normal weight. Most of the subjects in both groups were diagnosed of type 2 diabetes mellitus for less than 5 years. The existing disease mostly found in the control group was diabetes with both hypertension and dyslipidemia (33.3%) whereas in the SPI group was diabetes with hypertension (33.3%). Approximately 80% in both groups took sulfonylureas in combination with biguanides. In the current study, all characteristics data of the subjects were not different between groups.

TABLE 12 Characteristics of the subjects

Characteristics	Control (n=18) number (%)	SPI (n=18) number (%)	Total (n=36) number (%)
Sex			
Male	8 (44.4)	6 (33.3)	14 (38.9)
Female	10 (55.6)	12 (66.7)	22 (61.1)
	$\chi 2 = 0.468$	df=1	p=0.494
Age			
35-44 y	0 (0.0)	0 (0.0)	0 (0.0)
45-54 y	3 (16.7)	3 (16.7)	6 (16.7)
55-64 y	5 (27.8)	6 (33.3)	11 (30.6)
≥ 65 y	10(55.6)	9 (50.0)	19 (52.8)
	$\chi^2 = 0.144$	df=2	p=0.931
BMI (kg/m^2)			
18.5-22.9	4 (22.2)	2 (11.1)	6 (16.7)
23.0-24.9	7 (38.9)	4 (22.2)	11 (30.6)
25.0-29.9	7 (38.9)	12 (66.7)	19 (52.8)
	$\chi^2 = 2.801$	df=2	p=0.247
Duration of diabetes			
<5 y	10 (55.6)	10 (55.6)	20 (55.6)
5-10 y	3 (16.7)	5 (27.8)	8 (22.2)
>10 y	5 (27.8)	3 (16.7)	8 (22.2)
	χ^2 =1.000	df=2	p=0.607
Existing disease			
Diabetes only	4 (22.2)	8 (44.4)	12 (33.3)
Diabetes with hypertension	5 (27.8)	6 (33.3)	11 (30.6)
Diabetes with dyslipidemia	3 (16.7)	2 (11.1)	5 (13.9)
Diabetes with hypertension and dyslipidemia	6 (33.3)	2 (11.1)	8 (22.2)
	χ^2 =3.352	df=3	p=0.341
Use of oral antihyperglycemic drugs			
Sulfonylureas	3 (16.7)	2 (11.1)	5 (13.9)
Biguanides	2 (11.1)	2 (11.1)	4 (11.1)
Sulfonylureas + Biguanides	13 (72.2)	14 (77.8)	27 (75.0)
	χ^2 =0.237	df=2	p=0.888

n = number, SPI = soy protein isolate, df = degrees of freedom, χ^2 = compare frequency between groups by Chi-square test, y = year, BMI = body mass index, kg/m^2 = kilogram per metre square

4.1.1 Eating and Physical Activity Behaviors

The eating and physical activity behaviors were no significantly different between groups (Table 13). More than 70% of the subjects in both groups consumed meat and meat product 3-4 times/wk. The subjects in both groups consumed high saturated fatty food (77.8% in control group and 72.2% in SPI group). Most of the subjects in both groups did not consume fast food, while the consumption of fresh fruit and vegetable were more than 3-4 times/week in both groups (100% and 94.5% in SPI and control group, respectively).

The present study showed that 66.7% of the subjects in control group and 88.9% of the subjects in SPI group had physical activity including cycling, shopping, walking to work, housework and childcare more than 3 hr/wk. Physical activity index (PAI) was classified by type of present work and level of physical activity. It was found that most of the subjects in both groups (66.7% in control group and 77.8% in SPI group) were active.

4.1.2 Baseline Clinical Characteristics of Subjects

The baseline clinical characteristics of the subjects in both groups are summarized in Table 14. At baseline, none of the measured variables differed between groups ($p > 0.05$). Plasma folate and vitamin B_{12} levels were within normal range (3 - 7 ng/ml, 220 – 960 pg/ml, respectively) in all subjects both SPI and control groups (Kurowska et al., 2008).

TABLE 13 Eating and physical activity behaviors of subjects

Parameters	Control (n=18) number (%)	SPI (n=18) number (%)
Type of food consumption		
Meat and meat product		
none	1 (5.6)	1 (5.6)
1-2 day/week	4 (22.2)	3 (16.7)
3-4 day/week	4 (22.2)	6 (33.3)
5-6 day/week	5 (27.8)	2 (11.1)
every day	4 (22.2)	6 (31.3)
χ^2 =2.229	df=4	*p*=0.694
High saturated fatty acid food		
none	7 (38.9)	6 (33.3)
1-2 day/week	7 (38.9)	7 (38.9)
3-4 day/week	2 (11.1)	2 (11.1)
5-6 day/week	0 (0.0)	1 (5.6)
every day	2 (11.1)	2 (11.1)
χ^2 =1.077	df=4	*p*=0.898
Fast food		
none	16 (88.9)	17 (94.4)
1-2 day/week	2 (11.1)	0 (0.0)
3-4 day/week	0 (0.0)	1 (5.6)
5-6 day/week	0 (0.0)	0 (0.0)
every day	0 (0.0)	0 (0.0)
χ^2 =3.030	df=2	*p*=0.220
Fresh fruit and vegetable		
none	0 (0.0)	0 (0.0)
1-2 day/week	1 (5.5)	0 (0.0)
3-4 day/week	4 (22.2)	1 (5.6)
5-6 day/week	3 (16.7)	6 (33.3)
every day	10 (55.6)	11 (61.1)
χ^2 =3.848	df=3	*p*=0.278
Physical activity behaviors		
Physical activity		
< 1 hr/wk	3 (16.7)	0 (0.0)
1-3 hr/wk	3 (16.7)	2 (11.1)
> 3 hr/wk	12 (66.7)	16 (88.9)
χ^2 =3.771	df=2	*p*=0.152
PAI		
Inactive	0 (0.0)	0 (0.0)
Moderately inactive	3 (16.7)	0 (0.0)
Moderately active	3 (16.7)	4 (22.2)
Active	12 (66.7)	14 (77.8)
χ^2 =3.297	df=2	*p*=0.192

n = number, SPI = soy protein isolate, df = degrees of freedom, χ^2 = compare frequency between groups by Chi-square test, PAI = physical activity index

TABLE 14 Baseline clinical characteristics of subjects [1]

Characteristics	**Control** (n=18)	**SPI** (n=18)	**Difference between groups** (*p*-value) [2]
Age (year)	62.11 ± 10.07	62.00 ± 7.87	0.971
BMI (kg/m^2)	24.80 ± 2.50	25.55 ± 1.58	0.350
Glycemic control			
Fasting plasma glucose (mmol/l)	6.39 ± 1.29	6.63 ± 0.98	0.527
HbA1c (mg%)	7.07 ± 0.90	6.99 ± 1.12	0.820
Lipid profiles **(mmol/l)**			
Total cholesterol	5.16 ± 0.68	5.13 ± 0.76	0.887
LDL cholesterol	3.06 ± 0.80	3.12 ± 0.98	0.836
HDL cholesterol	1.37 ± 0.27	1.43 ± 0.38	0.558
Triglyceride	1.56 ± 0.54	1.53 ± 0.46	0.833
Blood pressure **(mmHg)**			
Systolic blood pressure	122.56 ± 15.64	121.83 ± 13.73	0.973
Diastolic blood pressure	75.00 ± 10.43	75.11 ± 7.36	0.971
Biomarkers of CVD			
hs-CRP (mg/l)	1.44 ± 0.65	1.59 ± 1.11	0.637
tHcy (µmol/l)	12.72 ± 3.50	12.92 ± 4.06	0.200
Folate (ng/ml)	7.53 ± 4.10	6.91 ± 3.99	0.647
Vitamin B_{12} (pg/ml)	433.78 ± 104.19	445.82 ± 112.55	0.741
Renal function			
GFR (ml/min)	72.16 ± 16.35	71.74 ± 19.74	0.953

[1] Data were expressed as mean ± SD

[2] statistic analysis by Independent *t*-test

n = number, SPI = soy protein isolate, BMI = body mass index, kg/m^2 = kilogram per metre square, mmol/l = millimole per litre, mg% = milligram percentage, LDL = low density lipoprotein, HDL = high density lipoprotein

4.2 Dietary Intake Assessment

Dietary intakes of the subjects were assessed by 3-day food records. The average dietary intakes of the subjects at week 0 and week 6 of the study are presented in Table 15. The result demonstrated that total energy, carbohydrate, fat, cholesterol, sugar and dietary fiber were not significantly different between groups at baseline and at the end of the study. However, after 6-week SPI supplementation, the amount of total protein and vegetable protein were significantly increased in SPI group compared with baseline and the control group ($p < 0.05$). Folate content in SPI was determined by microbiological assay using *Lactobacillus casei*. The result showed that 100 g of SPI provided 210.7 µg of folate**.**

4.3 Effects of SPI Supplementation on Glycemic Control, Blood Pressure and Lipid Profile

Significant differences between groups were not found for fasting plasma glucose and HbA1c after SPI supplementation (Table 16). HbA1c of the SPI group was significantly decreased from baseline ($p = 0.003$). In SPI group, fasting plasma glucose was decreased from baseline, but no statistical significance was found.

Serum lipid profiles and blood pressure were not significantly different between groups (Table 16). However, the decrease in TC, LDL-C and HDL-C were greater in the SPI group than those in the control group. The concentrations of TG tended to increase in the control group but decrease in the SPI group.

TABLE 15 Energy and nutrient intakes of subjects at baseline and at week 6 after soy protein isolate supplementation [1]

Variables	Control (n=18)		SPI (n=18)	
	Week 0	**Week 6**	**Week 0**	**Week 6**
Total energy (kcal/day)	1382.58 ± 233.93	1391.03 ± 222.09	1321.32 ± 219.25	1351.14 ± 213.09
Macronutrient				
Protein (g/d)	61.44 ± 13.58	59.15 ± 9.79†	56.32 ± 13.66	79.62 ± 15.88†*
Animal protein	36.64 ± 5.63	32.68 ± 8.27	32.99 ± 13.23	28.83 ± 10.44
Vegetable protein	16.10 ± 3.17	16.50 ± 6.27†	18.28 ± 6.10	43.52 ± 5.29†*
Carbohydrate (g/d)	185.70 ± 38.05	200.21 ± 36.62	196.95 ± 34.64	184.61 ± 30.75
Fat (g/d)	44.42 ± 11.24	37.91 ± 12.10	34.19 ± 11.62	35.03 ± 11.66
Cholesterol (mg/d)	192.40 ± 75.13	175.82 ± 77.40	162.59 ± 59.84	166.78 ± 69.14
Sugar (g/d)	21.35 ± 8.14	25.43 ± 13.29	18.78 ± 9.56	20.09 ± 9.56
Dietary fiber (g/d)	8.11 ± 1.19	8.64 ± 2.95	7.34 ± 2.56	7.96 ± 3.17

[1] Data were expressed as mean ± SD

†significant difference between groups ($p < 0.05$) * significant difference from baseline ($p < 0.05$)

n = number, SPI = soy protein isolate, kcal/day = kilocalories per day, g/d = gram per day, mg/d = milligram per day

TABLE 16 Effects of soy protein isolate supplementation on glycemic control blood pressure and lipid profiles[1]

Parameters	Control (n=18)			SPI (n=18)			(*p*-value) [2]
	Week 0	Week 6	Mean change	Week 0	Week 6	Mean change	
Fasting plasma glucose (mmol/l)	6.39 ± 0.96	6.99 ± 1.03	0.60 ± 1.89	6.63 ± 0.98	6.49 ± 1.01	-0.14 ± 1.01	0.153
HbA1c (mg%)	7.28 ± 1.57	6.97 ± 1.17	-0.32 ± 0.92	6.99 ± 1.12	6.65 ± 0.93*	-0.34 ± 0.42	0.374
Systolic blood pressure (mmHg)	122.56 ± 15.64	120.67 ± 15.69	-1.89 ± 12.28	122.39 ± 13.42	122.33 ± 8.05	-0.06 ± 11.76	0.693
Diastolic blood pressure (mmHg)	75.00 ± 10.43	73.89 ± 7.78	-1.11 ± 9.00	75.11 ± 7.36	75.28 ± 7.16	0.17 ± 8.96	0.581
Total cholesterol (mmol/l)	5.16 ± 0.68	5.06 ± 1.05	-0.11 ± 1.28	5.13 ± 0.76	4.70 ± 0.88	-0.43 ± 0.97	0.281
LDL cholesterol (mmol/l)	3.06 ± 0.80	2.99 ± 0.90	-0.07 ± 1.25	3.12 ± 0.98	2.79 ± 0.78	-0.33 ± 1.15	0.489
HDL cholesterol (mmol/l)	1.37 ± 0.27	1.27 ± 0.34	-0.09 ± 0.24	1.43 ± 0.38	1.33 ± 0.31	-0.11 ± 0.25	0.637
Triglyceride (mmol/l)	1.56 ± 0.54	1.67 ± 0.53	0.11 ± 0.42	1.53 ± 0.46	1.38 ± 0.52	-0.15 ± 0.52	0.102

[1] Data were expressed as mean ± SD

[2] Statistic analysis by Independent *t*-test at week 6

*significant difference from baseline ($p < 0.01$)

n = number, SPI = soy protein isolate, mmol/l = millimole per liter, mg% = milligram percentage, mmHg = millimeter of mercury, LDL = low density lipoprotein, HDL = high density lipoprotein

4.4 Effects of SPI Supplementation on Other Biomarkers of Cardiovascular Disease

The effects of SPI supplementation on biomarkers of CVD are represented in Table 17. The levels of hs-CRP, tHcy, folate and vitamin B_{12} were not significantly different between groups at the end of study. In the SPI group, plasma tHcy levels significantly decreased from baseline (p = 0.005) while folate levels significantly increased from baseline (p = 0.002) after 6-week of SPI supplementation. The levels of hs-CRP tended to decrease from baseline in the SPI group. At the end of 6-week SPI supplementation, this value of Scr, GFR, BUN, albumin and uric acid were not significantly different from baseline and between groups (Table 18).

4.5 Correlation of Biomarkers of Cardiovascular Disease

The correlation between baseline tHcy and hs-CRP with other biomarkers of CVD are shown in Table 19-20. Circulating tHcy was significantly inversely correlated with folate (r = -0.783, p < 0.01), vitamin B_{12} (r = -0.390, p = 0.019) and GFR (r = -0.753, p < 0.01) but significantly positively correlated with age (r = 0.565, p < 0.01). In addition, hs-CRP was significantly negatively correlated with GFR (r = -0.457, p = 0.005), and positively correlated with age (r = 0.399, p = 0.016) and HbA1c (r = 0.332, p = 0.048).

4.6 Compliance and Adverse Effects

At the end of study, the compliance was 92.6%. Adverse effects during the intervention period were examined. Overall, 4 of 18 subjects (22.2%) experienced mild constipation, and 3 subjects (16.7%) had flatulence. Indigestion, diarrhea, nausea, vomiting and other serious adverse effects from SPI supplementation such as heartburn, stomach pain were not found.

TABLE 17 Effects of soy protein isolate supplementation on other biomarkers of cardiovascular disease [1]

Parameters	Control (n=18)			SPI (n=18)			(*p*-value) [2]
	Week 0	Week 6	Mean change	Week 0	Week 6	Mean change	
hs-CRP (mg/l)	1.44 ± 0.65	1.42 ± 0.77	-0.02 ± 1.10	1.59 ± 1.11	1.10 ± 0.32	-0.41 ± 1.07	0.118
tHcy (µmol/l)	12.72 ± 3.50	13.60 ± 3.67	0.88 ± 2.07	12.92 ± 4.06	11.63 ± 3.22*	-1.29 ± 1.72	0.090
Folate (ng/ml)	7.53 ± 4.10	8.42 ± 5.33	0.88 ± 7.71	6.91 ± 3.99	11.44 ± 6.47*	4.53 ± 5.18	0.353
Vitamin B_{12} (pg/ml)	433.78 ± 104.19	429.71 ± 112.97	-4.07 ± 95.17	445.82 ± 112.55	444.64 ± 85.11	-1.18 ± 85.10	0.657

[1] Data were expressed as mean ± SD

[2] Statistic analysis by Independent *t*-test at week 6

* significant difference from baseline ($p < 0.01$)

n = number, SPI = soy protein isolate, hs-CRP = high sensitivity C-reactive protein, tHcy = total homocysteine , mg/l = milligram per litre, µmol/l = micromole per litre, ng/ml = nanogram per millilitre, pg/ml = picrogram per millilitre

TABLE 18 Effects of soy protein isolate supplementation on renal function [1]

Parameters	Control (n=18)			SPI (n=18)			(*p*-value) [2]
	Baseline	Week 6	Mean change	Baseline	Week 6	Mean change	
Serum creatinine (mg/dl)	0.88 ± 0.19	0.87 ± 0.25	-0.02 ± 0.15	0.89 ± 0.21	0.87 ± 0.21	-0.02 ± 0.11	0.960
GFR (ml/min)	72.16 ± 16.35	70.89 ± 25.36	-1.27 ± 15.95	71.96 ± 18.97	71.53 ± 18.80	-0.44 ± 5.92	0.975
BUN (mg/dl)	14.83 ± 3.50	14.24 ± 5.42	-0.59 ± 3.55	16.22 ± 3.66	16.24 ± 4.59	0.02 ± 3.72	0.242
Serum albumin (g/dl)	4.59 ± 0.26	4.55 ± 0.29	-0.04 ± 0.24	4.56 ± 0.21	4.46 ± 0.24	-0.11 ± 0.25	0.299
Uric acid (mg/dl)	5.54 ± 1.46	5.63 ± 1.57	0.14 ± 0.96	5.64 ± 1.50	5.48 ± 1.16	-0.16 ± 0.73	0.667

[1] Data were expressed as mean ± SD
[2] Statistic analysis by Independent *t*-test at week 6

n = number, SPI = soy protein isolate, mg/dl = milligram per deciliter, GFR = glomerular filtration rate, ml/min = milliliter per minute, g/dl = gram per deciliter, BUN = blood urea nitrogen

TABLE 19 Correlation between baseline homocysteine and C-reactive protein with other biomarkers of cardiovascular disease[1]

		tHcy	hs-CRP	Folate	Vitamin B_{12}	age	BMI	GFR
tHcy								
	***r* value**	1	0.322	-0.783	-0.390	0.565	-0.198	-0.753
	***P* value**	-	0.055	0.000**	0.019*	0.000**	0.246	0.000**
hs-CRP								
	***r* value**	0.322	1	-0.276	-0.108	0.399	0.036	-0.457
	***P* value**	0.055	-	0.103	0.531	0.016*	0.834	0.005**

[1] Statistic analysis by Pearson correlation coefficients in all subjects (n=36) at baseline

* significant correlation $p < 0.05$, ** significant correlation $p < 0.01$

hs-CRP = high sensitivity C-reactive protein, tHcy = total homocysteine , BMI = body mass index, GFR = glomerular filtration rate

TABLE 20 Correlation between baseline homocysteine and C-reactive protein with other biomarkers of cardiovascular disease (continued)[1]

		Fasting plasma glucose	HbA1c	Systolic blood pressure	Diastolic blood pressure	Total cholesterol	LDL cholesterol	HDL cholesterol	triglyceride
tHcy									
	***r* value**	0.091	-0.135	0.403	0.108	0.156	0.315	0.083	-0.041
	***P* value**	0.598	0.431	0.333	0.532	0.365	0.061	0.629	0.813
hs-CRP									
	***r* value**	0.132	0.332	0.099	0.066	0.042	0.106	0.174	0.086
	***P* value**	0.443	0.048*	0.567	0.703	0.808	0.537	0.310	0.620

[1] Statistic analysis by Pearson correlation coefficients in all subjects (n=36) at baseline

* significant correlation $p < 0.05$

hs-CRP = high sensitivity C-reactive protein, tHcy = total homocysteine , LDL = low density lipoprotein, HDL = high density lipoprotein

CHAPTER V

DISCUSSION

The present study was conducted to investigate the effects of SPI supplementation on biomarkers of cardiovascular disease (CVD) including glycemic control, lipid profile, high sensitivity C-reactive protein (hs-CRP), total homocysteine (tHcy), folate and vitamin B_{12} in type-2 diabetes mellitus.

5.1 Demographics and Subject Characteristics

In the present study, more than 50% of the subjects were female and approximately 80% the subjects were older than 55 years old. This was consistent with the age group of the majority of type 2 diabetic population in Thailand (Aekplakorn et al., 2007). Most of the subjects were overweight or obese, which were also consistent with previous studies in Thai population. The prevalence of obesity (BMI $\geq$ 25 kg /m^2) among those with and without diabetes were 43.4% and 27.5 %, respectively (Aekplakorn et al., 2006). Aekplakorn et al. (2003) reported that the proportion of diabetes was substantially greater for older people than younger people (63% for $\geq$ 55 years vs. 37% for $\leq$ 54 years). In Thai population, the prevalence of diabetes in women was higher than that in men (7.4 vs. 6.0%) (Aekplakorn et al., 2007).

Type 2 diabetic subjects in this study had hypertension (30.6%), dyslipidemia (13.9%) and both (22.2%). Hypertension and dyslipidemia are an extremely common co-morbidity of diabetes (Hinnen et al., 2004). Of those with diabetes, 72.7% had high blood pressure (BP) and 33.0% had high total cholesterol (TC) (Aekplakorn et al., 2007). The patients who have the coexistent diabetes and hypertension had risk of CVD up to 75%, leading to guidance for aggressive

treatment (reducing blood pressure to < 130/85 mmHg) (Sowers, Epstein, and Frohlich, 2001).

5.2 Effects of SPI Supplementation on Glycemic Control

This study showed that SPI supplementation in type 2 diabetic subjects caused favorable effects on glycemic control demonstrated by significant reduction in glycosylate hemoglobin (HbA1c) from baseline. The findings agreed with Li et al. (2005) who demonstrated that replacement animal protein with soy protein for 1 years was significantly decreased HbA1c compared to the control diet. Supplementation with soy protein (30 g/day) with isoflavones (132 mg/day) for 12 weeks significantly reduced mean values of fasting insulin, insulin resistance and HbA1c in type 2 diabetic subjects (Jayagopal et al., 2002a). In contrast, the most recent trial showed that daily supplementation with 132 mg purified isoflavones over 12 weeks had no effect on plasma glucose, HbA1c and insulin resistance (Gonzalez et al., 2007). Isoflavones were found to have a positive effect on glycemic control only when combination with soy proteins (Bartlett and Eperjesi, 2008).

When compared to the control group, this study showed that SPI supplementation gave a small improvement in FPG and HbA1c, but the results were not significant. Hermansen et al. (2001) also found that daily supplementation with isoflavones (165 mg/day) in combination with soy protein (50 g/day) for 6 weeks in type 2 diabetic patients showed no differences in plasma glucose, insulin and HbA1c, compared with placebo. The consumption of SPI (0.5 g/kg BW) compared with casein for 8 weeks in type 2 diabetic patients with nephropathy did not improve glycemic control (Teixeira et al., 2004). However, the results from previous studies (Jayagopal et al., 2002b; Li et al., 2005) showed that effects mediated by the SPI were too modest

to be detected over the 12-week study period. It was possible that no significant differences between groups found in this study may be because the 6-week of SPI supplementation was too short to observe the improvement glycemic control. The exact mechanism of soy proteins and isoflavones on glycemic control is not known. It is possible that hypoglycemic effects of SPI are due to the increased level of serum insulin and the improve of peripheral metabolism of glucose (Skim et al., 1999)

5.3 Effects of SPI Supplementation on Lipid Profile

This study showed that after SPI containing isoflavones supplementation for 6 weeks in type 2 diabetic subjects, there were no significant differences in serum TC, LDL-C, HDL-C and TG concentrations between groups. This may be expected because many studies (Erdman, 2000; Zhan and Ho, 2005) have shown that SPI supplementation significantly reduces blood lipid concentrations only in individuals with initial TC higher than normal concentrations. Subjects with hypercholesterolemia (TC > 6.22 mmol/l) had greater reductions of TC than normal subjects. The reduction of TC in hypercholesterolemia and normal subjects were 0.25 and 0.17 mmol/l, respectively (Zhan and Ho, 2005). Soy protein showed cholesterol-lowering effect only in subjects with moderate to severe hypercholesterolemia who had initial values above 8.66 mmol/l, while the results of subjects with normal to mild hypercholesterolemia (initial TC = 5.2 - 6.6 mmol/l) did not show this effect (Anderson et al, 1995). The current study, showed no significant differences in serum lipids after SPI supplementation because the mean baseline TC concentration of the subjects in this study was almost normal (5.1 ± 0.7 mmol/l [198.8 ± 27.5 mg/dl]).

Hall et al. (2006) reported that consumption of cereal bars containing isoflavones for 8 weeks in postmenopausal women did not have a significant

beneficial effect on blood lipids. Daily supplementation with isoflavones (132 mg/day) over 12 weeks did not show any effect on lipid profiles (Gonzalez, et al., 2007). Gardner et al. (2001) showed that isoflavone-rich soy protein reduced TC to a greater extent than did isoflavones-depleted soy protein. The plasma lipid level was not decreased when the isoflavones were removed from soy protein (Greaves et al., 1999). The intake of soy protein with isoflavones in hypercholesterolemia subjects for 4–12 weeks showed significantly decrease in serum LDL-C concentrations more than soy protein without isoflavones supplement (Taku et a., 2007). It is possible that isoflavones may provide cardioprotective benefits when contained in soy protein only.

Daily supplementation of soy protein with isoflavones ≥ 80 mg had greater lipid lowering effects than that with isoflavones 40 - 80 mg (Zhan and Ho, 2005). The result implied that the hypocholesterolemic effect of soy protein was likely dependent on the presence of the isoflavones contained in soy protein. The strongest lowering effects of soy protein containing isoflavones on TC, LDL-C and TG occurred within 4–12 weeks of SPI supplementation, whereas longer interventions (> 12 weeks) were associated with a greater improvement in HDL-C (Taku et al., 2007; Zhan and Ho, 2005). The results from previous studies have noted that variability in plasma cholesterol response might result from variable isoflavones content of soy preparations and duration of SPI supplementation (Anderson et al., 1995). Therefore, factors that may have contributed to the lack of significant improve lipid profile effects in current study may be the insufficient of isoflavones (32 mg isoflavones) in SPI and the relatively short exposure time (only 6-week of SPI supplementation).

5.4 Effects of SPI Supplementation on hs-CRP

The present study found no effect of isoflavones-containing SPI supplementation on hs-CRP levels in type 2 diabetic subjects. Previous studies also found no effect of soy protein or isoflavones on hs-CRP levels (Jenkins et al., 2002a; Maskarinec et al., 2009; Nikander et al., 2003). In contrast, some reports described lower hs-CRP levels with soy protein or isoflavone supplement, when mean baseline hs-CRP level was higher than 3 mg/l (Azadbakht et al., 2007; Chan et al., 2008). Mean baseline hs-CRP level in this study was lower than 1.6 mg/l. It was possible that soy protein or isoflavones may have hs-CRP lowering effect only in subjects with elevated hs-CRP (hs-CRP > 3 mg/l). Therefore, the effect of SPI on hs-CRP levels should be further investigated among subjects with elevated inflammation (arthritis, inflammatory bowel disease, end-stage renal disease etc.).

5.5 Effects of SPI Supplementation on tHcy, Folate and Vitamin B_{12}

The SPI group in current study showed significant decrease in tHcy and increase in folate concentrations from baseline at the end of supplementation period. These findings agreed with previous studies that ingestion soy protein or isoflavones significantly reduced plasma tHcy levels (Marini et al., 2010; Nagata et al., 2003). Furthermore, this study demonstrated that plasma tHcy levels were significantly inversely correlated with serum folate. Jacques et al. (2001) suggested that plasma folate was an important determination of plasma tHcy. As soybean is an excellent source of folate, it is possible that tHcy lowering effect of soy and soy products may be the result of high folate content (Nagata et al., 2003). Han et al. (2009) reported that, the soy-formula-fed infants had the highest serum folate compared with human milk and casein-formula fed infants. The results showed that infants fed with soy

formula had significantly higher plasma folate and lower tHcy concentrations than infants fed with human milk.

The current study found that 100 g of SPI contained 210.7 µg of folate. Soybean is an important source of folate, when compared with other beans. Folate content in black beans, kidney beans and green beans were 149, 144 and 37 µg per 100 g, respectively (Bailey, 1995). In addition to folate content, other nutrient components in SPI such as vitamin B_6 also play roles in decreasing plasma tHcy. Many studies investigated the effect of SPI containing soy protein and isoflavones, rather than isolated isoflavones on plasma tHcy (Hermansen et al., 2001; Jenkins et al., 2002b; Tonstand et al., 2002). The levels of plasma tHcy in hyperlipidemic subjects who received SPI containing either 10 or 73 mg isoflavones/day for 1 month reduced when compared with those in hyperlipidemic subjects who consumed dairy food control diet (Jenkins et al., 2002b). In patients with type 2 diabetes, daily intake of 50 g SPI with high levels of isoflavones (165 mg/day) reduced plasma tHcy concentrations compared with casein (Hermansen et al., 2001). Tonstand, et al. (2002) studied in hyperlipidemic subjects and found that plasma tHcy concentrations significantly decreased in groups receiving 30 and 50 g SPI containing 111 and 185 mg isoflavones respectively, compared with the casein. Casein is an animal protein, containing high methionine while methionine is the limiting amino acid in soy protein (Friedman and Brandon, 2001). Plasma tHcy could to increased with dietary methionine (Shoveller et al., 2004). Therefore, increasing methionine intake increased plasma tHcy concentrations.

This study found that vegetable protein intake in the SPI group was significantly increased, compared with baseline and the control group. This result agreed with the previous study that vegetable protein intake during SPI consumption

was higher than that in washout period (Teixeira et al., 2004). Animal protein intake in the SPI group was found to decrease from baseline greater than in control group. The SPI supplementation may increase vegetable protein and decrease animal protein intake (Friedman and Brandon, 2001). When dietary methionine intake is restricted, plasma tHcy concentrations decreased. Thus, the levels of plasma tHcy were significantly decreased from baseline after SPI supplementation.

The effect of SPI on plasma vitamin B_{12} was not examined in this study because vitamin B_{12} could not be found in SPI powder. It appeared that most of the subjects in both SPI and control groups (more than 80%) took metformin, and vitamin B_{12} levels tended to decrease from baseline in both groups. This agreed with the previous study showing that type 2 diabetic patients on long term (more than 5 years) metformin therapy had vitamin B_{12} deficiency because the absorption of vitamin B_{12} was decreased (Pflipse et al., 2009). In addition, subjects in this study included diabetic subjects who were older than 55 years. It was found that levels of plasma vitamin B_{12} decreased with age. This possibly resulted from dietary deficiency or declining vitamin absorption related to the aging process (Seshadri and Robinson, 1999). Therefore, metformin use and aging may be associated with decreased vitamin B_{12} in type 2 diabetic patients.

5.6 Correlation Between Baseline tHcy, hs-CRP with Other Biomarkers of CVD

Plasma tHcy concentrations in male were significantly higher than in female (Mazza et al., 2004). In normal healthy Thai subjects, the mean plasma tHcy concentrations of males and females were 11.5 and 8.6 μmol/l, respectively (Leowattana et al., 2001). The reason for the differences in plasma tHcy levels between genders is unclear. It could be due to different hormone profile as estrogen

inhibits the progression of atherosclerosis (Wouters et al., 1995). This study examined the relation of tHcy and hs-CRP and other biomarkers of CVD at baseline and found that age was positively correlated with tHcy. The finding confirmed that plasma tHcy levels increased with age. In Danish women, median tHcy levels were 7.6 µmol/l in younger and 9.4 µmol/l in older subjects (Rasmussen et al., 1996). The reasons for the higher tHcy concentrations observed in older age may be due to decreased B-vitamins absorption, depressed renal function, and decreased activity of methionine synthase, methylene tetrahydrofolate reductase and Cystathione beta synthase, which are the enzymes involving in tHcy metabolism.

This study showed that plasma tHcy levels were significantly negatively correlated with plasma folate, vitamin B_{12} and glomerular filtration rate (GFR). These findings agreed with previous study, which showed that low levels of folate and vitamin B_{12} and reduced renal functions (GFR) were responsible for the majority of elevation of tHcy levels (Akalin et al., 2008). Low folate and vitamin B_{12} levels may contribute to CVD by elevating tHcy levels. Therefore, supplementation with these vitamins may be beneficial in reducing CVD. However, B-vitamins supplementation appeared both to decrease (Clarke et al., 2002) and increase (Bonaa et al., 2006) rates for CVD. A meta-analysis study suggested that lowering tHcy level by 25% (~3 µmol/l) was associated with 11% reduction in coronary heart disease (CHD) risk and 9% reduction in stroke risk (Clarke et al., 2002). Norwegian Vitamin Trial (NORVIT) reported a marginally significant 22% increased risk of recurrent myocardial infarction (MI), stroke and sudden death among patients assigned to take the combination of 0.8 mg folic acid, 0.4 mg vitamin B_{12} and 40 mg vitamin B_6 over 40 months (Bonaa et al., 2006). A large clinical trial demonstrated that daily supplementation with 2.5 mg folic acid, 1 mg vitamin B_{12} and 50 mg vitamin B_6 in a

combined pill for 7.3 years could lower plasma tHcy levels by 18.5%, but it did not reduce CVE occurrence and morbidity in patients with vascular disease (Albert et al., 2008). Therefore, further research on CVE-lowering by B-vitamins therapy is needed.

In the present study, plasma tHcy levels were significantly inversely related to blood folate and vitamin B_{12} concentrations; however, vitamin B_6 was not significantly related to basal tHcy. Brattstrom et al (1998) found that 5 mg of folic acid supplementation significantly reduced plasma tHcy in healthy subjects, but not in those supplemented with either 1 mg vitamin B_{12} or 40 mg vitamin B_6. The greater effect of folic acid on plasma tHcy compared to vitamin B_{12} and vitamin B_6 was explained by the role of folic acid as a co-substrate rather than by the vitamin B_{12} and vitamin B_6, which act as enzyme cofactors (Brattstrom et al., 1998). Increasing intake of foods with naturally high folate contents or fortified with folic acid (200-400 µg/day) also raised plasma folate and reduced tHcy levels (Ashfield-Watt et a., 2004). Therefore, high folate intakes seem to be protective for CVD in individuals with elevated plasma tHcy.

This study found that both hs-CRP and tHcy concentrations were affected directly by the renal functions and increased substantially with the decrease in GFR, which is the indicator of renal function. According to Mezzano et al. (2001), both plasma tHcy and inflammation markers (especially hs-CRP) were found to be elevated in chronic renal failure patients. Renal function was strongly correlated with plasma tHcy levels, and the levels of plasma tHcy increased 2-4 fold in chronic renal failure patients (Seshadri and Robinson, 1999). In present study, hs-CRP concentrations were significantly positively correlated with age. A decline in GFR with age might be linked to increase in plasma tHcy and hs-CRP concentrations (Wollesen et al., 1999). The results suggested that nephropathy caused elevated

circulating tHcy and hs-CRP levels because of the reduction in clearance of both CVD biomarkers (Audelin and Genest, 2001; Razeghi et al., 2008). Long-term study indicated that substituting soy protein for animal protein protected against development of kidney disease and was effective in reversing or slowing the progression of established kidney disease in diabetic subjects (Anderson, Smith, and Washnock, 1999)

The serum levels of hs-CRP are a direct participant in the progression of atherosclerosis and important biomarker of CVD. Serum hs-CRP levels were increased in patients with type 2 diabetes higher than that in non-diabetes patients (Akalin et al., 2008). The present study found that hs-CRP was positively correlated with HbA1c values. According to Khan and Qayyum (2009), there was a significant positive correlation between hs-CRP and HbA1c. Furthermore, the HbA1c was significantly greater in the high hs-CRP (3-10 mg/l) group compared to the average (1-3 mg/l) and the low (<1 mg/l) hs-CRP groups. The hs-CRP rose as HbA1c increased in type 2 DM.

Several mechanisms such as nonenzymatic glycosylation of protein and inflammation have been proposed to explain the relation between the chronic hyperglycemia and development of diabetic complications (Aronson, 2008). The HbA1c or protein glycation is formed by nonezymatic glycosylation between glucose and proteins in arterial walls. Glycated proteins are recognized by receptors presented on macrophage and they can cause the atherosclerotic plaques (Robert and David, 2011; Smolin and Grosvenor, 2008). When blood glucose level is high, it can promote inflammation through the induction of cytokine secretion by several cell types, such as monocytes and adipocytes. In monocytes, chronic hyperglycemia causes a dramatic increase in the release of cytokines. Moreover, hyperglycemia leads to the secretion of

acute phase reactants (CRP) from adipocytes by promoting intracellular oxidative stress (Aronson, 2008). Therefore, long term hyperglycemia might promote cardiovascular damage in diabetic patients (Gonzalez-Clemente et al., 2002; Holly, 2007). These results suggested that good glycemic control may be able to reduce the systemic inflammation that contributes to atherosclerosis (Fukuhara et al., 2006). The present study found that hs-CRP levels trended to be lower with no statistical difference, after SPI supplementation. In SPI group, HbA1c values were significantly decreased from baseline.

In this study, significant relationship between hs-CRP and tHcy levels in type 2 diabetic subjects were not found. Akalin et al (2008) found no correlation between plasma tHcy levels and inflammatory biomarkers (hs-CRP, IL-6 and fibrinogen etc.). The result suggested that the connection of atherosclerosis to plasma tHcy may be not through inflammation.

CHAPTER VI

CONCLUSION

The present study investigated the effects of SPI supplementation on biomarkers of CVD, which included blood glucose, HbA1c, lipid profiles, tHcy, folate, vitamin B_{12} and hs-CRP in type 2 diabetic patients. After daily supplementation of 30-g SPI containing 32 mg of isoflavones for 6 weeks, there was no significant difference in biomarkers of CVD between groups. However, HbA1c and tHcy levels were significantly decreased and folate level was significantly increased from baseline. Lipid profiles and hs-CRP levels were not statistically different after intervention although a trend towards lower levels was noted in the SPI supplementation. Moreover, plasma tHcy levels were significantly negatively correlated with plasma folate, vitamin B_{12} and renal function (GFR) and significantly positively correlated with age. It appeared that hs-CRP levels were significantly negatively correlated with GFR and significantly positively correlated with age and HbA1c. Serious adverse effects were not occurred during SPI supplementation.

In conclusion, this study showed that SPI containing isoflavones may have the additional benefits including improving glycemic control (HbA1c reduction), increasing plasma folate and decreasing tHcy concentrations to type 2 diabetic patients. Therefore, it is reasonable to suggesting that SPI supplementation may help to effectively prevent and delay the development of chronic complications especially cardiovascular complications in patients with type 2 diabetes.

Recommendations for further study

1. Further double-blind, randomized, placebo-controlled trial should be conducted to reduce the bias of the study and confirm these findings.
2. The duration of the study may be extended.
3. The effect of amount of soy protein and isoflavones on biomarkers of CVD should be evaluated.
4. The effects of soy protein on biomarkers of CVD must be further investigated, particularly among subjects with severely hypercholesterolemia, hypertension or end-stage renal disease to potentially maximize the effect of soy protein.
5. The role of soy protein supplementation in the prevention of the increased risk of CVE is being actively explored.

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Appendices

Appendix A

Approval of Certificate from Ethics Committee
for Researches Involving Human Subjects,
the Bangkok Metropolitan Administration

Protocol Review No. 10-33-จฬม. 010

Study Protocol Approval

The Ethics Committee of The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand has approved the following study to be carried out according to the protocol dated and/ or amended as follows:

Study Title: Effects of soy protein isolate supplementation on biomarkers of cardiovascular disease in type 2 diabetic patients at Public Health Center 66, Health Department, Bangkok Metropolitan Administration

Study Code: -

Centre: CHULALONGKORN UNIVERSITY

Principal Investigator : Em-on Chaiprateep

Protocol Date : **August 26, 2011**

A list of the Ethics Committee members and positions present at the Ethics Committee meeting on the date of approval of this study has been attached.

This Study Protocol Approval Form will be forwarded to the Principal Investigator.

Chairman of Ethics Committee: Parkpoom Tengamnuay

(Parkpoom Tengamnuay, Ph.D.)

Secretary of Ethics Committee: Suyanee Pongthananikorn

(Suyanee Pongthananikorn, Ph.D.)

Date of Approval: **August 26, 2010**

คำชี้แจง

เอกสารข้อมูลคำอธิบาย/ คำชี้แจงสำหรับอาสาสมัครที่เข้าร่วมการวิจัย

(Patient or Participant Information Sheet)

ชื่อโครงการศึกษาวิจัยเรื่อง (ภาษาไทย) ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร

(ภาษาอังกฤษ) EFFECTS OF SOY PROTEIN ISOLATE SUPPLEMENTATION ON BIOMARKERS OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS AT PUBLIC HEALTH CENTER 66, HEALTH DEPARTMENT, BANGKOK METROPOLITAN ADMINISTRATION

ชื่อผู้วิจัยหลัก (ภาษาไทย) นางสาวเอมอร ชัยประทีป

(ภาษาอังกฤษ) Miss Em-on Chaiprateep

หน่วยงานที่ทำการศึกษาวิจัย ภาควิชาอาหารและเภสัชเคมี คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ (สามารถติดต่อได้ 24 ชั่วโมง) โทรศัพท์เคลื่อนที่ 080-066-6091

โทรศัพท์บ้าน 02-593-1506

ท่านได้รับเชิญให้เข้าร่วมการศึกษาวิจัยเรื่อง "ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร" ก่อนที่ท่านจะตัดสินใจให้ความยินยอมเข้าร่วมการศึกษาวิจัยนี้ ผู้วิจัยใคร่ขอชี้แจงรายละเอียดของโครงการวิจัยให้ท่านทราบ และขอให้ท่านทำความเข้าใจขั้นตอนที่ผู้วิจัยจะขอให้ท่านปฏิบัติ ขั้นตอนนี้เป็น "**กระบวนการให้คำยินยอม**"

กรุณาอ่านข้อมูลต่อไปนี้ด้วยความรอบคอบ และสอบถามถึงข้อสงสัยต่างๆ โดยไม่ลังเล

1. บทนำ

โรคเบาหวานเป็นกลุ่มโรคทางเมแทบอลิซึมที่มีระดับน้ำตาลในเลือดสูง เกิดจากความผิดปกติในการทำงานของตับอ่อน เป็นผลให้ร่างกายเกิดความบกพร่องในการหลั่งอินซูลินหรือการออกฤทธิ์ของอินซูลิน หรือทั้งสองประการ ในผู้ป่วยเบาหวานมีการทำงานของอินซูลินผิดปกติ ส่งผลให้ร่างกายมีระดับน้ำตาลในเลือดสูงเป็นเวลานาน จนทำให้เกิดภาวะแทรกซ้อนเรื้อรังเกิดการทำลายและเสื่อมสมรรถภาพการทำงานของอวัยวะที่เกี่ยวข้อง เช่น ตา ไต หลอดเลือดแดง และหัวใจ เป็นต้น ผู้ป่วยเบาหวานจึงมีความเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือด มากกว่าคนปกติ 2-6 เท่า และร้อยละ 80 ของผู้ป่วยเบาหวานชนิดที่ 2 มักเสียชีวิตจากโรคหัวใจและหลอดเลือด โดยเฉพาะผู้ป่วยเบาหวานที่มีความผิดปกติ เช่น ระดับไขมันในเลือดสูง ความดันโลหิตสูง อ้วนลงพุง ร่วมด้วยจะมีความเสี่ยงมากขึ้นในการเกิดโรคหัวใจและหลอดเลือด ดังนั้น การรักษาโรคเบาหวานจึงมีเป้าหมายสำคัญเพื่อควบคุม น้ำหนักตัว ระดับน้ำตาล ไกลโคซิเลทฮีโมโกลบิน (HbA1c) ไขมัน C-reactive protein (CRP) โฮโมซิสเทอีน (homocysteine: Hcy) วิตามินบี 12 และโฟเลตในเลือด ซึ่งเป็นปัจจัยเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือด ให้อยู่ในระดับที่เหมาะสม ซึ่งต้องอาศัยวิธีการรักษาหลายวิธีร่วมกัน เช่น การใช้ยาลดระดับน้ำตาลในเลือด การรับประทานอาหารและออกกำลังกายอย่างเหมาะสม เป็นต้น

ในปัจจุบันมีการศึกษาวิจัยต่างๆ เพิ่มมากขึ้น เพื่อหาแนวทางในการป้องกัน รักษาโรคเบาหวาน รวมทั้งลดความเสี่ยงในการเกิดโรคหัวใจและหลอดเลือดในผู้ป่วยโรคเบาหวาน จากการศึกษาที่ผ่านมาพบว่าโปรตีนสกัดจากถั่วเหลืองมีฤทธิ์ทางชีวภาพที่อาจมีประโยชน์ต่อสุขภาพ เช่น ลดน้ำหนักตัว ระดับน้ำตาล ไขมัน CRP และ โฮโมซิสเทอีน และเพิ่มระดับโฟเลตในเลือด เป็นต้น ทั้งนี้การศึกษาต่างๆ ในมนุษย์ที่ผ่านมาซึ่งมีการเสริมโปรตีนสกัดจากถั่วเหลืองในขนาด 20–90 กรัมต่อวัน พบว่าไม่มีผลข้างเคียงต่อสุขภาพที่รุนแรง และในปัจจุบันยังไม่มีรายงานถึงผลข้างเคียงที่รุนแรงต่อสุขภาพจากการรับประทานโปรตีนสกัดจากถั่วเหลือง อีกทั้งองค์การอาหารและยาของสหรัฐอเมริกาจัดให้โปรตีนสกัดจากถั่วเหลืองเป็นอาหารที่มีความปลอดภัยในการบริโภค อย่างไรก็ตามยังมีการศึกษาถึงผลของการรับประทานหรือการเสริมโปรตีนสกัดจากถั่วเหลืองในผู้ป่วยโรคเบาหวานค่อนข้างจำกัดโดยเฉพาะในประชากรที่เป็นคนไทย ผู้วิจัยจึงสนใจที่จะศึกษาถึงผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 หากผลการศึกษาพบว่าการเสริมโปรตีนสกัดจากถั่วเหลืองสามารถลดตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือดในผู้ป่วยโรคเบาหวานชนิดที่ 2 ก็อาจเป็นเป็นประโยชน์ในการวางแผนการรักษา เพื่อลดอุบัติการณ์การเกิดภาวะแทรกซ้อน และลดความเสี่ยงในการเกิดโรคหัวใจและหลอดเลือดในผู้ป่วยเบาหวานชนิดที่ 2 ต่อไป

2. วัตถุประสงค์ของการวิจัย

ศึกษาความสัมพันธ์ และผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ซึ่งจะเป็นประโยชน์ในการวางแผนการรักษาเพื่อลดอุบัติการณ์การเกิดภาวะแทรกซ้อน และลดความเสี่ยงในการการวิจัยนี้ผู้เข้าร่วมการวิจัยประกอบด้วย 2 กลุ่ม คือ

กลุ่มควบคุม ไม่ได้รับการเสริมโปรตีนสกัดจากถั่วเหลือง

กลุ่มทดลอง เสริมโปรตีนสกัดจากถั่วเหลืองชนิดผงละลายน้ำ วันละ 30 กรัม รับประทาน ทุกวัน เป็น เวลา 6 สัปดาห์ โดยวิธีการผสมผงโปรตีนสกัดจากถั่วเหลือง เช่น ผสมในน้ำเปล่า นม น้ำผลไม้ เครื่องดื่มต่างๆ ที่ชอบ นำไปปั่นผสมเป็นมิลค์เชค (milk shake) หรือ ผสมในซุปและโจ๊ก เป็นต้น

อย่างน้อยกลุ่มละ 20-40 คน

ตามที่ท่านเคยเข้าร่วมโครงการวิจัยเรื่อง “ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อภาวะดื้ออินซูลิน ระดับน้ำตาลและรูปแบบระดับไขมันในเลือดหลังอดอาหาร ในผู้ป่วยนอกโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร” ของนางสาวดาวรุ่ง คำวงศ์ ในช่วงระหว่างเดือนเมษายนถึงกรกฎาคม พ.ศ. 2552 ในงานวิจัยดังกล่าวได้รับการอนุมัติจากคณะกรรมการพิจารณาและควบคุมการวิจัยในคนของกรุงเทพมหานคร ซึ่งได้มีการเจาะเลือดทั้งหมด 2 ครั้ง ในสัปดาห์ที่ 4 และสัปดาห์ที่ 10 ของการวิจัย แต่ละครั้งห่างกัน 6 สัปดาห์ และได้ทำการวิเคราะห์ระดับน้ำตาล ไกลโคซิเลทฮีโมโกลบิน (HbA1c) ไขมัน โดยผู้วิจัยจะนำตัวอย่างเลือดที่แยกเก็บไว้จากการวิจัยดังกล่าว ซึ่งเก็บภายใต้อุณหภูมิ -80 องศาเซลเซียส ที่คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย นำไปตรวจวิเคราะห์ค่าชีวเคมีในเลือด ดังนี้

- เลือดส่วนที่ 1 ปริมาณ 500 ไมโครลิตร จะถูกนำไปวิเคราะห์พลาสมาโฮโมซิสเทอีน ด้วยวิธี fluorescence polarization immunoassay (FPIA) ที่คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
- เลือดส่วนที่ 2 ปริมาณ 300 ไมโครลิตร จะถูกนำไปวิเคราะห์พลาสมาวิตามินบี 12 ด้วยวิธี electro-chemiluminescence immunoassay(ECLIA) ที่คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล
- เลือดส่วนที่ 3 ปริมาณ 200 ไมโครลิตร จะถูกนำไปวิเคราะห์พลาสมาโฟเลต ด้วยวิธีทางจุลชีววิทยา ที่คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล
- เลือดส่วนที่ 4 ปริมาณ 5 มิลลิลิตร จะถูกนำไปวิเคราะห์ CRP ด้วยวิธี immunoturbid assay ที่บริษัทกรุงเทพ พยาธิ-แลป จำกัด

3. ความเสี่ยง ความไม่สบาย และผลข้างเคียงที่อาจเกิดขึ้น

ไม่มี

4. ผลประโยชน์ที่อาจจะได้รับ

5.1 ผู้เข้าร่วมการวิจัยทุกท่านจะได้รับคำแนะนำโภชนบำบัดและการดูแลโรคเบาหวานด้วยตนเอง ซึ่งอาจช่วยให้ท่านมีความรู้ความเข้าใจเกี่ยวกับโรคเบาหวานมากขึ้น สามารถเลือกรับประทานอาหารได้อย่างเหมาะสม นอกจากนี้ท่านจะได้จะได้รับการตรวจทางห้องปฏิบัติการเพิ่มเติมจากที่มีการตรวจปกติ ได้แก่ ระดับ CRP โฮโมซิสเทอีน วิตามินบี 12 และโฟเลตในเลือด ซึ่งจะทำให้ท่านทราบถึงภาวะโภชนาการ และความเสี่ยงในการเกิดโรคหัวใจและหลอดเลือด

นอกจากนี้ยังเป็นประโยชน์ต่อทีมสหวิชาชีพที่ทำการรักษาท่าน เพื่อกำหนดแนวทางการรักษาและให้โภชนบำบัดที่เหมาะสมแก่ท่านต่อไป

5.2 ผลการศึกษาวิจัยนี้จะได้ข้อมูลพื้นฐานหรือองค์ความรู้ด้านวิชาการถึงความสัมพันธ์ และผลของโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือดในผู้ป่วยโรคเบาหวานชนิดที่ 2 ที่เป็นประชากรคนไทยซึ่งมีอยู่ค่อนข้างน้อย

5. ทางเลือกอื่นในการรักษา

ไม่มี

6. ค่าใช้จ่ายและค่าชดเชย

ไม่มี

7. เงินชดเชยสำหรับการบาดเจ็บหรืออันตรายที่อาจเกิดขึ้น

ไม่มี

8. สิทธิในการถอนตัวออกจากการศึกษาวิจัย

การจะเข้าร่วมโครงการวิจัยนี้หรือไม่ขึ้นอยู่กับการตัดสินใจและความสมัครใจของท่านเอง ถ้าท่านไม่สะดวกที่จะเข้าร่วมโครงการวิจัยนี้หรือแม้ว่าท่านเข้าร่วมโครงการวิจัยนี้แล้ว ท่านมีสิทธิที่จะบอกเลิกการเข้าร่วมโครงการวิจัยเมื่อใดก็ได้ และการบอกเลิกการเข้าร่วมโครงการวิจัยนี้จะไม่มีผลกระทบต่อการรักษาที่ท่านพึงได้รับตามปกติแต่อย่างใด ท่านจะได้รับการรักษาต่อไปตามที่แพทย์และท่านพิจารณาร่วมกันว่ามีความเหมาะสมสูงสุด

9. การรักษาความลับของบันทึกทางการแพทย์ และข้อมูลการศึกษาวิจัย

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับตัวท่านไว้เป็นความลับและเปิดเผยเฉพาะผลสรุปการวิจัยเท่านั้น โดยไม่มีการระบุชื่อหรือข้อมูลส่วนตัวของท่านแต่อย่างใด

10. การเปิดเผยข้อมูลการศึกษาวิจัย (ระบุว่า บุคคลใดบ้างที่มีสิทธิ์เข้าถึงข้อมูลการวิจัย)

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับตัวท่านไว้เป็นความลับและเปิดเผยเฉพาะผลสรุปการวิจัยเท่านั้น โดยไม่มีการระบุชื่อหรือข้อมูลส่วนตัวของท่านแต่อย่างใด

11. การสอบถามข้อสงสัย

ถ้าท่านมีข้อสงสัยสามารถติดต่อผู้วิจัยได้ตลอด 24 ชั่วโมง ติดต่อที่

ชื่อผู้วิจัย นางสาวเอมอร ชัยประทีป

ที่อยู่ 29 หมู่ 1 ต.ท้ายเกาะ อ.สามโคก จ.ปทุมธานี 12160

โทรศัพท์เคลื่อนที่ 080-066-6091

โทรศัพท์บ้าน 02-593-1506

“หากผู้วิจัยไม่ปฏิบัติตามที่ชี้แจงในเอกสารข้อมูลคำอธิบาย ท่านสามารถร้องเรียนมายังคณะกรรมการพิจารณาจริยธรรมการศึกษาวิจัยในมนุษย์ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทร.02-218-8256

หนังสือแสดงความยินยอม
(Consent Form)

การศึกษาวิจัยเรื่อง ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร

EFFECT OF SOY PROTEIN ISOLATE SUPPLEMENTATION ON BIOMARKERS OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS AT PUBLIC HEALTH CENTER 66, HEALTH DEPARTMENT, BANGKOK METROPOLITAN ADMINITRATION

วันให้คำยินยอม

ข้าพเจ้า (นาย/ นาง/ นางสาว) ..... **นามสกุล** ..... **อยู่บ้านเลขที่** ..... **ซอย** ..... **ถนน** ..... **แขวง/ ตำบล** ..... **เขต/ อำเภอ** ..... **จังหวัด** ..... **รหัสไปรษณีย์** .....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ **ข้าพเจ้าได้รับเอกสารข้อมูลคำอธิบาย/ คำชี้แจงสำหรับอาสาสมัครที่เข้าร่วมการวิจัย** จากผู้วิจัยให้ทราบถึงวัตถุประสงค์ของการวิจัย วิธีวิจัย อันตรายหรืออาการข้างเคียงที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่เกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยได้ตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบัง ซ้อนเร้น จนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการนี้โดยความสมัครใจและมีสิทธิที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ โดยการบอกเลิก
จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะได้รับต่อไป

ผู้วิจัยรับรองว่า **"จะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าไว้เป็นความลับ และจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย"** เท่านั้น

ในการวิจัยครั้งนี้ ข้าพเจ้ายอมให้ผู้วิจัยนำตัวอย่างเลือดของข้าพเจ้าที่ได้เจาะไว้เมื่อครั้งที่เข้าร่วมโครงการวิจัยเรื่อง "ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อภาวะดื้ออินซูลิน ระดับน้ำตาลและรูปแบบระดับไขมันในเลือดหลังอดอาหาร ในผู้ป่วยนอกโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร" นำไปวิเคราะห์หาระดับน้ำตาล ไกลโคซิเลทฮีโมโกลบิน (HbA1c) ไขมัน (คอเลสเตอรอล แอลดีแอลคอเลสเตอรอล เอชดีแอลคอเลสเตอรอล และ ไตรกลีเซอไรด์) C-reactive protein (CRP) โฮโมซิสเทอีน วิตามินบี 12 และโฟเลตในเลือดหลังอดอาหาร

ผู้วิจัยรับรองว่า **การวิจัยดังกล่าวไม่มีความเสี่ยง อาการข้างเคียง และอันตรายใดๆ** ที่จะเกิดขึ้นกับผู้เข้าร่วมการวิจัย

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

พร้อมนี้ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมการวิจัยที่ข้าพเจ้าได้ลงนามและวันที่แล้วและเอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมโครงการวิจัยแล้ว 1 ชุด

ลงนาม ..... **ผู้ให้ความยินยอม**
(.....) ชื่อตัวบรรจง

ลงนาม ..... **ผู้รับผิดชอบการวิจัย**
(.....) ชื่อตัวบรรจง

ลงนาม ..... **พยาน**
(.....) ชื่อตัวบรรจง

ลงนาม ..... **พยาน**
(.....) ชื่อตัวบรรจง

หมายเหตุ 1. ในกรณีที่ผู้เข้าร่วมการวิจัยไม่สามารถอ่านออกเขียนได้ จะให้ใช้พิมพ์ลายนิ้วมือ โดยมีพยานลงนาม 2 คน

Appendix B

Questionnaire

แบบบันทึกข้อมูลผู้ป่วยที่เข้าร่วมการวิจัย

งานวิจัยเรื่อง ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพที่เป็นปัจจัยเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยนอกโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร

EFFECTS OF SOY PROTEIN ISOLATE SUPPLEMENTATION ON BIOMARKERS OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS AT PUBLIC HEALTH CENTER 66, HEALTH DEPARTMENT, BANGKOK METROPOLITAN ADMINISTRATION

คำชี้แจง ผู้วิจัยขอความร่วมมือจากท่าน กรุณาตอบแบบสอบถามทุกข้อตามความเป็นจริง โดยทำเครื่องหมาย ✓ ลงใน ☐ และเติมข้อความในช่องว่าง
ซึ่งประกอบด้วย

ส่วนที่ 1 แบบสอบถามข้อมูลทั่วไป
ส่วนที่ 2 แบบบันทึกประวัติผู้ป่วยและผลตรวจทางห้องปฏิบัติการ
ส่วนที่ 3 แบบสอบถามข้อมูลการบริโภคอาหารและการออกกำลังกาย
ส่วนที่ 4 แบบประเมินภาวะโภชนาการ
ส่วนที่ 5 แบบประเมินภาวะโภชนาการโดยการวัดสัดส่วนของร่างกาย

โครงการวิจัยเรื่อง “ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพของการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร”

วันที่ ...../...../..... **รหัส**.....

ชื่อ – สกุล.....

HN.....

อยู่บ้านเลขที่ ..... **หมู่**.....**ซอย** ..... **ถนน**

..... **แขวง/ ตำบล** .....**เขต/ อำเภอ**

..... **จังหวัด** ..... **รหัสไปรษณีย์** .....

เบอร์โทรศัพท์ที่ติดต่อได้.....

ผู้ที่สามารถติดต่อได้เมื่อมีเหตุฉุกเฉิน.....**เกี่ยวข้องเป็น**

.....

เบอร์โทรศัพท์ที่ติดต่ได้

.....

หมายเหตุ แยกเก็บข้อมูลส่วนนี้เป็นความลับ

(ส่วนที่ 1) **รหัส.....**

แบบสอบถามข้อมูลทั่วไป

1. เพศ ☐ 1. ชาย ☐ 2. หญิง
2. อายุ
   ☐ 1. 35-39 ☐ 2. 40-44 ☐ 3. 45-49
   ☐ 4. 50-54 ☐ 5. 55-60 ☐ 6. 60 ปี ขึ้นไป
3. สถานภาพสมรส
   ☐ 1. โสด ☐ 2. คู่ /สมรส
   ☐ 3. หย่าร้าง/ แยกกันอยู่ ☐ 4. หม้าย (คู่สมรสเสียชีวิต)
4. ระดับการศึกษาสูงสุด
   ☐ 1. ไม่ได้เรียนหนังสือ ☐ 2. ประถมศึกษา
   ☐ 3. มัธยมศึกษา ☐ 4. ปวช./ ปวส./ อนุปริญญา
   ☐ 5. ปริญญาตรี/ สูงกว่าปริญญาตรี
5. อาชีพ
   ☐ 1. ไม่ได้ประกอบอาชีพ ☐ 2. รับราชการ
   ☐ 3. พนักงานรัฐวิสาหกิจ ☐ 4. ค้าขาย/ธุรกิจส่วนตัว
   ☐ 5. อื่นๆ โปรดระบุ.....
6. สิทธิการรักษา
   ☐ 1. ชำระเงินเอง ☐ 2. เบิกต้นสังกัด/ โครงการเบิกจ่ายตรง
   ☐ 3. ประกันสังคม ☐ 4. โครงการหลักประกันสุขภาพถ้วนหน้า
   ☐ 5. อื่นๆ โปรดระบุ.....
7. ท่านเคยได้รับความรู้เกี่ยวกับโรคเบาหวานหรือไม่
   ☐ 1. เคย ☐ 2. ไม่เคย

(ส่วนที่ 2) **รหัส.....**

แบบบันทึกประวัติผู้ป่วย

2.1 แบบบันทึกประวัติผู้ป่วย

ผู้ป่วย ได้รับการวินิจฉัยว่าเป็นโรคเบาหวานชนิดที่ 2 ระยะเวลาที่เป็น.....ปี

ประวัติการรักษาโรคเบาหวาน (ย้อนหลัง 3 เดือน)

วัน เดือน ปี ยาและขนาดยาที่ได้รับ

หมายเหตุ

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ประวัติการแพ้ยา ☐ไม่มี ☐มี.....อาการ

.....

ประวัติการแพ้อาหาร ☐ไม่มี ☐มี.....อาการ

.....

ประวัติการเจ็บป่วยหรือการผ่าตัด

.....

โรคประจำตัวอื่นๆ

.....

อาการแทรกซ้อนจากโรคเบาหวานที่เคยเกิดขึ้น

☐ มือสั่น/ใจสั่น ☐ กระหายน้ำบ่อย ☐ ปัสสาวะบ่อย (ตอนกลางคืน)

☐ ตาพร่ามัวเวลาทำงาน ☐ ชาปลายมือ/เท้า ☐ เจ็บแน่นหน้าอกด้านซ้าย

☐ มีแผลที่เท้า ☐ เท้าเย็น/ เป็นตะคริว ☐ ปวดร้อนตามปลายมือ/เท้า

☐ เคยเป็นอัมพาตบ่อย ☐ เป็นฝีพุพองบ่อย ☐ มีผื่นคันตามตัว/มีเชื้อรา

☐ กระเพาะปัสสาวะอักเสบ ☐ ไอเรื้อรัง/ วัณโรค ☐ อื่นๆ .....

อาการเจ็บป่วยที่เกิดขึ้นเป็นประจำ (ไม่ใช่สาเหตุจากโรคเบาหวาน)

.....

.....

ความร่วมมือในการรับประทานยา และโปรตีนสกัดจากถั่วเหลือง

การรับประทานยา	**สัปดาห์ที่ 0** วันที่..../..../....	**สัปดาห์ที่ 4** วันที่..../..../....	**สัปดาห์ที่ 10** วันที่..../..../....
ในช่วง 1 เดือนที่ผ่านมา ลืมรับประทานยากี่ครั้ง (ระบุจำนวนครั้ง)	.....	.....	.....
การรับประทานโปรตีนสกัดจากถั่วเหลือง			
ในช่วง 1 เดือนที่ผ่านมา ลืมรับประทานโปรตีนสกัดจากถั่วเหลืองกี่ครั้ง (ระบุจำนวนครั้ง)	.....		
จำนวนซองโปรตีนสกัดจากถั่วเหลืองที่รับประทาน	.....		
จำนวนซองโปรตีนสกัดจากถั่วเหลืองที่เหลือจากการรับประทาน	.....		
อาการไม่พึงประสงค์จากการรับประทานโปรตีนสกัดจากถั่วเหลือง (ระบุอาการ และจำนวนครั้งที่มีอาการ)	.....		
การแก้ไขอาการไม่พึงประสงค์	.....		

2.2 ผลทางห้องปฏิบัติการ

ข้อมูล	ค่าปกติ	ช่วงก่อนการวิจัย			สัปดาห์ที่ 0	สัปดาห์ที่ 4	สัปดาห์ที่ 10	หมายเหตุ
วันนัดตรวจ (วัน/เดือน/ปี)								
ความดันโลหิต (มม.ปรอท)								
FPG (mg/dl)								
TG (mg/dl)								
Total Cholesterol (mg/dl)								
RBC ($x10^6$ mm^3)								
WBC (cell/mm^3)								
Hb (g%)								
Hct (%)								
PMN (%)								
Lymphocytes (%)								
Monocytes(%)								
Eosinophils (%)								
Basophils (%)								
Albumin (g/dl)								
CRP-hs (mg/dl)								
Homocysteine (µmol/l)								
Folate (nmol/l)								
Vitamin B12 (pmol/l)								

(ส่วนที่ 3) **รหัส**

แบบสอบถามข้อมูลการบริโภคอาหาร การทำงานและการออกกำลังกาย

<u>ข้อมูลเกี่ยวกับแบบแผนการบริโภคอาหาร</u> (ดัดแปลงจากแบบสอบถามพฤติกรรมการบริโภคอาหาร สำนักงานสถิติ, 2549)

1. ส่วนใหญ่ท่านรับประทานอาหารแบบใด
   - ☐ 1. ทำรับประทานที่บ้าน
   - ☐ 2. ซื้ออาหารถุงสำเร็จรูป รับประทานที่บ้าน
   - ☐ 3. ซื้ออาหารปรุงสำเร็จรูป รับประทานนอกบ้าน
   - ☐ 4. อาหารกระป๋อง
   - ☐ 5. อื่นๆ .....
2. ปกติท่านรับประทานอาหารมื้อหลักวันละกี่มื้อ
   - ☐ 1. รับประทานครบ 3 มื้อ ☐ 4. รับประทาน 2 มื้อ งดมื้อเย็น
   - ☐ 2. รับประทาน 2 มื้อ งดมื้อเช้า ☐ 5. รับประทานเพียง 1 มื้อเท่านั้น
   - ☐ 3. รับประทาน 2 มื้อ งดมื้อกลางวัน ☐ 6. รับประทานมากกว่า 3 มื้อ
3. ปกติท่านชอบรับประทานอาหารรสชาติใดมากที่สุด
   - ☐ 1. จืด ☐ 2. หวาน ☐ 3. เค็ม
   - ☐ 4. เผ็ด ☐ 5. เปรี้ยว ☐ 6. อื่นๆ ระบุ .....
4. ปกติท่านชอบรับประทานอาหารที่ปรุงด้วยวิธีใดมากที่สุด
   - ☐ 1. ต้มหรือลวกสุก ☐ 2. ตุ๋น ☐ 3. ผัด ☐ 4. ทอด
   - ☐ 5. สุกๆ ดิบๆ ☐ 6. นึ่ง ☐ 7. ปิ้งย่าง ☐ 8. อื่นๆ ระบุ.....
5. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานอาหาร<u>กลุ่มเนื้อสัตว์และผลิตภัณฑ์</u> เช่น ไข่ เนื้อสัตว์ เครื่องในสัตว์ เบคอน กุนเชียง หมูยอ เป็นต้น โดยเฉลี่ยสัปดาห์ละกี่วัน
   - ☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์
   - ☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

6. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานอาหารกลุ่มอาหารไขมันสูง ได้แก่ อาหารที่ปรุงด้วยวิธี
การผัด ทอด แกงที่ปรุงด้วยกะทิ ขนมเบเกอรี่ เช่น ขนมเค้ก โดนัท คุกกี้ เป็นต้น โดยเฉลี่ยสัปดาห์ละกี่วัน

☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์
☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

7. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานอาหารกลุ่มขนมสำหรับกินเล่นหรือขนมกรุบกรอบ
โดยเฉลี่ยสัปดาห์ละกี่วัน

☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์
☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

8. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานอาหารกลุ่มอาหารประเภทจานด่วน เช่น พิซซ่า แซนวิช
แฮมเบอร์เกอร์ เป็นต้น โดยเฉลี่ยสัปดาห์ละกี่วัน

☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์
☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

9. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานอาหารกลุ่มผักและผลไม้โดยเฉลี่ยสัปดาห์ละกี่วัน

☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์
☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

10. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานกลุ่มผลไม้แปรรูป เช่น ผลไม้เชื่อม กวน แช่อิ่ม ดอง เป็นต้น โดยเฉลี่ยสัปดาห์ละกี่วัน

☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์
☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

11. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานเครื่องดื่มประเภทน้ำอัดลมและเครื่องดื่มที่มีรสหวาน เช่น โกโก้ น้ำผลไม้ที่ใส่น้ำตาล เป็นต้น โดยเฉลี่ยสัปดาห์ละกี่วัน

☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์
☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

12. ในระหว่าง 1 เดือน ก่อนวันสัมภาษณ์ ท่านได้รับประทานอาหารแปรรูปต่างๆ ได้แก่ อาหารประเภทแปรรูปประเภทใส่เกลือเป็นหลัก เช่น เนื้อเค็ม หมูเค็ม ปลาร้า บูดู เป็นต้น โดยเฉลี่ยสัปดาห์ละกี่วัน

☐ 1. ไม่รับประทานเลย ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์

☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ทุกวัน

13. โดยปกติ ท่านดื่มน้ำ (ยกเว้น ชา กาแฟ แอลกอฮอล์) วันละประมาณเท่าไร

☐ 1. น้อยกว่า 8 แก้วต่อวัน ☐ 2. มากกว่า 8 แก้วต่อวัน

14. ท่านดื่มชาหรือไม่ ☐ 1. ไม่ดื่ม ☐ 2. ดื่ม ประมาณ .....แก้วต่อวัน

15. ท่านดื่มกาแฟหรือไม่ ☐ 1. ไม่ดื่ม ☐ 2. ดื่ม ประมาณ.....แก้วต่อวัน

16. ท่านดื่มเครื่องดื่มที่มีแอลกอฮอล์หรือไม่

☐ 1. ไม่ดื่ม ☐ 2. 1-2 วันต่อสัปดาห์ ☐ 3. 3-4 วันต่อสัปดาห์

☐ 4. 5-6 วันต่อสัปดาห์ ☐ 5. ดื่มทุกวัน

ข้อมูลการทำงานและการออกกำลังกาย (National Institute for Health and Clinical Excellence, 2006)

1. ลักษณะกิจกรรมที่เกี่ยวข้องกับการทำงานของท่าน

☐ไม่ได้ทำงาน (เกษียณอายุ, เหตุผลทางสุขภาพ, ต้องได้รับการดูแลตลอดเวลา เป็นต้น)

☐ใช้เวลาส่วนใหญ่นั่งทำงาน เช่น พนักงานบัญชี ธุรการ พนักงานออฟฟิช เป็นต้น

☐ใช้เวลาส่วนใหญ่ในการยืนหรือเดิน แต่ไม่ต้องใช้แรงมาก เช่น พนักงานขาย ช่างตัดผม ครู เป็นต้น

☐งานที่ใช้กำลังปานกลาง หรือใช้เครื่องมือ เช่น พนักงานทำความสะอาด ช่างไม้ ช่างไฟฟ้า พยาบาล เป็นต้น

☐งานที่ใช้กำลังมาก หรือยกสิ่งของที่มีน้ำหนักมาก เช่น พนักงานขนย้ายของ งานก่อสร้าง เกษตรกร เป็นต้น

2. ในช่วง 1 สัปดาห์ที่ผ่านมา ท่านใช้เวลาเท่าไหร่ในการทำกิจกรรมเหล่านี้

	ไม่ทำเลย	<1 ชั่วโมง	1- <3 ชั่วโมง	≥3 ชั่วโมง
(1) ออกกำลังกาย เช่น ว่ายน้ำ วิ่งเหยาะๆ แอโรบิก ฟุตบอล เทนนิส โยคะ เป็นต้น	☐	☐	☐	☐
(2) ขี่จักรยาน	☐	☐	☐	☐
(3) เดินไปทำงาน ซื้อของ เดินเล่น	☐	☐	☐	☐
(4) ทำงานบ้าน ดูแลเด็ก	☐	☐	☐	☐
(5) งานสวน	☐	☐	☐	☐

1. ความเร็วในการก้าวเดินตามปกติของท่านเป็นเช่นใด

☐ เดินช้า ☐ ปานกลาง

☐ เดินไว กระฉับกระเฉง ☐ เดินเร็วมาก

physical activity index (PAI)

Inactive	ผู้ที่มีการทำงานแบบนั่งโต๊ะ เคลื่อนไหวน้อย หรือใช้กำลังน้อย
Moderately inactive	ผู้ที่มีการทำงานแบบนั่งโต๊ะและออกกำลังกายบ้างแต่น้อยกว่า 1 ชั่วโมง/สัปดาห์ หรือการทำงานแบบยืนและไม่มีการออกกำลังกาย
Moderately active	ผู้ที่มีการทำงานแบบนั่งโต๊ะและออกกำลังกาย 1- 2.9 ชั่วโมง/สัปดาห์ หรือ การทำงานแบบยืนและมีการออกกำลังกายบ้างแต่น้อยกว่า 1 ชั่วโมง/สัปดาห์ หรือ การทำงานที่ใช้กำลังปานกลางและไม่มีการออกกำลังกาย
Active	ผู้ที่มีการทำงานแบบนั่งโต๊ะและมีการออกกำลังกาย ≥3 ชั่วโมง/สัปดาห์ หรือ การยืนทำงานและมีการออกกำลังกาย 1- 2.9 ชั่วโมง/สัปดาห์ หรือการทำงานที่ใช้กำลังปานกลางและมีการออกกำลังกายบ้างแต่น้อยกว่า 1 ชั่วโมง/สัปดาห์ หรือการทำงานประจำที่มีการใช้กำลังมากหรือยกสิ่งของที่มีน้ำหนักมา

Appendix C

Analysis Report of Total Isoflavones

หน่วยเครื่องมือกลาง/Central Instrument Facility

อาคารเฉลิมพระเกียรติ ชั้น 6 ห้อง K629 คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล 272 ถ. พระราม 6 แขวงทุ่งพญาไท เขตราชเทวี กทม. 10400

Chaloempraklet Building, 6th Floor, Room K629, Faculty of Science, Mahidol University, 272 Rama VI Road, Rajthevee, Payathai, Bangkok, 10400 Thailand

Analysis Report of Total Isoflavone

ใบรายงานผลเลขที่ CIF.SA052/2552

ชื่อและที่อยู่ผู้ขอรับบริการ	คุณดาวรุ่ง คำวงศ์ ภาควิชาอาหารและเภสัชเคมี คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ใบรับตัวอย่างเลขที่	CIF.SA052/2552
วันที่รับตัวอย่าง	16 มีนาคม 2552
วันที่ทำการทดสอบ	24 มีนาคม 2552
ชื่อตัวอย่าง	Soy Protein Isolate
รายละเอียดตัวอย่าง	Soy Protein Isolate บรรจุซองฟลอยด์อลูมิเนียม
ชนิดการทดสอบ	Total isoflavone
วิธีทดสอบ	HPLC-UV

ISOFLAVONE	mg/100g
glycitin	4.79
genistin	42.03
daidzein	27.14
glycitein	2.07
genistein	30.51
Total isoflavone*	106.55

2 ซ้ำ; *Total isoflavone = glycitin + genistin + daidzein + glycitein + genistein

รายงานนี้มีผลเฉพาะกับตัวอย่างที่นำมาทดสอบเท่านั้น และรายงานผลต้องไม่ถูกทำสำเนาเฉพาะเพียงบางส่วน ยกเว้นทำทั้งฉบับ โดยไม่ได้รับความยินยอมเป็นลายลักษณ์อักษรจากห้องปฏิบัติการ

(นางสาวนันทนาถ กิติศรีวรพันธุ์)

นักวิทยาศาสตร์

25 / มี.ค. / 52

Phone: +66 (0)2-201-5970, +66 (0)2-201-5985-7 Fax: +66 (0)2-201-5972 Email: cif.scmu.customer@gmail.com Website:http://www.sc.mahidol.ac.th/scre/cif

Appendix C

A 3-day Food Record

แบบบันทึกการบริโภคอาหาร 3 วัน

งานวิจัยเรื่อง “ผลของการเสริมโปรตีนสกัดจากถั่วเหลืองต่อตัวบ่งชี้ทางชีวภาพที่เป็นปัจจัยเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือด ในผู้ป่วยโรคเบาหวานชนิดที่ 2 ณ ศูนย์บริการสาธารณสุข 66 สำนักอนามัย กรุงเทพมหานคร”

คำชี้แจง ผู้วิจัยขอความร่วมมือจากท่าน กรุณาบันทึกข้อมูลตามความเป็นจริง ทำการจดบันทึกการบริโภคอาหาร 3 วัน โดยเลือกบันทึกในช่วงวันจันทร์ถึงศุกร์ 2 วัน และเลือกบันทึกวันเสาร์หรืออาทิตย์อีก 1 วัน ข้อมูลที่สำคัญของการจดบันทึกการบริโภคอาหาร 3 วัน ประกอบด้วย

1. **ชนิดของมื้ออาหาร:** ทำการบันทึกมื้ออาหารที่รับประทานพร้อมทั้งระบุเวลาที่รับประทานโดยประมาณ เช่น เช้า-กลางวัน-เย็น-อาหารว่าง เป็นต้น
2. **รายการอาหารและเครื่องดื่ม:** ทำการบันทึกรายการอาหาร รวมทั้งเครื่องดื่มทุกชนิดที่รับประทานตั้งแต่ตื่นนอนจนกระทั่งเข้านอน ต่อเนื่องกัน 3 วัน เช่น ก๋วยเตี๋ยวเส้นเล็กลูกชิ้นปลา น้ำมะตูม เป็นต้น
3. **ส่วนประกอบ:** ทำการบันทึกส่วนประกอบต่างๆ ของอาหารและเครื่องดื่มที่รับประทาน เช่น ข้าวต้มปลา มีส่วนประกอบด้วย ข้าวสวย เนื้อปลาสด ผักชี หัวหอม กระเทียม เป็นต้น
4. **ปริมาณที่รับประทาน:** ระบุปริมาณอาหารและเครื่องดื่มที่รับประทาน โดยระบุปริมาณอาหารในหน่วยวัดระดับครัวเรือน ดังต่อไปนี้

ข้าว และผักต่างๆ	ระบุหน่วยเป็น ทัพพี
เส้นก๋วยเตี๋ยวต่างๆ	ระบุหน่วยเป็น ก้อน
เนื้อสัตว์	ระบุหน่วยเป็น ช้อนโต๊ะ
ผลไม้	ระบุหน่วยเป็น ผล หรือจำนวนชิ้นคำ
เครื่องดื่ม	ระบุหน่วยเป็น แก้ว กล่อง หรือกระป๋อง (1 แก้ว = 240 มิลลิลิตร)
น้ำปลา น้ำมัน น้ำตาล	ระบุหน่วยเป็น ช้อนชา หรือช้อนโต๊ะ

5. **วิธีการเตรียมหรือวิธีการปรุงอาหารและเครื่องดื่ม:** ระบุวิธีการประกอบอาหารและเครื่องดื่มที่รับประทานตัวอย่างเช่น ต้ม ตุ๋น ผัด ทอด ลวก ปิ้ง ย่าง แกง แช่แข็ง รับประทานสด อาหารกระป๋อง
6. **สถานที่รับปะทานอาหารและเครื่องดื่ม:** ระบุสถานที่รับประทานอาหารและเครื่องดื่ม เช่น บ้าน ที่ทำงาน ร้านอาหาร
7. **ผลิตภัณฑ์เสริมอาหาร:** ระบุผลิตภัณฑ์เสริมอาหารที่รับประทาน เช่น วิตามิน แร่ธาตุ พร้อมทั้งระบุจำนวน เวลา และวิธีการรับประทาน

ตัวอย่างการจดบันทึกรายการอาหารและเครื่องดื่ม

บันทึกวันที่ 1 วันที่..2...เดือน...ธันวาคม...ปี..2552... (เลือกบันทึกวันใดวันหนึ่งในช่วงจันทร์ถึงศุกร์)

มื้ออาหาร/เวลา	รายการอาหาร/เครื่องดื่ม	ส่วนประกอบ (คร่าวๆ)	ปริมาณที่รับประทาน	วิธีการเตรียม/ปรุงอาหาร	สถานที่รับประทานอาหาร
เช้า/8.00 น.	- ข้าวต้มไก่	- ข้าวสวย - เนื้อไก่ไม่มีหนังติด	- 2 ทัพพี - 2 ช้อนโต๊ะ	ต้ม	บ้าน
	- นมพร่องมันเนย	- นมพร่องมันเนย	- 240 มิลลิลิตร (1 กล่อง)	กล่อง/กระป๋อง	บ้าน
กลางวัน/12.00 น.	- มักกะโรนีน้ำหมูสับ	- เส้นมักกะโรนี - เนื้อหมูสับไม่ติดมัน - น้ำซุบ	- 2 ก้อน - 2 ช้อนโต๊ะ - 1 ถ้วยตวง	ต้ม	บ้าน
	- ส้มเขียวหวาน	- ส้มเขียวหวาน	- 1 ผลกลาง	สด	บ้าน
	- น้ำเปล่า	- น้ำเปล่า	- 240 มิลลิลิตร (1 แก้วน้ำ)	-	บ้าน
อาหารว่าง/15.00 น.	- ขนมปังขาว	- ขนมปังขาวไม่ใส่แยม	- 2 แผ่น	ซื้อ (ยี่ห้อ: ฟาร์มเฮ้าส์)	บ้าน
	- น้ำสับปะรดปั่น	- น้ำสับปะรดปั่นสดไม่ใส่เกลือ	- 120 มิลลิลิตร (1/2 แก้วน้ำ)	ปั่น	บ้าน
เย็น/18.00 น.	- ข้าวสวย - ผัดผักกาดขาวใส่หมูสับ	- ข้าวสวย - ผักกาดขาว - เนื้อหมูสับไม่ติดมัน - น้ำมันถั่วเหลือง - ซีอิ้วขาวถั่วเหลือง	- 2 ทัพพี - 1/2 ถ้วยตวง - 2 ช้อนโต๊ะ - 1 ช้อนโต๊ะ - 1 ช้อนชา	หุง ผัด	บ้าน บ้าน
	- แอปเปิ้ล	- แอปเปิ้ล	- 1 ผล	สด	บ้าน

แบบบันทึกการบริโภคอาหาร 3 วัน

<u>บันทึกวันที่ 1</u> วันที่.....เดือน.....ปี..... (เลือกบันทึกวันใดวันหนึ่งในช่วง<u>จันทร์ถึงศุกร์</u>)

มื้ออาหาร/เวลา	รายการอาหาร/เครื่องดื่ม	ส่วนประกอบ (คร่าวๆ)	ปริมาณที่รับประทาน	วิธีการเตรียม/ปรุงอาหาร	สถานที่รับประทานอาหาร

<u>บันทึกวันที่ 2</u> วันที่.....เดือน.....ปี..... (เลือกบันทึกวันใดวันหนึ่งในช่วง<u>จันทร์ถึงศุกร์</u>)

มื้ออาหาร/เวลา	รายการอาหาร/เครื่องดื่ม	ส่วนประกอบ (คร่าวๆ)	ปริมาณที่รับประทาน	วิธีการเตรียม/ปรุงอาหาร	สถานที่รับประทานอาหาร

<u>บันทึกวันที่ 3</u> วันที่.....เดือน.....ปี..... (เลือกบันทึกวัน<u>เสาร์หรือวันอาทิตย์</u>วันใดวันหนึ่ง)

มื้ออาหาร/เวลา	รายการอาหาร/เครื่องดื่ม	ส่วนประกอบ (คร่าวๆ)	ปริมาณที่รับประทาน	วิธีการเตรียม/ปรุงอาหาร	สถานที่รับประทานอาหาร

Appendix E

Folate Analysis in Plasma and SPI

Method (Herbert, 1966; Keowkase, 2000)

Plasma folate levels were determined by microbiological assay using *Lactobacillus casei* as the test organism. Deionized-distilled water was used for preparation of all media and buffers. It was free from folate and the heavy metal which might have the undesirable ability to accelerate oxidation of the ascorbic acid in the buffer.

All glasswares used in the present experimental must be free from folate by boiling in Teepol 1:200 for at least 30 minutes. They were then soaked overnight in the cleansing solution and rinsed well with tap water and distilled water.

1. Preparation of plasma samples

Two millilitres of plasma were obtained from fasting subjects and centrifuged at 1,000 xg for 5 minutes. This supernatant was aliquated and frozen at -80 ^{o}C until assay.

2. Preparation of micro inoculum broth (Maintenance medium)

Maintenance medium was prepared by dissolving 18.5 g of microinoculum broth in distilled water and adjusted volume to 500 ml, stirred and filtered through filter paper. Then, 10 ml of this medium was dispensed into each screw-capped tube. These tubes were autoclaved at 15 lbs/inch^2 for 15 minutes. They were left to cool at room temperature. One sampled tube was incubated at 37 ^{o}C overnight for sterility check. The sterile set was then stored at 4^{o}C until used.

3. Preparation of folate casei medium (Double strength)

Dissolve 9.4 g of folic acid casei media in 100 ml of deionized water, to make double strength medium. Add 50 mg of ascorbic acid and filter through the filter paper. Let it cool, and dispense 3 ml into assay vials.

4. Preparation of stock culture of *Lactobacillus casei*

Lactobacillus casei ATCC (American Type Culture Collection No 7469) was kept in the maintenance media and stored in the refrigerator (4^{o}C). The organism must be subcultured every 2 weeks by adding aseptically one drop of a 0.5 ml pipette from the last stock culture to the tube containing fresh maintenance medium. This tube was incubated at 37^{o}C for 18 hour for the next subculture for the preparation of inoculums.

Occasionally, the stock was checked for the purity by plating on the blood agar medium and was incubated at 4 ^{o}C

5. Preparation of inoculums

In the afternoon before the assayed day, 1 drop of the stored culture was added to 10 ml of maintenance medium and incubated at 37 ^{o}C for 18 hours. Then 0.5 ml of this fresh 18 hours culture was added to 10 ml of maintenance medium and incubated at 37 ^{o}C for 6 hours. The inoculums for the assay, called "L.C." (Lactobacillus casei), was prepared by adding 0.05 ml of this 6-hour culture to 18 ml of the single strength basal medium. One drop of the inoculums was added to each assay vials.

6. Preparation of Ascorbic acid phosphate buffer

This buffer must be freshly prepared by dissolving 150 mg ascorbic acid in 100 ml of phosphate buffer pH 6.1

7. Preparation of phosphate buffer pH 6.1

- Solution A (acid 0.2 M) was prepared by dissolving 31.2 g of sodium dihydrogen phosphate dihydrates ($NaH_2PO_4.2H_20$) in the deionized-distilled water and made up to 1 litre.

- Solution B (base 0.2 M) was prepared by dissolving 28.4 g of disodium hydrogen phosphate (Na_2HPO_4) in the deionized-distilled water and made up to 1 litre.

Then 212.5 ml of solution A was mixed with 37.5 ml of solution B and made up to 1 litre with the deionized-distilled water. The pH of the solution should be 6.1. The solution was stored at the room temperature.

8. Preparation of standard folic acid stock solution. 1.0×10^{-5} g/ml

One hundred milligram of standard folic acid powder was weighed and dissolved in 20 ml of dionized-distilled water. The 0.1 N Sodium hydroxide solution was slowly added until the solution became clear yellow. It was made up to 100 ml with deionized-distilled water in the volumetric flask. The concentration of this solution was 1.0×10^{-3} g/ml. Then 1 ml of this solution was transferred into 100 ml volumetric flask and made up to 100 ml with 20% ethyl alcohol. The concentration of the final solution was 1.0×10^{-5} g/ml. The solution was divided into small tubes and stored at -40 ^{o}C.

9. Preparation of standard folic acid stock solutions. 1.0×10^{-9} g/ml and 1.0×10^{-10} g/ml (This solutions must be freshly prepared before use)

The standard folic acid stock solution (1.0×10^{-5} g/ml) was taken from the freezer, thawed and 1 ml of the solution was pipette into 100 ml volumetric flask. The solution was made up to 100 ml with deionized-distilled water. The concentration of this solution was 1.0×10^{-7} g/ml

To prepare 1.0×10^{-9} g/ml folic acid solution, 1 ml of the 1.0×10^{-7} g/ml folic acid solution was diluted to 100 ml with deionized-distilled water in the volumetric flask. Then 10 ml of the 1.0×10^{-9} g/ml folic acid solution was diluted to

100 ml with deionozed-distilled water in the volumetric flask. The concentration of the final folic acid solution was 1.0×10^{-10} g/ml.

10. Method of assay folic acid

- The vials for assay standard folic acid were numbered and prepared by adding deionized-distilled water, 1.0×10^{-9} g/ml standard folic acid solution and 1.0×10^{-10} g/ml standard folic acid solution to each vial as shown in Table E-1.
- With an automatic syringe, 3 ml of double strength assay medium (see 3) was added to each assay vial.
- Another 25 ml tube, 9 ml of deionized-distilled water and 9 ml of the double strength assay medium were added. This tube was marked “LC”
- All of the assay vials and L.C. with screw caps loosely affixed were autoclaved at 15 lbs/inch^2 pressure (121 ^{o}C) for 5 minutes, cooled to room temperature and the screw caps were tightened
- The unknown plasma samples were taken from freezer, thawed at room temperature and were aseptically added to each assay vial (0.05 ml) by micropipette.
- To prepare inoculums, 0.05 ml of 6 hours culture (see 5) was added to the “L.C.” bottle. One drop of this inoculums was incubated to the assay vials except the “control” vial. This must be done with aseptic technique. Then the assay vials were incubated at 37 ^{o}C for 40-48 hours.

- The density (absorbance) of the growth microorganisms in each vial was measured with Nepho-Colorimeter using a red filter (wave length 655 nm.
- The growth densities (absorbance) of each standard concentration were averaged and plotted on a graph paper to make the standard growth curve. The concentrations of unknown were calculated from this standard curve.
- Calculation of plasma folate concentrations by following formula:

  $$\text{Plasma folate conc.(ng/ml)} = \frac{\text{Folate conc. from standard (pg/ml)} \times F}{1000}$$

  Whereas F= Factor value

$$\text{Factor value} = \frac{\text{Total volume}}{\text{Volume of sample used}}$$

3.6.4 Folate in SPI analysis (Jansuittivechakul 1979; Sunthornthum, 1977)

- Preparation of conjugase

  Thirty milligram of chicken pancreas was dissolved in 10 ml of deionized- distilled water. The solution was centrifuged at 4,500xg for 10 minutes. The supernatant was kept in the freezer.

- Preparation of SPI samples with conjugase treatment

Two gram of SPI was dissolved in 50 ml of ascorbic acid phosphate buffer solution (see 6). The homogeneous SPI samples were then autoclave at 15 lbs/inch^2 pressure (121 ^oC) for 10 minutes. The solution was centrifuged at 4,500xg 10 minutes. The supernatant was prepared as follow.

(a) SPI without conjugase

9 ml of SPI samples solution were mix with 1 ml of deionized-distilled water. The mixture was kept in the freezer until assay (F).

(b) SPI with conjugase

9 ml of SPI sample solution was mixed with 1 ml of conjugase and 0.5 ml of toluene was added, then incubated at 37 ^{o}C for 16-18 hours or overnight. The reaction of the enzyme was stopped by boiling in water bath for 10 minutes and centrifuge at 4,500xg for 10 minutes. The supernatant was freezed until assay (C).

In the determination of folate in SPI sample, the sample were diluted as shown in Table E-2. Calculation the SPI sample in terms of the standard curve by the following formula:

$$\text{Folate conc. (pg/ml)} = \frac{\text{Folate from standard curve} \times \text{Dilution} \times \text{Total volume of assay media}}{\text{Volume of SPI sample added to assay media}}$$

Table E-1 Protocol for preparation of standard curve

Number	Final folate concentration (pg/ml)	Folic acid concentration (ml)		Deionized distilled water (ml)	Assay media (ml)	Total volume (ml)
		1.0×10^{-10} g/ml	1.0×10^{-9} g/ml			
Control	0	-	-	3	3.0	6.0
0	0	-	-	3	3.0	6.0
1	5	0.3	-	2.7	3.0	6.0
2	10	0.6	-	2.4	3.0	6.0
3	20	1.2	-	1.8	3.0	6.0
4	40	2.4	-	0.6	3.0	6.0
5	70	0.2	0.4	2.4	3.0	6.0
6	100	-	0.6	2.4	3.0	6.0
7	150	-	0.9	2.1	3.0	6.0
8	200	-	1.2	1.8	3.0	6.0
9	400	-	2.4	0.6	3.0	6.0

Table E-2 Volume adjustment of assay vials

Sample	Number	Deionized-distilled water	SPI without conjugase (ml)	SPI with conjugase (ml)
1	F^1	2.95	0.05	-
	F^1	2.90	0.10	-
	C^1	2.95	-	0.05
	C^1	2.90	-	0.10
2	F^2	2.95	0.05	-
	F^2	2.90	0.10	-
	C^2	2.95	-	0.05
	C^2	2.90	-	0.10
etc.				

Appendix F

Statistical Analysis

Statistical analysis

The statistics used in this study included Shapiro-Wilk, Skewness, and Kurtosis test for normal distribution data, Independent t-test for comparing mean between two groups, Pair-Sample t-test for comparing means before and after treatment in each group, Chi-Square test for comparing frequency of two groups and in each group and Pearson's correlation test for relating variables.

The variables of this study included fasting blood sugar, HbA1c, total cholesterol, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglyceride (TG), hs-CRP and anthropometry. The level of statistical significance was set at $p<0.05$.

1. Normal distribution test

One-sample Shapiro-Wilk test was used to investigate whether the variables were normally distributed. The data showed that *p*-value of some tested variables were more than 0.05 (Table F-1). However, *p*-value of some tested variables were less than 0.05, they were tested by Skewness or Kurtosis test. If the value of Skewness' statistic (or Kurtosis' statistic) ± 1.96 (standard error of Skewness or Kurtosis) cross zero, the variables were normally distributed (Table F-2). If the variables did not accept by Skewness or Kurtosis test, the variables were cut the outliners and tested normal distribution by Shapiro-Wilk test again (Table F-3). It suggested that parametric statistics could be used to compare mean of variables in this study.

Table F-1 Shapiro-Wilk's test of biomarkers of CVD

Variables	Groups	Shapiro-Wilk's Value	df	*P*
Age (years)	Control	0.974	18	0.884
	Treatment	0.947	18	0.406
SBP (mmHg)	Control (baseline)	0.896	18	0.049*
	Control (week 6)	0.922	18	0.137
	Treatment (baseline)	0.929	18	0.185
	Treatment (week 6)	0.911	18	0.089
DBP (mmHg)	Control (baseline)	0.867	18	0.016*
	Control (week 6)	0.868	18	0.016*
	Treatment (baseline)	0.715	18	0.000*
	Treatment (week 6)	0.875	18	0.022*
FPG (mg/dl)	Control (baseline)	0.730	18	0.000*
	Control (week 6)	0.777	18	0.001*
	Treatment (baseline)	0.920	18	0.130
	Treatment (week 6)	0.847	18	0.008*
HbA1c (mg/dl)	Control (baseline)	0.758	18	0.000*
	Control (week 6)	0.927	18	0.172
	Treatment (baseline)	0.869	18	0.017*
	Treatment (week 6)	0.805	18	0.002*
Total-C (mg/dl)	Control (baseline)	0.945	18	0.354
	Control (week 6)	0.952	18	0.465
	Treatment (baseline)	0.917	18	0.116
	Treatment (week 6)	0.964	18	0.689
HDL-C (mg/dl)	Control (baseline)	0.921	18	0.135
	Control (week 6)	0.861	18	0.013*
	Treatment (baseline)	0.931	18	0.200
	Treatment (week 6)	0.985	18	0.986
LDL-C (mg/dl)	Control (baseline)	0.958	18	0.559
	Control (week 6)	0.958	18	0.563
	Treatment (baseline)	0.906	18	0.072
	Treatment (week 6)	0.978	18	0.932

* *data* were *not normally distributed* ($p < 0.05$)

Table F-1 Shapiro-Wilk's test of biomarkers of CVD (continued)

Variables	Groups	Shapiro-Wilk's Value	df	*P*
TG (mg/dl)	Control (baseline)	0.949	18	0.405
	Control (week 6)	0.981	18	0.956
	Treatment (baseline)	0.942	18	0.310
	Treatment (week 6)	0.950	18	0.422
CRP (mg/l)	Control (baseline)	0.910	18	0.086
	Control (week 6)	0.932	18	0.214
	Treatment (baseline)	0.834	18	0.005*
	Treatment (week 6)	0.616	18	0.000*
hs-CRP (mg/l)	Control (baseline)	0.902	18	0.063
	Control (week 6)	0.734	18	0.000*
	Treatment (baseline)	0.834	18	0.005*
	Treatment (week 6)	0.614	18	0.000*
tHcy (µmol/l)	Control (baseline)	0.959	18	0.580
	Control (week 6)	0.966	18	0.718
	Treatment (baseline)	0.969	18	0.786
	Treatment (week 6)	0.981	18	0.955
Folate (ng/ml)	Control (baseline)	0.934	18	0.227
	Control (week 6)	0.908	18	0.079
	Treatment (baseline)	0.964	18	0.679
	Treatment (week 6)	0.938	18	0.263
Vitamin B_{12} (pg/ml)	Control (baseline)	0.936	18	0248
	Control (week 6)	0.946	18	0.364
	Treatment (baseline)	0.948	18	0.389
	Treatment (week 6)	0.964	18	0.690

* *data* were *not normally distributed* ($p < 0.05$)

Table F-1 Shapiro-Wilk's test of renal function

Variables	Groups	Shapiro-Wilk's Value	df	*P*
Albumin (g/dl)	Control (baseline)	0.967	18	0.734
	Control (week 6)	0.941	18	0.297
	Treatment (baseline)	0.897	18	0.051
	Treatment (week 6)	0.955	18	0.508
Uric acid (mg/dl)	Control (baseline)	0.975	18	0.889
	Control (week 6)	0.932	18	0.210
	Treatment (baseline)	0.978	18	0.923
	Treatment (week 6)	0.934	18	0.231
SCr (mg/dl)	Control (baseline)	0.946	18	0.289
	Control (week 6)	0.972	18	0.837
	Treatment (baseline)	0.919	18	0.116
	Treatment (week 6)	0.901	18	0.060
BUN (mg/dl)	Control (baseline)	0.953	18	0474
	Control (week 6)	0.901	18	0.060
	Treatment (baseline)	0.948	18	0.395
	Treatment (week 6)	0.937	18	0.259
GFR (ml/min)	Control (baseline)	0.987	18	0.995
	Control (week 6)	0.876	18	0.022*
	Treatment (baseline)	0.968	18	0.785
	Treatment (week 6)	0.975	18	0.890

* *data* were *not normally distributed* ($p < 0.05$)

Table F-1 Shapiro-Wilk's test of anthropometric parameters

Variables	Groups	Shapiro-Wilk Value	df	*P*
BMI (kg/m^2)	Control (baseline)	0.957	18	0.539
	Control (week 6)	0.973	18	0.846
	Treatment (baseline)	0.956	18	0.523
	Treatment (week 6)	0.966	18	0.710
Waist circumference (cm)	Control (baseline)	0.900	18	0.058
	Control (week 6)	0.908	18	0.078
	Treatment (baseline)	0.917	18	0.115
	Treatment (week 6)	0.931	18	0.200
Hip circumference (cm)	Control (baseline)	0.974	18	0.864
	Control (week 6)	0.973	18	0.860
	Treatment (baseline)	0.953	18	0.476
	Treatment (week 6)	0.930	18	0.192
WHR	Control (baseline)	0.969	18	0.778
	Control (week 6)	0.974	18	0.868
	Treatment (baseline)	0.924	18	0.155
	Treatment (week 6)	0.908	18	0.079
TSF (mm)	Control (baseline)	0.922	18	0.138
	Control (week 6)	0.972	18	0.841
	Treatment (baseline)	0.965	18	0.696
	Treatment (week 6)	0.938	18	0.273
MAC (cm)	Control (baseline)	0.920	18	0.128
	Control (week 6)	0.954	18	0.496
	Treatment (baseline)	0.919	18	0.123
	Treatment (week 6)	0.952	18	0.450
MAMC (cm)	Control (baseline)	0.982	18	0.969
	Control (week 6)	0.957	18	0.538
	Treatment (baseline)	0.924	18	0.152
	Treatment (week 6)	0.909	18	0.081

* data were not normally distributed ($p < 0.05$)

Table F-1 Shapiro-Wilk's test of energy intake data

Variables	Groups	Shapiro-Wilk's Value	df	*P*
Total energy intake (kcal)	Control (baseline)	0.951	18	0.443
	Control (week 6)	0.968	18	0.764
	Treatment (baseline)	0.939	18	0.282
	Treatment (week 6)	0.944	18	0.333
Total protein (g)	Control (baseline)	0.946	18	0.346
	Control (week 6)	0.961	18	0.624
	Treatment (baseline)	0.929	18	0.189
	Treatment (week 6)	0.895	18	0.048*
Protein from animal (g)	Control (baseline)	0.897	18	0.052
	Control (week 6)	0.986	18	0.990
	Treatment (baseline)	0.901	18	0.060
	Treatment (week 6)	0.900	18	0.058
Protein from vegetable (g)	Control (baseline)	0.944	18	0.342
	Control (week 6)	0.907	18	0.077
	Treatment (baseline)	0.949	18	0.417
	Treatment (week 6)	0.931	18	0.203
Fat (g)	Control (baseline)	0.966	18	0.789
	Control (week 6)	0.956	18	0.660
	Treatment (baseline)	0.981	18	0.974
	Treatment (week 6)	0.895	18	0.081
Cholesterol (mg)	Control (baseline)	0.971	18	0.874
	Control (week 6)	0.871	18	0.018*
	Treatment (baseline)	0.923	18	0.216
	Treatment (week 6)	0.904	18	0.068
Carbohydrate (g)	Control (baseline)	0.956	18	0.531
	Control (week 6)	0.972	18	0.827
	Treatment (baseline)	0.939	18	0.280
	Treatment (week 6)	0.933	18	0.222

* *Data* were *not normally distributed* ($p < 0.05$)

Table F-**2** Shapiro-Wilk's test of energy intake data (continued)

Variables	Groups	Shapiro-Wilk's Value	df	*P*
Sugar (g)	Control (baseline)	0.910	18	0.086
	Control (week 6)	0.897	18	0.051
	Treatment (baseline)	0.968	18	0.0767
	Treatment (week 6)	0.938	18	0.266
Dietary fiber (g)	Control (baseline)	0.968	18	0.850
	Control (week 6)	0.940	18	0.288
	Treatment (baseline)	0.954	18	0.498
	Treatment (week 6)	0.982	18	0.970

* *Data* were *not normally distributed* ($p < 0.05$)

Table F-**2** The Skewness and Kurtosis test of clinical outcome data

Variables	Groups	Skewness value	Kurtosis value
SBP (mmHg)	Control (baseline)	(-1.684) - (0.418)	(-2.526) - (1.542)
DBP (mmHg)	Control (baseline)	(-0.177) - (1.925)	(-2.677) - (1.391)
	Control (week 6)	(-1.058) - (1.044)	(-2.984) - (1.084)
	Treatment (baseline)	(0.072) – (2.174)*	(-2.181) - (1.887)
	Treatment (week 6)	(-1.224) - (0.878)	(-1.984) - (2.084)
FPG (mg/dl)	Control (baseline)	(0.334) - (2.436)*	(-0.624) - (3.444)
	Control (week 6)	(0.274) - (2.376)*	(-1.553) - (2.515)
	Treatment (week 6)	(0.411) - (2.513)*	(-0.133) - (3.955)
HbA1c (mg/dl)	Control (baseline)	(-0.036) - (2.066)	(-1.544) - (2.524)
	Treatment (baseline)	(-0.006) - (2.095)	(-1.965) - (2.103)
	Treatment (week 6)	(0.232) - (2.334)*	(-1.580) - (2.488)
HDL-C (mg/dl)	Control (week 6)	(0.200) - (2.301)*	(-1.197) - (2.871)
CRP (mg/l)	Treatment (baseline)	(0.052) - (2.153)*	(-2.009) - (2.059)
	Treatment (week 6)	(2.206) - (4.308)*	(10.150) - (14.22)*
GFR (ml/min)	Control (week 6)	(0.121) - (2.223)*	(-1.299) - (2.769)
Total protein (g)	Treatment (week 6)	(-1.940) - (0.162)	(1.808) - (5.876)*
	Control (baseline)	(0.567) - (2.669)*	(1.619) - (5.687)*
Cholesterol (mg)	Control (week 6)	(-1.703) - (0.399)	(-3.168) - (0.900)

* *Data* were *not normally distributed* ($p < 0.05$)

Table F-3 Normality test of clinical outcome data after excluding the outlier

Variables	Groups	Skewness value	Kurtosis value	Shapiro-Wilk Value	df	*p*
CRP (mg/l)	Treatment (week 6)	(-0.578) - (1.578)	(-2.152) - (2.014)	0.962	17	0.674

* *Data* were *not normally distributed* ($p < 0.05$)

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