EFFICACY AND SAFETY OF MULBERRY LEAVES ON GLYCEMIC CONTROL IN PATIENTS WITH OBESITY AND PATIENTS WITH TYPE 2 DIABETES

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ประสิทธิศักย์และความปลอดภัยของใบหม่อนในการควบคุมระดับน้ำตาลในเลือดในผู้ที่มีภาวะอ้วนและผู้ป่วยเบาหวานชนิดที่ 2

น.ส.ธัญชนิต ไทยพิทักษ์วงศ์

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Thesis Title	EFFICACY AND SAFETY OF MULBERRY LEAVES ON GLYCEMIC CONTROL IN PATIENTS WITH OBESITY AND PATIENTS WITH TYPE 2 DIABETES
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Field of Study	Pharmaceutical Care
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ธัญชนิต ไทยพิทักษ์วงศ์ : ประสิทธิศักย์และความปลอดภัยของใบหม่อนในการควบคุมระดับน้ำตาลในเลือดในผู้ที่มีภาวะอ้วนและผู้ป่วยเบาหวานชนิดที่ 2. (EFFICACY AND SAFETY OF MULBERRY LEAVES ON GLYCEMIC CONTROL IN PATIENTS WITH OBESITY AND PATIENTS WITH TYPE 2 DIABETES) อ.ที่ปรึกษาหลัก : ศ. ภญ. ดร.พรอนงค์ อร่ามวิทย์, อ.ที่ปรึกษาร่วม : พอ. นพ.อุปถัมภ์ ศุภสินธุ์

งานวิจัยนี้แบ่งเป็น 3 การศึกษา ประกอบด้วยการศึกษาทางคลินิกแบบสุ่มและมีกลุ่มควบคุม 2 ระยะและการศึกษาการแสดงออกของโปรตีนโดยเทคนิคโปรติโอมิกส์ การศึกษาทางคลินิกระยะที่หนึ่งมีวัตถุประสงค์เพื่อประเมินประสิทธิภาพและความปลอดภัยของใบหม่อนในการลดน้ำตาลในเลือดหลังการรับประทานสารละลายซูโครส 50 กรัมในอาสาสมัครสุขภาพดี และเพื่อประเมินขนาดรับประทานที่เหมาะสมของใบหม่อนโดยกำหนดจากปริมาณของ 1-ดีออกซีโนจิริไมซิน (ดีเอ็นเจ) ซึ่งเป็นสารสำคัญหลักในใบหม่อน ผลการศึกษาพบว่าใบหม่อนสามารถลดภาวะน้ำตาลสูงหลังมื้ออาหารได้อย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุมและสัมพันธ์กับขนาดของดีเอ็นเจ อาการข้างเคียงที่พบ ได้แก่ ท้องอืด ถ่ายเหลว และคลื่นไส้ และดีเอ็นเจขนาด 12 มิลลิกรัมเป็นขนาดรับประทานที่เหมาะสมที่สามารถลดระดับน้ำตาลในเลือดได้และทำให้เกิดอาการไม่พึงประสงค์น้อยที่สุด การศึกษาระยะที่สองมีวัตถุประสงค์เพื่อประเมินประสิทธิภาพในการควบคุมระดับน้ำตาลในเลือดและความปลอดภัยในระยะยาวของใบหม่อนในผู้ที่มีภาวะอ้วนและผู้ป่วยเบาหวานชนิดที่ 2 ผลการศึกษาพบว่าการรับประทานใบหม่อนที่ประกอบด้วยดีเอ็นเจ 12 มิลลิกรัม วันละ 3 ครั้งทุกวันนาน 12 สัปดาห์ทำให้การควบคุมระดับน้ำตาลดีขึ้นเมื่อเทียบกับค่าเริ่มต้นก่อนรับประทาน และยังพบแนวโน้มที่ดีขึ้นของความไวต่ออินซูลินในกลุ่มทดลองอีกด้วย อย่างไรก็ดีไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม นอกจากนี้ใบหม่อนลดระดับไขมันในเลือดได้ โดยพบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่ม การศึกษานี้ไม่พบผลข้างเคียงเกี่ยวกับการทำงานของตับและไต อย่างไรก็ดีพบรายงานอาการท้องอืด ถ่ายเหลว และท้องผูกจากการรับประทานใบหม่อน การศึกษาระยะที่สามเป็นการศึกษาผลของใบหม่อนต่อการแสดงออกของโปรตีนในพลาสมาในกลุ่มที่ได้รับประทานใบหม่อนจากการศึกษาทางคลินิกระยะที่สองด้วยเทคนิคโปรติโอมิกส์ โดยพบว่าการแสดงออกของโปรตีนในกลุ่มเมทาบอลิก เมทริกซ์นอกเซลล์ ระบบภูมิคุ้มกัน และกระบวนการอักเสบเปลี่ยนแปลงในทางที่ดีขึ้นภายหลังการรับประทานใบหม่อน

สาขาวิชา	การบริบาลทางเภสัชกรรม	ลายมือชื่อนิสิต .....
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KEYWORD: Mulberry leaves, 1-Deoxynojirimycin, Clinical trial, Blood glucose, Adverse event

Thanchanit Thaipitakwong : EFFICACY AND SAFETY OF MULBERRY LEAVES ON GLYCEMIC CONTROL IN PATIENTS WITH OBESITY AND PATIENTS WITH TYPE 2 DIABETES. Advisor: Prof. Pornanong Aramwit, Ph.D. Co-advisor: Col. Ouppatham Supasyndh, M.D.

This work was divided into three study phases: the two-phase randomized controlled clinical trials and proteomics study. First, the phase I clinical trial aimed to determine efficacy and safety of mulberry leaves on postprandial glucose following the 50-g sucrose ingestion in healthy nondiabetic adults and to explore the optimal administered dose of 1-deoxynojirimycin (DNJ), the major antihyperglycemic compound of mulberry leaves. The results showed the alleviation of postprandial hyperglycemia by mulberry leaves in a dose-dependent fashion. Adverse effects of mulberry leaves included bloating and flatulence, loose stool, and nausea. In addition, 12 mg of DNJ was considered the optimal dose defined by the clinically effective dose with the minimal side effects. Second, the phase II clinical trial was conducted to determine efficacy and safety of the long-term administration of mulberry leaves on glycemic control in patients with obesity and patients with type 2 diabetes. Daily administration of mulberry leaves containing 12 mg of DNJ thrice daily before meals resulted in the improvement in glycemic control as well as insulin sensitivity in the mulberry leaves-treated group; however, there was no difference between the treatment group and the control. Moreover, mulberry leaves were capable of reducing blood lipids when compared with the control group. Our study did not observe the changes in hepatic and renal function by mulberry leaves administration. Nonetheless, it caused bloating and flatulence, loose stool, and constipation. Last, effects of mulberry leaves on expressions of plasma proteins of persons who enrolled the phase II clinical study were further determined using proteomics analysis. In response to mulberry leaves treatment, the analysis found modulation in expressions of proteins involved in metabolic regulation, extracellular matrix constituents and organization, immunity, and inflammatory response.

Field of Study:	Pharmaceutical Care	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....
		Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ACO	Acyl-CoA oxidase
AGE	Advanced glycation end product
Akt	Protein kinase B
ALT	Alanine aminotransferase
AMPK	Adenosine monophosphate-activated protein kinase
AST	Aspartate aminotransferase
AP-1	Activator protein-1
AUC	Area under the curve
A1C	Glycated hemoglobin
BHT	Butylated hydroxytoluene
BMI	Body mass index
BUN	Blood urea nitrogen
CI	Confidence interval
cm	Centimetre
COX-2	Cyclooxygenase-2
CPT	Carnitine palmitoyltranferase
Cr	Serum creatinine
CRP	C-reactive protein
DAG	Diacylglycerol
DNJ	1-Deoxynojirimycin
DPP	Diabetes Prevention Program
DPPH	1,1-Diphenyl-2-picrylhydrazyl
ECM	Extracellular matrix
emPAI	Exponentially Modified Protein Abundance Index
ER	Endoplasmic reticulum
FAS	Fatty acid synthase
FFA	Free fatty acid
FPI	Fasting plasma insulin

FPG	Fasting plasma glucose
g	Gram
GABA	γ-Aminobutyric acid
GLUT	Glucose transporter
GO	Gene ontology
GR	Glutathione reductase
GSK-3β	Glycogen synthase kinase-3β
GST	Glutathione-S-transferase
GPAT	Glycerol-3-phosphate acyltransferase
GPx	Glutathione peroxidase
G6Pase	Glucose-6-phosphatase
G-6-PD	Glucose-6-phosphase dehydrogenase
g/mol	Grams per mole
HDL-C	High-density lipoprotein cholesterol
HMG-CoA	β-hydroxy β-methylglutaryl-CoA
HOMA-IR	Homeostasis model assessment of insulin resistance
HPLC	High-performance liquid chromatography
HR	Hazard ratio
Hz	Hertz
IC_{50}	50% Inhibitory concentration
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IKK	Inhibitor of nuclear factor-κB kinase
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IPAQ	International Physical Activity Questionnaire
IRS	Insulin receptor substrate
ISO	Isoproterenol
IU/L	International units per litre
IκB	Inhibitor of NF-κB
JAK-STAT	Janus kinase-signal transducer

JNK	Jun N-terminal kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
kg	Kilogram
kGray	Kilogray
kg/m^2	Kilograms per square metre
LC	Liquid chromatography
LDL-C	Low-density lipoprotein cholesterol
LXR	Liver X receptor
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
MD	Mean difference
mg/dL	Milligrams per decilitre
mg/g	Milligrams per gram
min	Minute
mIU/L	Milli-International Units per litre
mL	Millilitre
MS	Mass spectrometry
mTORC1	Mammalian target of rapamycin complex 1
NCD	Non-communicable diseases
NEMO	NF-κB essential modulator
NH_4HCO_3	Ammonium bicarbonate
NF-κB	Nuclear factor-κB
NLRP	NOD-like receptor protein
NLRP-7	NACHT, LRR and PYD domains-containing protein 7
NOD-2	Nucleotide-binding oligomerization domain-containing protein 2
OGTT	Oral glucose tolerance test
PEPCK	Phosphoenolpyruvate carboxykinase
PIP3	Phosphatidylinositol 3,4,5-trisphosphate
PI3K	Phosphatidylinositol-3-kinase

PPAR	Peroxisome proliferator-activated receptor
PPG	Postprandial plasma glucose
RBP-4	Retinol-binding protein 4
RCT	Randomized controlled trial
ROS	Reactive oxygen species
RR	Relative risk
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SMD	Standardized mean difference
SOD	Superoxide dismutase
SREBP	Sterol regulatory element-binding protein
STZ	Streptozotocin
sVCAM-1	sSoluble vascular cell adhesion molecule-1
TC	Total cholesterol
TG	Triglycerides
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNF-R1	TNF receptor superfamily member 1A
UPR	Unfolded protein response
US	United States
V	Volt
WC	Waist circumference
WMD	Weighed mean difference
µg/mL	Micrograms per millilitre
µIU/mL	Micro- international units per millilitre
µL	Microlitre
µm	Micrometre
µmol/L	Micromoles per litre
1,5-AG	1,5-Anhydroglucitol
^{o}C	Degree Celsius

CHAPTER I
INTRODUCTION

1.1 Background and rationale

Type 2 diabetes is one of the most challenging public health problems in recent decades. Persistent hyperglycemia, the major manifestation of diabetes, leads to life-threatening complications and related mortality [1-3]. Over 600 million adults worldwide predict to have type 2 diabetes in the next 25 years [4]. In addition to the rapidly growing prevalence, type 2 diabetes imposes substantially high economic costs in the health care system [5]. Beyond optimizing glycemic control in known diabetic patients, clinical management nowadays focuses on preventing and delaying the disease onset in persons at high risk of type 2 diabetes to limit the numbers of new diabetic cases [6, 7].

Prediabetes, also known as intermediate hyperglycemia [8], refers to a crucially high-risk state for the development of type 2 diabetes [7]. It is an ongoing stage of insulin resistance and β-cell dysfunction, which are the key defects in glucose homeostasis in type 2 diabetes [9], and related to metabolic syndromes and cardiovascular events [8]. It is documented that impaired glucose metabolism in prediabetes could be reversed to normal condition by proper therapeutic approaches. Strategies that improve insulin sensitivity and preserve β-cell function would be the cornerstone for the management of prediabetes [8, 9].

Development of prediabetes as well as type 2 diabetes results from an integration of genetic susceptibility and environmental factors [6]. Obesity, poor eating habits, and sedentary lifestyle can be corrected in order to lessen individual diabetic risk [10, 11]. Obesity is established as the most crucial risk factor for insulin resistance and the fundamental of metabolic disturbance [12]. Obese persons are almost 10 times more likely to develop type 2 diabetes than lean persons [13]. Worldwide prevalence of obesity is projected to be dramatic in parallel with the growing of type 2 diabetes as well as metabolic syndrome and cardiovascular diseases [13, 14].

Poor eating habits are also the leading cause of prediabetes and type 2 diabetes in modern society [14, 15]. Carbohydrate-rich foods and high glycemic index carbohydrates directly aggravate postprandial hyperglycemia, which is an independent risk factor for diabetic complications and cardiovascular diseases [15, 16]. Moreover, chronic stimulation of insulin secretion by carbohydrate-rich diets provokes β-cell disruption [14]. Asian meals commonly have a greater proportion of carbohydrates with a higher glycemic index when compare with Western foods. For example, white rice is considered a staple food of Asians. A robust association between white rice consumption and an increased diabetic risk was found in previous meta-analyses [17, 18]. Moreover, regarding genetic disposition, Southeast Asians populations had the higher severity of postprandial hyperglycemia and impaired glucose metabolism in relative to people in other regions [19].

Several approaches intervene the progression of prediabetes in different populations. Two large randomized controlled trials showed that intensive lifestyle intervention including weight reduction, eating healthy diets, and increased physical activity decreased the risk for type 2 diabetes by 58% in individuals who were diagnosed with prediabetes [10, 11]. Its advantages are easily accessible and affordable, but long-term maintenance of lifestyle modification remains challenging in the real life. Pharmacotherapy also exhibited favorable effects on diabetic risk [2]. Alpha-glucosidase inhibitor is one of many drug classes that effectively prevent the development of type 2 diabetes. Acarbose and voglibose reduced the rate of type 2 diabetes by 25% and 40%, respectively, in persons with prediabetes [20, 21]. The drugs suppressed postprandial hyperglycemia through their major mechanism of action, resulting in the better control in A1C [22]. Acarbose also alleviated excessive insulin secretion, β-cell disruption, and impaired vascular homeostasis and coagulation that involved in metabolic disturbance [23-25]. Furthermore, acarbose lowered the risk for cardiovascular events by 35% in patients with type 2 diabetes [26]. These suggest rationales for consideration of α-glucosidase inhibitors in the management of prediabetes. Nonetheless, the synthetic α-glucosidase inhibitors caused significant gastrointestinal events such as flatulence, diarrhea, and abdominal pain [27], contributing to the high discontinuation rate in clinical and surveillance studies [22, 28].

Mulberry (*Morus* spp.; family Moraceae) is a multi-functional plant widely distributed in Asian countries. Leaves of mulberry play the important role in sericulture industry because it is the only food of silkworm. In addition, they are known as traditional medicine and functional food with no report of toxicity and serious adverse event. Mulberry leaves contain a cluster of bioactive compounds such as alkaloids, phenolic acids, and flavonoids [29]. 1-deoxynojirimycin (DNJ) is the most notable antihyperglycemic agent of mulberry leaves exhibiting an inhibitory effect on α-glucosidase enzymes, resulting in the decrease in postprandial plasma glucose (PPG) [30]. Mulberry leaves have been therefore gained the attention as a natural source of a promising α-glucosidase inhibitor.

Antihyperglycemic efficacy of mulberry leaves is widely demonstrated in clinical trials. A single administration of mulberry leaves attenuated postprandial hyperglycemia during carbohydrate tolerance test [31-33]. Furthermore, daily supplementation of mulberry leaves improved glycemic control in persons with prediabetes and poorly controlled patients with type 2 diabetes [32, 34, 35]. Mulberry leaves reduced fasting plasma glucose (FPG) and increased 1,5-anhydroglucitol (1,5-AG). The level of glycated hemoglobin (A1C) tended to be improved by mulberry leaves treatment [34]. Mulberry leaves also ameliorated the impairments in glucose homeostasis in several pathways: reversing insulin resistance [36], suppressing hepatic gluconeogenesis [37-39], and restoring β-cell viability [40, 41]. In terms of safety, mulberry leaves are safe and well tolerated with the most common side effects including gastrointestinal discomfort [34, 35]. As a result, mulberry leaves could have potentials for glycemic control and delay the progression of type 2 diabetes.

To date, there are difficulties in the implementation of mulberry leaves in evidence-based practice. First, antihyperglycemic effect of mulberry leaves against postprandial hyperglycemia has been well established; however, it is questionable whether mulberry leaves can effectively reduce the concentrations of FPG and A1C [32, 34, 35]. Second, mulberry leaves can improve insulin sensitivity [36-39] and preserve β-cell function in animals [40, 41], yet these effects have not been determined in clinical trials. Third, beyond the direct lowering effect on postprandial glucose, little is known about mechanisms of action of mulberry leaves on glucose

and insulin homeostasis at the molecular level, particularly in human. Fourth, the optimal dose of mulberry DNJ was clearly defined in few studies [32, 33, 35]. In general, the effective dose of administration was mainly considered based on the glucose-lowering effectiveness, but not the safety issues. It remains unclear whether the dosage regimen is safe and tolerable in the long-term use. Last, a huge variation in response to α-glucosidase inhibitors regarding the different eating habits and ethnicity was reported [27, 42]. A greater magnitude of reduction in PPG by acarbose was observed in persons who consume Eastern foods than those consuming Western ones [43]. In addition, the Southeast Asian population is more likely to have high PPG and insulin resistance than other ethnic groups eating the same foods [44]. At present, there has been no study investigating effects of mulberry leaves on glycemic responses in Southeast Asians, including Thai population.

The ultimate purpose of this work is to highlight potentials of the product derived from mulberry leaves, an easily accessible medicinal plant in Thailand, for the management of type 2 diabetes, particularly for the early phases of the disease. Two randomized controlled clinical studies were consecutively conducted. First, a multi-dose study was performed in order to explore the optimal dose of mulberry DNJ as defined by the effective dose with minimal adverse effects. This study also aimed to ensure efficacy and safety of our mulberry leaf product as a preliminary data for the further clinical investigations. Second, a 12-week, two-parallel group study was conducted to determine efficacy and safety of daily mulberry leaves supplementation on glycemic control and insulin resistance profile in Thai adults who had impaired glucose metabolism, including obese persons with prediabetes and patients with early-stage type 2 diabetes. Beyond clinical outcomes evaluation, effect of mulberry leaves on plasma protein expression was also evaluated using proteomic analysis in order to better understand the possible molecular mechanisms of action of mulberry leaves against metabolic impairments in prediabetes and type 2 diabetes.

1.2 Objectives

Study I

1. To determine efficacy of mulberry leaves on lowering postprandial hyperglycemia in response to carbohydrate load in healthy non-diabetic adults

2. To determine safety of mulberry leaves in healthy non-diabetic adults

3. To explore the optimal dose of mulberry DNJ on lowering postprandial hyperglycemia in response to carbohydrate load

Study II

1. To determine efficacy of mulberry leaves on glycemic control in obese persons who have prediabetes and patients with early-stage type 2 diabetes regarding effects on the following variables;

(i) PPG excursion over 2 hours following the standard 75-g OGTT

(ii) A1C

(iii) FPG

(iv) Insulin resistance: FPI and HOMA-IR

2. To determine effects of mulberry leaves on lipid profiles in obese persons who have prediabetes and patients with early-stage type 2 diabetes regarding effects on the following variables;

(i) Total cholesterol (TC)

(ii) Triglycerides (TG)

(iii) High-density lipoprotein cholesterol (HDL-C)

(iv) Low-density lipoprotein cholesterol (LDL-C)

3. To determine effects of mulberry leaves on anthropometric profiles (body weight, waist circumference, and body mass index; BMI) in obese persons who have prediabetes and patients with early-stage type 2 diabetes

4. To determine possible adverse events of the daily mulberry leaves supplementation in obese persons who have prediabetes and patients with early-stage type 2 diabetes regarding effects on the following variables;

(i) Hepatic function: aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

(ii) Renal function: serum creatinine (Cr)

(iii) Self-report adverse events

Study III

To determine effects of mulberry leaves on protein expression in plasma of obese persons who have prediabetes and patients with early-stage type 2 diabetes using proteomic analysis

1.3 Benefits of this study

1. Provides additional information about potentials of mulberry leaves and mulberry DNJ for the management of prediabetes and type 2 diabetes

1.1 Verifies the optimal dose of mulberry DNJ for the long-term administration

1.2 Verifies glucose-lowering effect of mulberry leaves against the elevated PPG, A1C and FPG concentration

1.3 Suggests modulating effects of mulberry leaves on glucose intolerance and insulin resistance

1.4 Suggests the possible molecular mechanisms of action of mulberry leaves

2. Highlights the promising roles of Asian medicinal plant in evidence-based practice for diabetes management

CHAPTER II
LITERATURE REVIEW

Content of the review

2.1 Prediabetes and type 2 diabetes

2.2 Postprandial hyperglycemia

2.3 Evaluation of glycemic control and insulin resistance

2.4 Determination of protein expression using proteomic analysis

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2.1 Prediabetes and type 2 diabetes

2.1.1 Overview

Type 2 diabetes is one of the most common non-communicable diseases (NCD) and ranked the seventh leading cause of death [1]. The disease is associated with the dysregulation of glucose metabolism, leading to persistent hyperglycemia [3]. Prolonged uncontrolled hyperglycemia subsequently causes morbidity and mortality because of irreversible dysfunction and failure of several vital organs including eyes, nerves, kidneys, and blood vessels [3]. These clinical consequences diminish quality of life of diabetic patients inevitably [45].

The global prevalence of type 2 diabetes has been rising at alarming rate. It is estimated that 415 million of adults were living with diabetes and the numbers of the patients could be reach to 640 million in the next 25 years [4]. In Thai population, the prevalence of type 2 diabetes was comparable to the projection of diabetes worldwide.

The recent National Health Examination Survey (NHES) reported the increasing prevalence from 7.0% in 2004 to 9.7% in 2014 among Thai adults aged over 20 years [46]. The survey additionally showed that 40% of these patients remained undiagnosed and were not treated properly [46]. It is noteworthy that the diabetes prevalence varies across the different geographical areas and socioeconomic statuses. An increasing trend of type 2 diabetes remains observed in developing countries [47]. In parallel with the dramatic growth of disease prevalence, health care related costs of type 2 diabetes have been rising over years. In 2017, the total national estimated cost of diabetes was $327 billion in the United States (U.S.), which was 26% higher than the costs in the past five years [5].

Prediabetes is a medical condition refers to an intermediate stage between normal glucose metabolism and type 2 diabetes [7]. Individuals with prediabetes are at high risk of developing type 2 diabetes. Approximately 70% of those with prediabetes can progress to type 2 diabetes [48]. It is one of the independent risk factors of microvascular- and macrovascular complications [45]. One-tenth of prediabetic patients had retinopathy, nephropathy, and neuropathy even signs and symptoms of diabetes are not yet present [9]. Furthermore, prediabetes is strongly associated with metabolic syndromes, including insulin resistance, abdominal obesity, dyslipidemia, and hypertension [8]. It has been hypothesized that the mild impairments in glucose metabolism in prediabetes can be cured. Considerations of the early detection and treatment for prediabetes are well evident because it provides benefits on prevention and delaying the onset of type 2 diabetes [6, 7].

The prevalence of prediabetes is largely epidemic in alignment with the trend of type 2 diabetes. A recent estimation showed that 352 million adults worldwide at the age of 20 to 79 years had prediabetes [49]. More than 400 million adults are predicted to develop prediabetes in 2030 [49]. In similar, 8.2 million Thais aged over 15 years were classified as prediabetes in 2014 [50].

2.1.2 Pathophysiology and pathogenesis

Type 2 diabetes is a multifactorial disease involved multi-organ dysfunction [3]. Insulin resistance and pancreatic β-cell dysfunction are well established as the major

pathophysiology of the disease [2, 3]. Defects in glucose metabolism silently and gradually develops at the molecular level for years before the overt diabetes can be diagnosed [3]. In other words, the early phases of type 2 diabetes are commonly asymptomatic; nonetheless, the disease progression is irreversible and endless [2, 9]. The significant signs and symptoms of diabetes are detected when the impairments extremely worsen [9].

Liver, skeletal muscle, and adipocytes are the peripheral target of insulin and the vital components of metabolic regulation [3]. Hepatic cells take part in glucose storage, gluconeogenesis, and glucose liberation under the regulations of insulin and glucagon in order to normalize the basal blood glucose level during the fasting state [3, 9]. Meanwhile, skeletal muscle and adipocytes are responsible for glucose clearance from blood circulation. In response to the increased blood glucose after meals, the processes of glucose uptake in skeletal muscle and adipocytes are up-regulated to alleviate postprandial hyperglycemia [8, 9]. Moreover, adipose tissue is the key source of adipocytokine production, which significantly involved in various metabolic controls and inflammatory responses [51].

Dysregulation and dysfunction of these metabolic tissues play the central roles in the development of insulin resistance and the subsequent β-cell dysfunction, which are well established as the major pathophysiology of type 2 diabetes [2, 3]. Insulin resistance, an insufficient response of the insulin-target tissues to insulin, is the hallmark of prediabetes and the early stage of diabetes [3]. Hepatic insulin resistance contributes to fasting hyperglycemia due to glucose dysregulation during basal state [52]. Meanwhile, the impairments in glucose uptake into the resistant skeletal muscle and adipocytes lead to postprandial hyperglycemia [9]. Figure 1 demonstrates the development and progression of prediabetes and type 2 diabetes. When insulin sensitivity of the target cells slightly diminishes, healthy β-cells are able to compensate by increasing the cell mass and insulin production in order to maintain normoglycemic condition [8, 9]. Thus, it is general to observe hyperinsulinemia in the early stages of type 2 diabetes [52]. Peripheral insulin resistance continuously aggravates over time concurrently with the compensatory processes by β-cells. It causes β-cells exhaust and no longer fully compensate. In this phase, insulin secretion

is not sufficient to regulate blood glucose, contributing to the emerge of mild hyperglycemia [9]. After a long period of cellular adaptation, the extremely exhausted β-cells are unable to overcome the elevated blood glucose because of the critical loss of β-cell mass and function [8, 9]. Consequently, persistent and marked hyperglycemia emerges. Moreover, since insulin plays the essential roles in fat and fatty acid metabolism, the mentioned circumstances inevitably lead to the dysregulation of lipid homeostasis It is not only the root cause of the overt dyslipidemia and excessive lipid accumulation, but also lipotoxicity-induced metabolic inflammation [53].

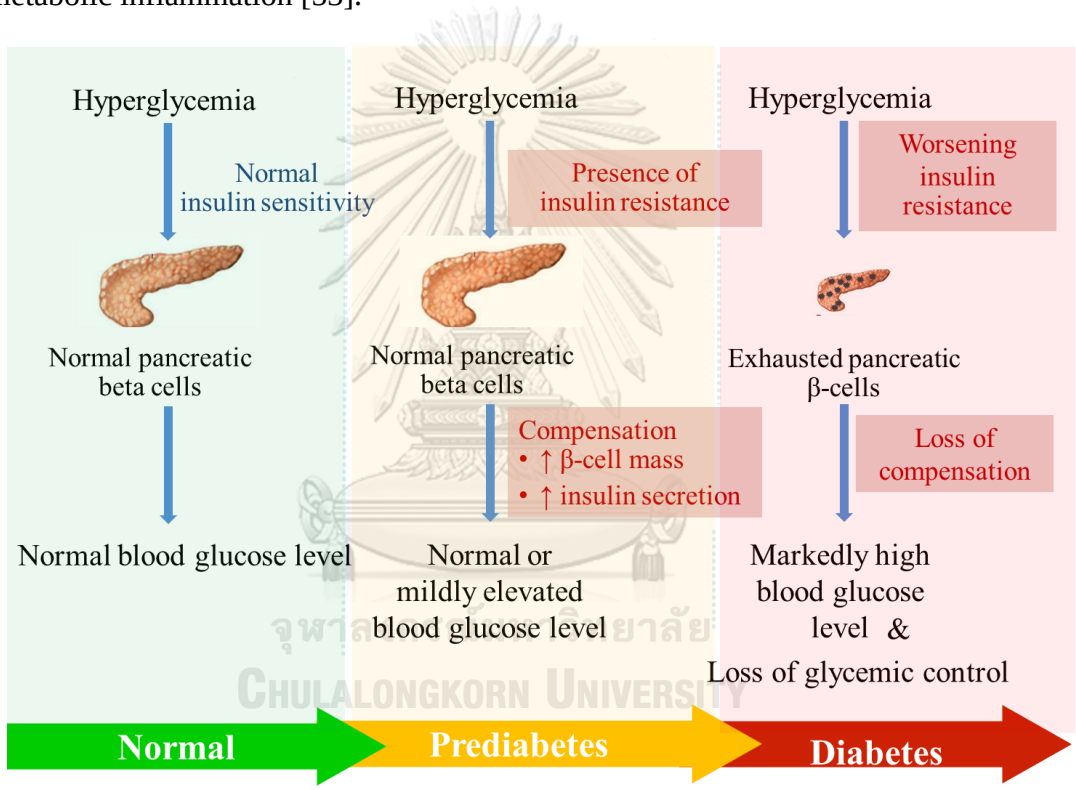


Figure 1 The development and progression of prediabetes and type 2 diabetes

Various changes at the molecular level have been established as pathogenesis of insulin resistance and β-cell dysfunction Herein, we reviewed the underlying mechanisms that extensively proposed in scientific studies and summarized in Figure 2.

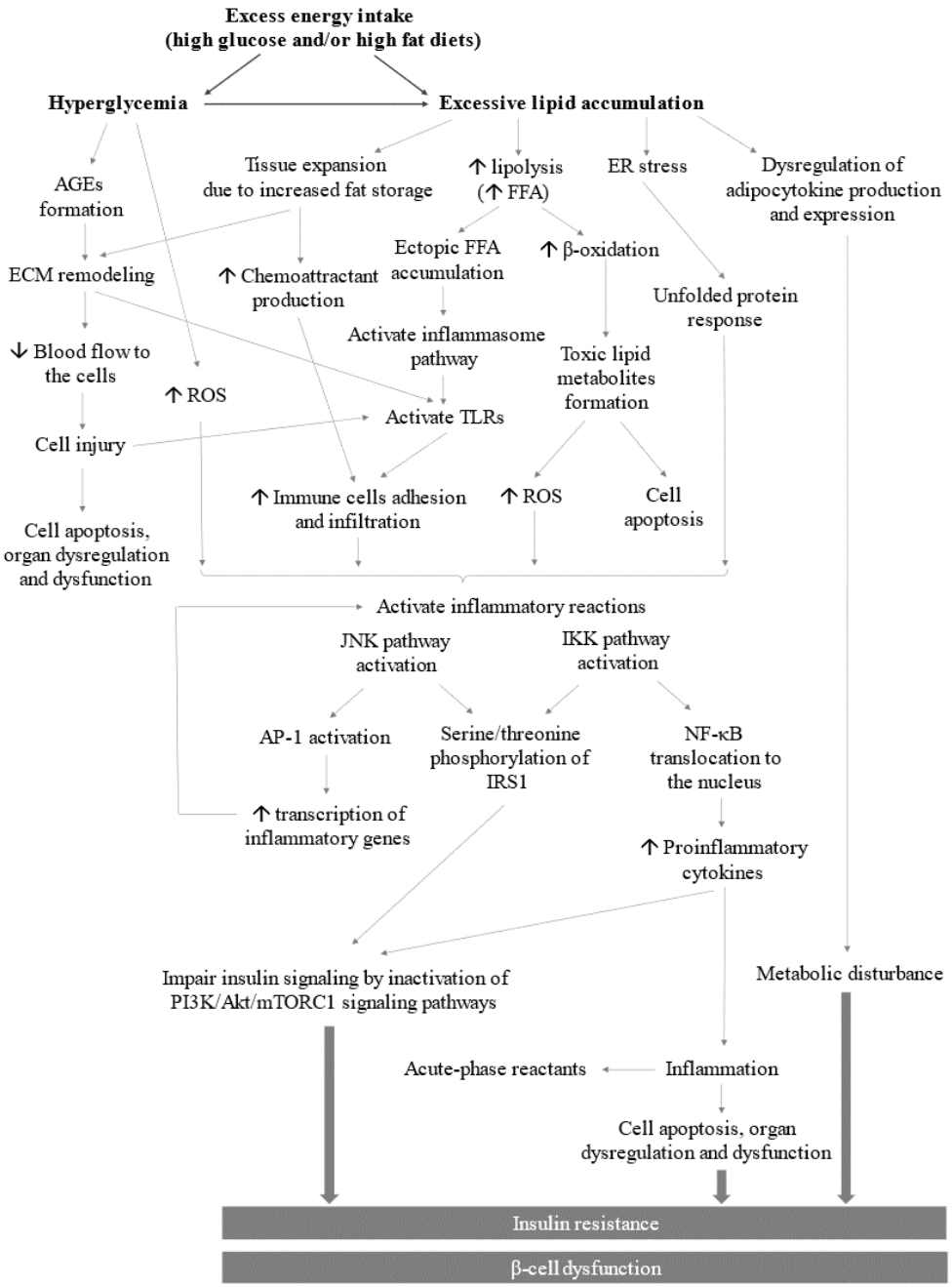


Figure 2 Molecular pathogenesis of insulin resistance and β-cell dysfunction (AGE: advanced glycation end product; FFA: free fatty acid; ER: endoplasmic reticulum; ROS: reactive oxygen species; TLR: Toll-like receptor; JNK: c-Jun N-terminal kinase; IKK: inhibitor of nuclear factor-κB kinase; AP-1: activator protein-1; IRS1: insulin receptor substrate 1; NF-κB: nuclear factor-κB; PI3K: phosphatidylinositol 3-kinase; Akt: protein kinase B: mTORC1: mammalian target of rapamycin complex 1)

1) Extracellular matrix remodeling

Persistent hyperglycemia directly aggravates non-enzymatic glycation of blood glucose and protein, leading to the formation of advanced glycation end products (AGEs) [54]. The deleterious role of AGEs in insulin resistance is that the substances vitally affect compositions and organization of extracellular matrix (ECM), which is also known as ECM remodeling, in the metabolic tissues [55]. ECM refers to the non-cellular components that provide structural support to the cells. It is composed of a linking network of glycoproteins (i.e. collagens, elastin, laminin, and fibronectin) and proteoglycans [56, 57]. ECM is a highly dynamic biological substance. Its compositions and structure alter in response to stimuli to maintain cell integrity and homeostasis. Furthermore, ECM regulates cellular processes, including cell proliferation and cell differentiation. The interactions between ECM molecules and cell surface receptors facilitate intercellular communication [57].

In addition, high fat consumption is another cause of ECM remodeling. Due to the excessive lipid storage, the enlarged adipocytes promote ECM production and deposition in the cell surface [58]. The structure acts as a physical barrier for angiogenesis that decreases blood supply to the cells and results in adipocyte necrosis, which is the leading cause of adipocytokine dysregulation [58, 59]. Also, dysregulation of ECM organization serves as the trigger of innate immune system. It induces macrophage infiltration into the cells, contributing to the elevated inflammatory responses. The elevation of inflammatory mediators subsequently impair insulin signaling of insulin receptor substrate (IRS)-1 and cause insulin resistance [54, 55, 58].

Previous studies observed the massive alterations in structural components and excessive deposition of ECM in liver, skeletal muscle, and adipose tissue during the development of insulin resistance and type 2 diabetes in animals and human [58-60]. The findings indicate the strong association between ECM remodeling and metabolic impairments.

2) Pattern recognition proteins and inflammasome

Besides ECM remodeling, there are several factors taking the important parts in the immune activation that related to insulin resistance and β-cell dysfunction. It has been established that the development of insulin resistance is linked to innate immune system via the overexpression of pattern recognition proteins, particularly Toll-like receptor (TLR)-4, and inflammasome. TLR-4 is the transmembrane protein sensor detecting lipopolysaccharides and lipopeptides derived from the elevated body fat and blood glucose and microbial pathogens [61, 62]. Meanwhile, inflammasome, also known as NOD-like receptor protein (NLRP), refers to the complex of intracellular proteins located in the cytoplasm of immune cells. Studies showed that free fatty acid (FFA), ceramides, and hyperglycemia are considered the major triggers of TLR-4 and inflammasome responses [62-64]. In general, to maintain cellular homeostasis, oligomerization of pattern recognition proteins and the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) of the inflammasome complex activates immune system [65]. However, the overinduction of pattern recognition proteins and inflammasome pathways causes tissue inflammation and tissue damage via the caspase-1 activation [66]. The activated caspase-1 eventually contributes to the secretion of interleukin (IL)-1β, which mediates macrophage accumulation in the peripheral tissues [67]. Deposition of macrophage, especially in adipose tissue, provokes the production of other inflammatory cytokines, including tumor necrosis factor (TNF)-α and IL-6. The up-regulated TLR-4 is additionally involved in the insulin signaling impairment due to the increased serine/threonine phosphorylation of IRS-1 [61].

Moreover, the dysregulation of pattern recognition proteins might be associated with adaptive immune system; however, the certain mechanism is poorly understood at present. It has been also suggested that ligation of pattern recognition proteins in dendritic cells can aggravate biological responses of T cells and B cells [63]. The hyperactivated pattern recognition proteins and inflammasome are the risk factors of insulin resistance as well as impaired glucose homeostasis [67]. Many experiments have consistently supported that depletion of the genes encoding TLR-4 and

inflammasome modulated metabolic inflammation and insulin resistance in obese animals and high-calorie diet-induced insulin-resistant mice [64-66].

3) Chemoattractant

Chemoattractant, including monocyte chemoattractant protein-1 (MCP-1), is considered as another explanation of immune activation during the development of metabolic impairments. MCP-1 is formed under the condition with excessive fat accumulation. It attracts the adhesion of monocytes and macrophages to the cell surface and mediates immune cells infiltration [61]. The increased macrophage level was observed in adipocytes of mice overexpressing MCP-1. The transgenic mice also developed insulin resistance afterwards. On the other hand, knockout MCP-1 mice exhibited the decreased macrophage accumulation in adipocytes [68]. Studies further found that MCP-1 activates the production inflammatory cytokines as well as tissue inflammation by provoking inflammatory reactions, which exert the deleterious effects on insulin signaling pathway [61, 68].

4) Oxidative stress

Excessive reactive oxygen species (ROS) accumulation is regarded as the common trigger of inflammatory signaling pathways. A primary origin of metabolic tissue inflammation is hyperglycemia-induced ROS formation, resulting from autooxidation of glucose [69]. Similarly, saturated FFA serves as the major deleterious molecule that directly aggravates cellular oxidative stress [70]. Furthermore, it is involved in the production of lipid metabolites: ceramide and diacylglycerol (DAG). The metabolites are toxic to the cells because they do not stimulate ROS formation only, but they also deteriorate endogenous antioxidative activities [70].

Under the stress conditions such as hyperglycemia and obesity, the unfolded/misfolded proteins are generated in the lumen of endoplasmic reticulum (ER) [69]. Unfolded protein response (UPR) is also considered as the inflammatory mediator. It refers to a cellular mechanism resulting from accumulation of the unfolded protein in response to ER stress [71]. The UPR is normally activated in order to restore cell homeostasis and cell survival [71]. The increased expression of

UPR was found in adipocytes and skeletal muscle of obese persons and glucose intolerant individuals, whereas their expressions were modulated by weight reduction [72, 73]. The intimate link between the increased UPR activity and the impaired insulin signaling transduction has been addressed. Like ROS and lipid metabolites, the UPR induces inflammatory signaling via Jun N-terminal kinase (JNK) and inhibitory-κB kinase (IKK)/nuclear factor (NF)-κB signaling pathways, contributing to serine/threonine phosphorylation of IRS-1 [69]. Moreover, the prolonged ER stress promotes β-cell apoptosis, resulting in the decreased insulin secretion in pancreas [69].

5) Inflammatory signaling pathways associated with insulin resistance and β-cell dysfunction

Insulin resistance are believed to be the chronic, low-grade inflammation [74]. The JNK and IKK/NF-κB signaling pathways are the primary regulatory mechanisms of metabolic inflammation as well as metabolic diseases. First, the activated JNK, one of the downstream components belonging to mitogen-activated protein kinase (MAPK) signaling pathway, primarily promotes serine/threonine phosphorylation of IRS-1 and results in the impaired insulin signaling [75]. The JNK pathway is also associated with the reduced insulin secretion due to the inhibitory effect of IL-1 [76]. It has been proposed that the up-regulation of JNK causes insulin resistance by facilitating the phosphorylation of the activator protein (AP-1) transcription factor and subsequently promoting inflammatory gene transcription [61]. Studies supported that suppression of the JNK pathway improved insulin sensitivity and glucose tolerance [76]. JNK knockout mice were protected from diet-induced insulin resistance. Also, depletion of JNK in adipose tissue exerted the protective effect against hepatic steatosis and increased insulin clearance [75].

Second, the activation of IKK/NF-κB follows a parallel pathway to the JNK responses. Under the normal condition, the inactive NF-κB is stabilized by interacting with the inhibitory molecules termed inhibitor of NF-κB (IκB). In response to stimuli, the IKK complex (IKK-α/β/γ) is up-regulated which leads to phosphorylation and degradation of the IκB proteins. Subsequently, the free form of NF-κB localizes to the nucleus, resulting in the up-expression of inflammatory cytokines, for example, TNF-

α, IL-1β, and IL-6 [77, 78]. The activated IKK also inhibits the expression of anti-inflammatory cytokines such as leptin and adiponectin [70]. On the other hand, IKK deficiency results in the lowered expression of TNF-α and IL-6 in transgenic mice when compared with controls. In addition, the deletion of IKK improved glucose tolerance and insulin sensitivity [77].

6) Inflammatory cytokines and acute-phase reactants

The elevation of inflammatory cytokines was found in various models of obesity, insulin resistance, and type 2 diabetes and a strong relationship between multiple cytokines and insulin resistance has been evident [61, 74]. TNF-α plays a central role in inflammatory responses by mediating adipocyte lipolysis and promoting serine/threonine phosphorylation of IRS-1 [79, 80]. Furthermore, it impairs glucose uptake in skeletal muscle by interfering glucose transporter (GLUT)-4 translocation [79]. Previous studies found the impaired insulin action and glucose tolerance in animals fed with TNF-α when compared with controls [80]. By contrast, the treatment of TNF-α antagonist exerted the favorable effects against insulin resistance in animals and human [80, 81]. IL-6, the key regulator of Janus kinase-signal transducer (JAK-STAT) signaling pathway, is directly involved in the activation of TLR-4 expression and the decreased expression of IRS-1 signaling [74]. IL-6 also disturbs glucose homeostasis in the insulin-targeted tissues by disrupting GLUT4 expression and inhibiting transcription genes related to hepatic glycogenenolysis [61]. Meanwhile, IL-1β is associated with the inflamed pancreatic β-cells, subsequently contributing to the impaired insulin secretion [74, 82]. Also, it mediates macrophage accumulation in the peripheral insulin-target tissues, leading to the decreased responses to insulin action [67].

In addition to the overexpression of inflammatory cytokines, the increase in positive acute-phase reactants is the indicator of inflammation [61]. It refers to the circulating proteins that considerably elevate during the inflammatory processes [83]. The positive acute-phase reactants, for example, C-reactive protein (CRP), fibrinogen, hepcidin, and serum amyloid A, are primarily synthesized by hepatocytes, whereas the few amounts are also produced by adipocytes, macrophage, and endothelial cells [84]. They are considered the indicators of inflammation both in the short- and long-

term states [83]. It has also believed that the presences of acute-phase reactants can last in the blood circulation for the whole duration of chronic inflammation. However, they are not specific to the certain inflammatory stimuli [83]. Besides inflammation, the changes in acute-phase reactants expression result from metabolic stress, infection, trauma, and autoimmune diseases [84].

Evidences has demonstrated the association between acute-phase reactants expression and metabolic impairments. Nonetheless, the casual roles of various inflammatory proteins in the development of insulin resistance as well as type 2 diabetes are largely varied among the studies. In the recent large cohort study, the elevated concentrations of CRP, orosomucoid, and haptoglobin, were suggested to be the risk factors of type 2 diabetes development with hazard ratio (HR)1.40 (95% confidence interval (CI): 1.01-1.95; p = 0.046), 1.18 (95%CI: 0.83-1.67; p = 0.51), and 1.19 (95%CI: 0.85-1.62; p = 0.10), respectively [85]. The increased CRP, erythrocyte sedimentation rate, fibrinogen, white blood cells count, and complement C3 were observed in prediabetic individuals [86]. Similarly, persons who diagnosed with type 2 diabetes exhibited the higher concentrations of CRP, ceruloplasmin, and total sialic acid than non-diabetic controls ($p < 0.01$) [87]. In addition, interleukin-1 receptor antagonist and hs-CRP were positively associated with impaired insulin sensitivity (HR = 1.13; 95% CI: 1.07-1.20), whereas glycoprotein acetyls was associated with impaired insulin secretion, hyperglycemia, and type 2 diabetes (HR = 1.37; 95% CI: 1.29-1.46) [88].

2.1.3 Diagnosis

Table 1 demonstrates the cut-off values of blood glucose for diagnosis of prediabetes and type 2 diabetes. Prediabetes is defined on the basis of the levels of FPG, 2-hour PPG following a standard oral glucose tolerance test (OGTT), and A1C [6, 7]. The levels of blood glucose among individuals with prediabetes are higher than the normal ranges; nonetheless, the levels do not reach the threshold of diabetes [9]. Similarly, diagnosis of type 2 diabetes relies on the mentioned glycemic indices. Random plasma glucose is additionally considered for persons who have significant

symptoms of hyperglycemia (i.e. increased thirst, increased hunger, and frequent urination) or hyperglycemic crisis [6, 7].

Table 1 Diagnosis of prediabetes and type 2 diabetes

Glycemic indices	Prediabetes	Type 2 diabetes
FPG (mg/dL)	100 – 125	≥ 126
2-hour PPG (mg/dL) [a]	140 – 199	≥ 200
A1C (%)	5.7 – 6.4	≥ 6.5
Random plasma glucose (mg/dL)	–	≥ 200

[a] 2-hour PPG following 75-g OGTT

FPG: fasting plasma glucose; PPG: postprandial glucose; A1C: glycated hemoglobin

2.1.4 Characteristics of impaired glucose metabolism

Impaired glucose metabolism is a metabolic disturbance in which the body losses normal regulation of glucose utilization and production as described previously, resulting in presentations of abnormally high blood glucose that comprises both prediabetes and type 2 diabetes.

Impaired glucose metabolism is categorized into impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and type 2 diabetes. Meanwhile, IFG and IGT are the subsets of prediabetes and characterized by the values of FPG (100-125 mg/dL) and 2-hour PPG (140-199 mg/dL), respectively [6]. In each individual, the abnormal glycemic indices can be present solely or in combination because there is no concordance between the two glycemic parameters [89]. For example, a person who have IFG may have either normal or impaired glucose tolerance.

There are characteristics differences among the types of impaired glucose metabolism. In individuals with IFG in which insulin resistance develops in hepatic cells, an increase in FPG could be detected [48]. In contrast to persons who have IGT, insulin resistance emerges in skeletal muscle cells; thus, the significant disturbance in glucose uptake into peripheral cells results in an elevation of PPG [48]. Meanwhile, patients with overt type 2 diabetes have the aggressive insulin resistance and β-cell dysfunction, resulting in prolonged hyperglycemia both in fasting and postprandial periods [8, 9].

2.1.5 Risk factors

There are several risk factors aggravating the development of prediabetes and type 2 diabetes [6, 7]. Table 2 summarizes the possible risk factors, which are classified into modifiable and non-modifiable factors. Non-modifiable risk factors are intrinsically unavoidable in any persons. Therefore, correction of modifiable one is the only possible way to lessen the risk for developing the disease [10, 11]. Relationship of modifiable risk factors and the development of prediabetes and type 2 diabetes are described below.

Table 2 Risk factors for prediabetes and type 2 diabetes

Modifiable risk factors	**Non-modifiable risk factors**
Obesity	Increased age
Poor eating habits	First-degree relatives to diabetic patients
Sedentary lifestyle	African American, American Indian, Hispanic/Latino, and Asian American

1) Obesity

Obesity refers to a condition of having excess body weight due to overstorage of adipose tissue inside body [12]. Asian adults who have BMI equal to 25.0 kg/m^2 or higher are considered obese [13]. In addition to genetics, an imbalance between energy intake and energy output primarily causes weight gain [12]. The condition is established as a crucial health problem because it is the fundamental of metabolic disturbance; thus, obesity is the common concomitant of many cardiometabolic disorders [13]. Obesity is the major predictor for insulin resistance and type 2 diabetes [51]. Evidences showed that the incidence rate of insulin resistance sharply rises when BMI over 25.0 kg/m^2 and increased waist circumference [90]. Compared with lean persons, obese adults had around seven times greater chance to have future type 2 diabetes [13]. Besides, region of fat accumulation is also associated with the disease development. Previous studies showed that visceral fat appears more predictive to insulin resistance than subcutaneous fat because of the highly diabetogenic property [91]. This indicates that individuals who have abdominal obesity have a higher tendency to have insulin resistance and type 2 diabetes when compare to others [12].

The numbers of obese persons have been dramatically growing. It was reported that approximately 35% of adults in the U.S. have BMI over 25.0 kg/m^2 in the recent decade [1]. A cross-sectional study revealed that Thais were the second-rank population having the highest average BMI among 10 Asian countries [92]. This study also supported that urban lifestyle including high-caloric intake and physical inactive are the significant leading causes of obesity in the study population [92]. The prevalence of obesity is in parallel with type 2 diabetes. More than 80% of type 2 diabetic cases worldwide were concurrently obese [51], whereas around 70% of Thai patients with type 2 diabetes were diagnosed with obesity [93].

Underlying pathways linking between obesity and insulin resistance were postulated. During obese state, numerous biological substances are produced by malfunctional adipocytes and they negatively affect insulin-target tissues, leading to insulin resistance [91]. First, overstorage of fat cells in adipose tissue contributes to FFA overproduction, which is a crucial substance disrupting insulin receptor functions and diminishing insulin sensitivity. Particularly, adipocytes in visceral fat are more likely to breakdown and release excess FFA to blood circulation subsequently [12]. Moreover, the oxidized products of FFA including diacylglycerol and ceramides are also cytotoxic. The substances induce β-cell apoptosis, impair β-cell function, and deteriorate insulin sensitivity of peripheral cells [51]. Second, obesity promotes biosynthesis of proinflammatory cytokines, for example, CRP, TNF-α, and IL-6 in adipocytes. The presences of cytokines are not only associated with chronic low-grade inflammation in obesity, but they also interrupt insulin signaling pathway and induce FFA production [51, 53]. Third, obesity-induced dysregulation of adipokines is also linked to development of insulin resistance [12]. Alterations in plasma concentration of adiponectin and leptin, the key adipokines regulating glucose and fat metabolism [91], were observed in individuals with overweight and obesity [93], resulting in the greater risk of developing insulin resistance, type 2 diabetes, as well as metabolic syndrome [94, 95].

2) Poor eating habits

Many analyses revealed a robust relationship between dietary pattern and the risks of NCDs, particularly in individuals who have metabolic disturbance at baseline

[14]. Excess carbohydrate and fat consumption contribute to obesity and impaired glucose metabolism. Dietary carbohydrates directly cause postprandial hyperglycemia, which activates insulin output by β cells in order to keep blood glucose in the normal range. Repeated stimulation of insulin secretion by chronic high carbohydrate intake accelerates β cell exhaustion [15]. Meanwhile, high-fat diets result in high lipid accumulation, especially TG, in peripheral cells. Lipolysis of TG by lipoprotein lipase eventually produces excess FFA that diminishes insulin signaling pathways as described in the previous session [12].

In general, the relatively high quantity of carbohydrate consumption, especially white rice, was found in Asian populations [43]. The average white rice consumption were three to four servings daily in Asians and one to two servings weekly in Westerns [17]. Evidence showed that high consumption of white rice was associated with the increased risk for type 2 diabetes with the relative risk (RR) 1.55 (95%CI: 1.20-2.01), especially in Asians [17]. Also, white rice consumption may induce the occurrence of metabolic syndrome with the pooled RR 1.44 (95%CI: 1.10-1.90; p = 0.02) [96]. It was proposed that the high glycemic index of white rice could lead to excessive compensatory hyperinsulinemia and lipogenesis, resulting in the development of insulin resistance [96]. Moreover, due to the transitions of socioeconomic factors among Asian countries in recent decades, the typical Asian diets are affected by the Western food styles, characterized by the increased proportion of processed meat, high-fat dairy products, and oils consumption [19]. According to the eating habits simultaneously with genetic susceptibility of Asians, it contributed to the higher severity of postprandial hyperglycemia and impaired glucose metabolism as observed in Asians, especially Southeast Asians, in relative to people in other regions [19, 43].

3) Sedentary lifestyle

Relationship of sedentary lifestyle and the development of type 2 diabetes was revealed in epidemiological studies. The incidence of type 2 diabetes was considerably higher among persons with sedentary lifestyle and lack of frequent exercise than those with higher exercise level [97-99]. It was also evident that

urbanization and economic growth are the major contributor to have less physically active in Asian population to date [19].

2.1.6 Management

Regarding scope of the present study, management for persons with prediabetes and untreated, newly diagnosed patients with type 2 diabetes who have FPG < 180 mg/dL and A1C < 8.0% were hereby reviewed.

1) Screening

American Diabetes Association (ADA) [6] suggests consideration of screening test for prediabetes and type 2 diabetes in asymptomatic adults who meet the following criteria.

(i) Persons age ≥ 45 years

(ii) Persons at any ages who have BMI ≥ 25 kg/m^2 or BMI ≥ 23 kg/m^2 (Asian Americans) together with at least one of the risk factors as followed;

- First-degree relative with diabetes
- African American, Latino, Native American, Asian American, Pacific Islander
- History of cardiovascular diseases
- Hypertension (defined by blood pressure ≥ 140/90 mmHg or treated with antihypertensive drug)
- HDL-C < 35 mg/dL and/or TG > 250 mg/dL
- Women with polycystic ovary syndrome and/or have history of gestational diabetes
- Physical inactivity
- Medical conditions that associated with insulin resistance i.e. severe obesity and acanthosis nigricans

Consistently, the Diabetes Association of Thailand [100] recommends using a risk score model to predict individual diabetic risk in the next 12 years for Thai

population. Screening test are strongly recommended for persons who have the score ≥ 6, indicating the high risk state for type 2 diabetes [101]. The test should be performed every three years or more frequently as needed in normal persons. Meanwhile, at least annual monitoring for the disease progression should be considered afterwards in persons with prediabetes. Levels of FPG, 2-hour PPG following 75-g OGTT, and A1C are equally appropriate to be used as glycemic parameters for diagnosis [6].

2) Diabetes self-management education and support (DSMES)

At the time of diagnosis, individuals with prediabetes and type 2 diabetes should be received DSMES, which is a fundamental element for diabetes care aiming to provide knowledge, skills, and ability of self-management of the disease as well as the related conditions for persons at high risk and known cases of diabetes [6]. It is an effective tool for improving health behaviors, individual goal-setting for glycemic control, self-monitoring blood glucose, and prevention and delay the disease progression [6, 100]. Previous report showed that DSMES can reduce A1C up to 1% [102]. The DSMES program is required specialized skills of health care professionals (i.e. physician, nurse, registered dietitian, and pharmacist) to educate and provide a patient-centered consultation to the patients [102].

3) Nutrition therapy

Nutrition therapy is an essential component of lifestyle intervention. Cornerstone of nutrition therapy includes reducing caloric intake and improving eating patterns in order to promote and maintain weight loss in overweight and obese persons, optimize glycemic control, and lessen cardiovascular risk [102]. It was evident that nutrition therapy by registered dietitian can result in the decrease in A1C by 0.5-2.0% [100]. Patient education and counseling should be based on individual metabolic profiles, personal preference, and cultural to reinforce the long term adherence [100]. Nutritional recommendations for the high-risk population as well as patients with type 2 diabetes are described below [103].

(i) Macronutrients (carbohydrate, protein, and fat) distribution: an ideal percentage of calories from macronutrients remains inconclusive. The optimal amount

of macronutrients intake should be based on eating habits, preferences, and metabolic goals.

(ii) Carbohydrate: increase consumption of fiber-rich foods (i.e. vegetables, fruits, legumes, and unprocessed grains), avoid sugar-sweetened beverages, and minimize the added sugar in foods

(iii) Protein: increase good protein sources (i.e. lean animal, tofu, and legumes), decrease fat dairy products, and avoid carbohydrate source with high protein content because the food can provoke insulin response without causing elevated PPG.

(iv) Fat: increase a Mediterranean-style diet rich in monounsaturated- and polyunsaturated fats, and long chain n-3 fatty acid, and avoid trans-fat and limit intake of processed- and fast food, red meat, and full-fat dairy products

(v) Micronutrients: roles of vitamins and minerals on glycemic control and prevention of cardiovascular diseases are unclear

(vi) Alcohol: no more than one drink per day for adult women and no more than two drinks per day for adult men.

In addition, benefits of restriction of carbohydrate intake on endogenous glucose metabolism have been demonstrated. The reduced carbohydrate consumption attenuated hyperglycemia, contributing to the positive impact on the management of type 2 diabetes [15, 104]. Evidence showed that dietary carbohydrate restriction is the most effective approach to improve glycemic control [15].

4) Physical activity

Physical activity is emphasized concurrently with nutritional therapy in order to balance between energy expenditure and caloric input. It is also one of the fundamentals of weight reduction and glycemic control management [7]. Evidences strongly suggested that sedentary lifestyle (i.e. prolonged sitting) should be minimized in all adults [7, 105, 106]. Moderate-to-vigorous intensity aerobic activity at least 150 min/week, distributing at least three days/week with no more than two consecutive days without activity or resistance exercises at least twice a week on nonconsecutive

days is recommended [5]. Previous meta-analysis of 16 randomized controlled trials (RCTs) supported that aerobic exercises and resistance exercises significantly lowered A1C by 0.66% ($p < 0.001$) in the intervention group when compared with the control group [107].

Benefits of increased physical activity on insulin resistance were proposed. Activities of adenosine monophosphate-activated protein kinase (AMPK)/GLUT-4 signaling pathway were enhanced by exercise, contributing to the improvement in glucose uptake in skeletal muscle [108, 109]. Moreover, fatty acids oxidation in insulin-target tissues was accelerated [110]. Favorable mechanisms were maintained for about 60 hours and returns to pre-exercise levels three to five days afterwards. Moreover, repeated moderate intensity exercise was capable of extending insulin sensitivity profile. Thus, it was proposed that repeating exercise within a time interval of 48 to 60 hours may help control blood glucose levels over the long term [111].

Treatment regimen for persons who are diagnosed with prediabetes and type 2 diabetes (only patients with FPG < 180 mg/dL and A1C < 8.0%) is presented in Figure 3. Lifestyle modification is established as the fundamental therapeutic approach regardless of the disease severity [6, 7] because it is strongly effective, easily affordable and cost-saving. Evidences also showed that clinical efficacy of lifestyle modification was superior to medications [10, 112, 113].

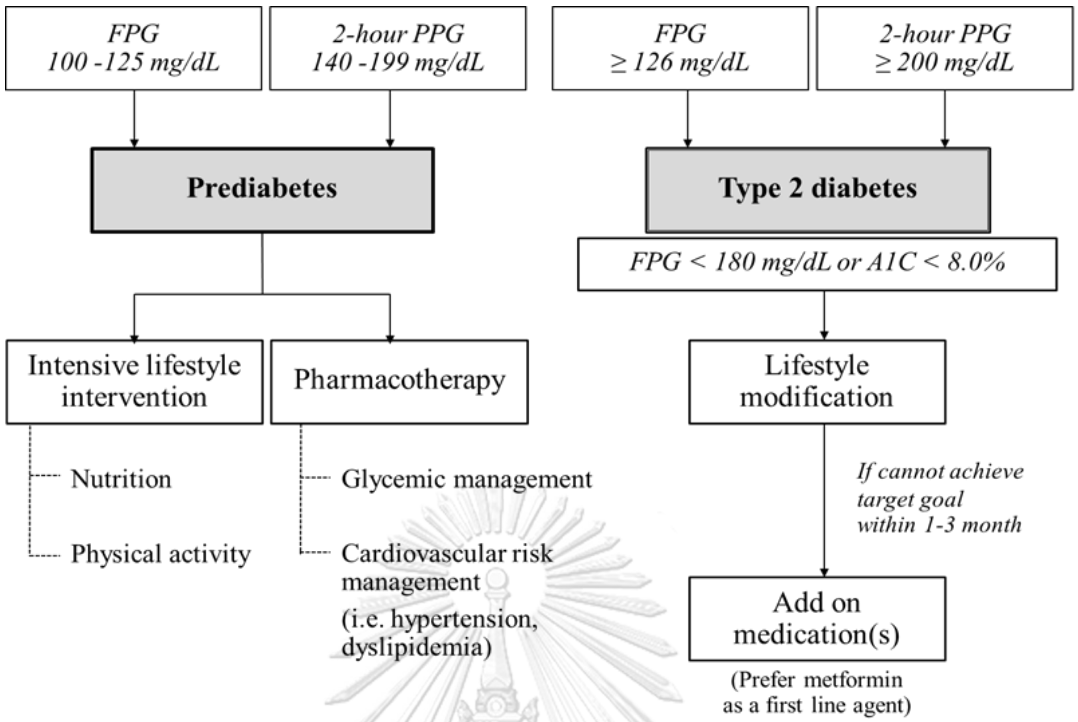


Figure 3 Treatment regimen for prediabetes and newly diagnosed type 2 diabetes (FPG: fasting plasma glucose; PPG: postprandial plasma glucose; A1C: glycated hemoglobin)

5) Management of prediabetes

Patients with prediabetes should be referred to intensive lifestyle intervention according to the Diabetes Prevention Program (DPP) study [10]. The following interventions are recommended to lessen diabetic risk in prediabetic persons [6].

(i) Healthy low-caloric eating patterns

(ii) At least 7% weight reduction of initial body weight and maintenance in obese persons

(iii) A minimum of 150 min a week of moderate-intensity physical activity (i.e. brisk walking), distributing at least three days/week with no more than two consecutive days without activity

Efficacy of pharmacotherapy on the prevention of type 2 diabetes and cardiovascular events in the high-risk population is well established. Purpose of pharmacotherapy is to optimize glycemic control. In addition, known cases of dyslipidemia and hypertension should be concurrently treated to modify cardiovascular risks. The ADA [6, 7] also suggests the consideration of metformin in specific population with prediabetes, including persons age older than 60 years, obese persons who have BMI $\geq$ 35 kg/m^2, and women with a history of gestational diabetes according to the findings of DPP study [10].

Importance of early detection and management of prediabetes on prevention and delay the onset of type 2 diabetes is well documented. Due to the disease pathophysiology, ultimate goals of the disease management are to improve insulin sensitivity and preserve β-cell function by correcting the modifiable risk factors [9]. Lifestyle modification [10, 11, 114-116] and medications [10, 20, 21, 112, 113, 117-119] provided favorable effects on the improvement in insulin sensitivity and the reduction in diabetic risk among persons who had prediabetes as summarized in Table 3.

Table 3 Efficacy of lifestyle modification and medications on the management of prediabetes

Interventions	Study duration	Participants (N)	Diabetes risk reduction [a]	References
- Diet control - Increase leisure physical activity (exercise) - Diet control plus exercise	6 years	Persons with IGT (577)	- 31% for diet control - 46% for exercise - 42% for diet control plus exercise	Da Qing study: Pan *et al.*, 1997 [114]
Intensive lifestyle intervention: ≥ 5% weight reduction, caloric restriction, and moderate-intensity physical activity ≥ 30 min/day	3.2 years	Obese persons with IGT (522)	58%	Finnish Diabetes Prevention study: Tuomilehto *et al.*, 2001 [11]
Intensive lifestyle intervention: low-caloric and low-fat diet to reduce and maintain ≥ 7% of the initial body weight and moderate-intensity physical activity for at least 150 min/week	2.8 years	Obese persons with FPG 95-125 mg/dL and/or 2-hour PPG 140-199 mg/dL (3,234)	58%	US DPP study: DPP research group, 2002 [120]
Metformin	2.8 years	Obese persons with FPG 95-125 mg/dL and/or 2-hour PPG 140-199 mg/dL (3,234)	31%	US DPP study: DPP research group, 2002 [120]

Table 3 Efficacy of lifestyle modification and medications on the management of prediabetes (cont.)

Interventions	**Study duration**	**Participants (N)**	**Diabetes risk reduction** [a]	**References**
Metformin	2.5 years	Obese persons with IGT (531)	26%	Indian DPP study: Ramachandran *et al.*, 2006 [112]
Pioglitazone	2.4 years	Persons with IGT (441)	70%	ACT NOW study: DeFronzo *et al.*, 2013 [121]
Rosiglitazone	3 years	Persons with IFG and/or IGT (4,999)	55%	DREAM study: Gerstein *et al.*, 2006 [117]
Acarbose	3.3 years	Persons with IGT (412)	25%	STOP-NIDDM study: Chiasson *et al.*, 2002 [20]
Voglibose	48 weeks	Persons with IGT (1,780)	40%	Kawamori *et al.*, 2009 [21]
Orlistat	4 years	Obese persons with IGT (3,305)	37%	XENDOS study: Torgerson *et al.*, 2004 [118]
Lorcaserin	1 year	Overweight and obese person (4,008)	38%	BLOSSOM: Fidler *et al.*, 2011 [119]

[a] compared with control group

FPG: fasting plasma glucose; PPG: postprandial glucose; IFG: impaired fasting glucose; IGT; impaired glucose tolerance

Limitations of lifestyle modification and pharmacotherapy for the management of prediabetes are addressed. Long term maintenance and good adherence to lifestyle changes remains challenging in real life setting. Meanwhile, therapeutic efficacy of medications is not durable after discontinuation. In addition, cost-effectiveness and severe drug adverse events limit the medication uses in clinical practice nowadays [2].

6) Management of newly diagnosed patients with type 2 diabetes

In newly diagnosed patients with type 2 diabetes who have FPG < 180 mg/dL, A1C < 8.0%, and without the significant hyperglycemia signs and symptoms, lifestyle intervention is recommended as the primary strategy. Pharmacotherapy is considered when the patients fail to response to lifestyle modification alone [6, 7].

2.2 Postprandial hyperglycemia

Postprandial hyperglycemia refers to an increase in blood glucose level after meals, defined by the 2-hour PPG ≥ 140 mg/dL [122]. Dietary carbohydrates are digested into monosaccharaides and absorbed through gastrointestinal brush-border membrane [122]. In non-diabetic persons, blood glucose elevates and reaches the peak level around 30 to 60 min after carbohydrate ingestion, generally not more than 140 mg/dL, and returns to the basal level within two to three hours [123].

2.2.1 Roles of postprandial hyperglycemia in the development of type 2 diabetes and cardiovascular diseases

Postprandial hyperglycemia is an important manifestation in prediabetes and early-phase type 2 diabetes [122]. In addition, it potentiates the risk of developing cardiovascular events and diabetic complications [25, 28]. Role of postprandial hyperglycemia in the development of type 2 diabetes and cardiovascular diseases is shown in Figure 4. Blood glucose raising, especially after consumption of carbohydrate-rich diets and high glycemic index carbohydrate, activates excessive insulin production and secretion in order to maintain normoglycemic state [8]. Chronic hyperinsulinemia aggravates impairments in insulin sensitivity and β-cell

function. When compensatory processes no longer overcome persistent hyperglycemia, type 2 diabetes can be diagnosed [8, 9]. Elevated PPG results in the progression of cardiovascular diseases via independent mechanisms. Postprandial hyperglycemia induces free radical production, diminishes vasodilating effect of nitric oxide, and interferes coagulation processes, eventually leading to endothelial dysfunction and atherosclerosis [24, 124].

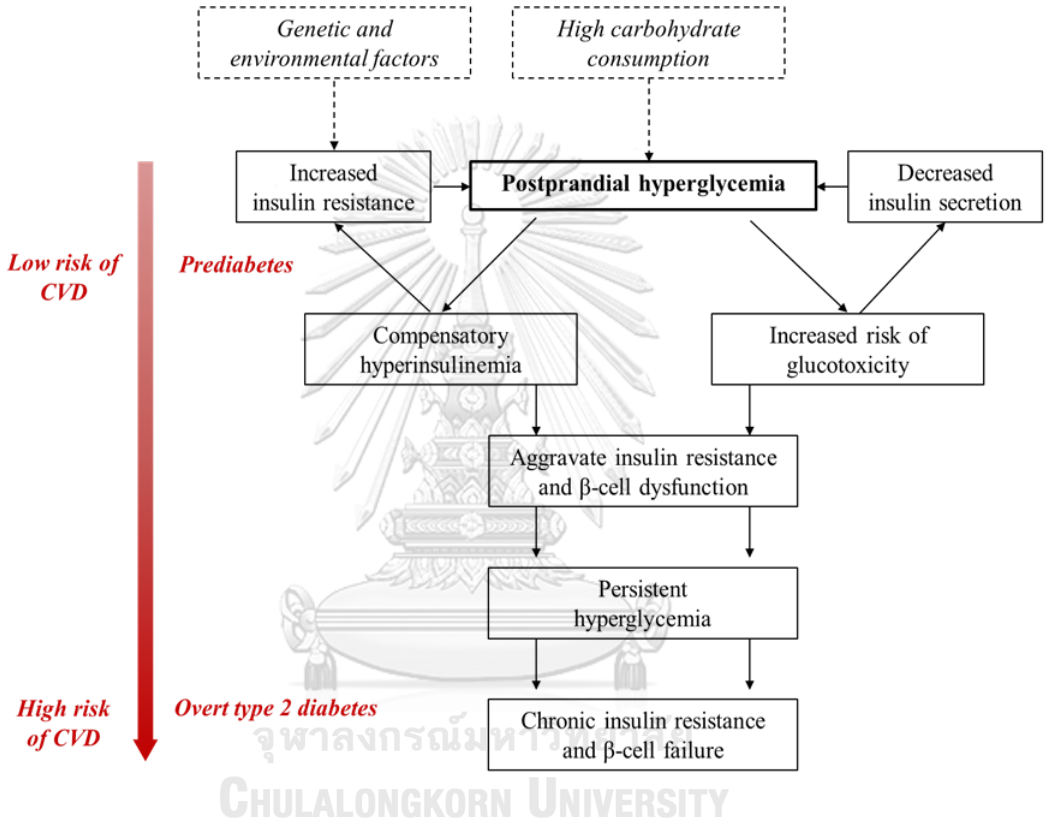


Figure 4 Roles of postprandial hyperglycemia in the development of type 2 diabetes and cardiovascular diseases(CVD: cardiovascular disease)

Previous clinical data supported that PPG-related variables including PPG at individual time points, 2-hour PPG, and area under the curve (AUC) of glucose were markedly correlated with atherosclerotic events [125]. These variables also showed the stronger correlation with the risk for atherosclerosis when compared with FPG and A1C [125]. The peak glucose during postprandial state possessed the most robust link with oxidative stress production, leading to the undesired consequences in cardiovascular system [25]. Epidemiological studies also found that chronic postprandial hyperglycemia promotes the risk for macrovascular complications and

related mortality [23, 25, 126]. This condition is more robustly related to cardiovascular diseases in relative to fasting hyperglycemia [127].

2.2.2 Factors influencing postprandial hyperglycemia

Excursion of postprandial glucose is influenced by internal and external determinants. First, beyond intestinal digestion and absorption, a rising in PPG involves physiological capacities of insulin and glucagon secretion, hepatic glucose synthesis, and glucose uptake by peripheral tissues [9] . Individuals who have any defects in glucose metabolism regulation could have abnormal postprandial glucose excursion, for example, the higher and delay in glucose peak, and more prolonged hyperglycemia, in relative to non-diabetic persons [123]. Second, diets influence postprandial hyperglycemia. High carbohydrate meals and high glycemic index carbohydrates aggravate the great magnitude of hyperglycemia [128], especially in persons who have baseline impaired glucose metabolism [14]. Complexity of diet composition also results in variation in glycemic responses. The mixed diets containing complex carbohydrates, fats, and proteins provoke the higher degree of postprandial hyperglycemia than glucose consumption alone [25]. Moreover, solid- and liquid meals affect glycemic excursion in the different ways regarding the time remaining in gastrointestinal tract. A longer gastric emptying time was observed after eating solid meals than liquid meals matched for volume and energy content [129]. For these reasons, eating habits also play an important role affecting patterns of postprandial excursion. Third, there is ethnic differences in the underlying pathophysiology that leads to the diversity of glycemic responses [19]. Southeast Asians had the highest PPG and the lowest insulin sensitivity in response to the standard carbohydrate load when compared with South Asians, East Asians, European Caucasians, and Arabic Caucasians who were matched for age, BMI, and waist circumference [19, 44].

2.2.3 Conventional α-glucosidase inhibitors in management of postprandial hyperglycemia

Scientific data suggested potentials of synthetic α-glucosidase inhibitors, including acarbose, voglibose, and miglitol, in the early management of type 2 diabetes. With respect to the local effect of α-glucosidase inhibitors against α-glucosidase enzymes along brush border of small intestine, the agents directly alleviate postprandial hyperglycemia [130]. A recent meta-analysis revealed that α-glucosidase inhibitors reduced 1-hour PPG by -2.16 mmol/L (95%CI: -3.37 to -0.95) and 2-hour PPG by -3.00 mmol/L (95%CI: -3.58 to -2.42) in comparison with placebo in Asian population [27]. Efficacy of the medications on PPG suppression in Asians and non-Asians was comparable [27]. Consistently, therapeutic effects of conventional α-glucosidase inhibitors on glycemic indices among patients with type 2 diabetes was reported in a large meta-analysis of 41 clinical trials [131]. The results showed that α-glucosidase inhibitors dose-dependently reduced 1-hour postload glucose (2.32 mmol/L; 95%CI 1.92-2.73 mmol/L for acarbose) and FPG (1.09 mmol/L; 95%CI: 0.83-1.06 mmol/L for acarbose and 0.52 mmol/L; 95%CI: 0.16-0.88 mmol/L for miglitol). On the other hand, these medications lowered the level of A1C (0.77%; 95%CI: 0.64-0.90% for acarbose and 0.68%; 95%CI: 0.44-0.93% for miglitol) without the dose-response relationship. Subgroup analysis further found the greater reduction in A1C in individuals with the higher baseline A1C level. In comparison with the 12-24 week- clinical trials, the less reduction in A1C was seen in the over 24-week study period. In addition, acarbose treatment led to a decrease in BMI by 0.17 kg/m^2 (95%CI: 0.08-0.26 kg/m^2), but no reduction in body weight was observed. The study also showed that there was no significant effect on fasting insulin, plasma lipids, and diabetes-related morbidity and mortality [131].

Management of postprandial hyperglycemia reduced the risk for cardiovascular events, including coronary heart disease, cardiovascular death, congestive heart failure, cerebrovascular event, and peripheral vascular disease [123]. Numerous RCTs showed beneficial effects of α-glucosidase inhibitors on cardiovascular events beyond glucose-lowering effect. A 49% reduction (HR 0.51; 95%CI: 0.28-0.95, p = 0.03) in cardiovascular events was a secondary result from

acarbose treatment over a mean follow-up of 3.3 years in persons with IGT (N = 1,429) [132]. A meta-analysis of seven double-blind, placebo-controlled trials (N = 2,180) consistently demonstrated that acarbose decreased the risk for cardiovascular events by 35% in patients with type 2 diabetes [26]. Delay in the progression of intima-media thickness were also reported among those with IGT treated with acarbose (N = 132) [133]. Moreover, when the presences of stroke, coronary revascularization, and angina were additionally included into the analysis, acarbose decreased these cardiovascular events by 35% [133]. It was postulated that the favorable effect of acarbose resulted from the reductions in body weight, BMI, and TG [26]. Meanwhile, a recent randomized, double-blind, placebo-controlled study reported that acarbose reduced the progression to type 2 diabetes by 18% in patients with coronary heart disease who had IGT (N = 6,522); however, no significant benefit on the overall cardiovascular events was observed in this study [134].

2.3 Evaluation of glycemic control and insulin resistance

2.3.1 Blood glucose

FPG, 2-hour PPG following 75-g OGTT, and A1C are considered the three conventional standard glycemic indices for diagnosis of diabetes and monitoring glycemic control [6, 7]. All of the three conventional tests are effective for evaluating blood glucose in the different contexts and purposes. Investigation of blood glucose is minimally invasive. Many quantitative assays are reliable, easily accessible, and well standardized in general laboratories at present [135].

1) FPG

FPG is related to hepatic glucose efflux because blood glucose is mainly under the regulation of liver cells during the fasting state [9]. It is the most common glucose measurement in routine practice due to its simplicity and inexpensiveness. An 8-hour overnight fast is required for the testing. A great day-to-day variation of FPG was evident. The level of FPG could be affected by stress, diurnal variation, diets, and

medications (i.e. corticosteroids, second-generation antipsychotics, thiazide diuretics, and β-blockers) [136].

2) A1C

A1C is well established as the gold standard marker for evaluating long-term glycemic control [6, 7, 105]. Since A1C is the amount of glucose molecules attached to hemoglobin in red blood cells, it reflects the average concentration of glucose over the past two to three months [135]. In other words, A1C is an integration of blood glucose in both fasting- and postprandial states. As a result, normalizations of FPG and PPG are the key components for achieving the goal of A1C and optimizing glycemic control [123].

Evidences showed that A1C is also a robust predictor for diabetic complications [135]. The measurement of A1C is convenient and there is no need for fasting blood sample [6]. However, its cost is considerably higher than FPG. Accuracy of A1C analysis is influenced by abnormal metabolism of red blood cells such as hemolytic anemia, iron deficiency, and renal dysfunction [135]. It was also found that the measurement of A1C appears unreliable in the conditions with rapid fluctuation of blood glucose level [137].

3) PPG following a standard OGTT

A standard OGTT refers to a test in which 75-g glucose is orally administered and blood glucose is monitored afterwards over the 2-hour period (i.e. at 30, 60, 90, and 120 min) [6]. The result directly reflects patterns of postprandial glucose excursion in response to glucose load. It also reveals the values of PPG at individual time points of blood collection, AUC, glucose peak, and time observed glucose peak [125]. The 2-hour PPG is one of the standard glycemic marker for diagnosis of type 2 diabetes [138, 139] and it is also the most robust predictor for atherosclerotic events when compared with the other glycemic indices [125]. Furthermore, the test is performed to determine glucose tolerance of insulin-target cells, predominantly of skeletal muscle [138]. Glucose tolerance is the ability of peripheral cells to maintain normoglycemia by absorption circulating glucose into the cells when blood glucose elevates during postprandial state [9].

The OGTT is set after at least 8-hour overnight fast [123]. The test is rarely performed for the diagnostic purpose in general population because of practical considerations. Patient preparations including restriction of carbohydrate consumption and heavy exercise few days before the test are required [122]. The procedure for measuring 2-hour PPG is also time-consuming. In addition, there is a high within-person variation [137]. However, the test has been used in many scientific researches for determining the existence of IGT [139].

2.3.2 Insulin resistance

Aims of the assessment of insulin resistance are to determine the initial presentation of type 2 diabetes and to screen the risk factor for metabolic syndromes [140]. At present, the test is not common for diagnostic purpose, on the other hand, it is mainly performed in human studies [138].

Hyperinsulinemic euglycemic glucose clamp is the gold standard method for assessing the whole body insulin resistance [141]. However, the method is impractical in the large-scale studies because of its labor, time-consuming, and expensiveness [138]. Surrogate markers were therefore developed and validated to overcome these limitations.

1) Fasting plasma insulin

Fasting plasma insulin (FPI) represents plasma concentration of insulin produced by β-cells during the basal state [139]. Fasting specimen is required for the assay. It is the simplest surrogate marker for evaluating insulin resistance, but assessment of insulin resistance using FPI should be in caution regarding pathophysiological heterogeneity of type 2 diabetes [138].

An increased FPI, also called as hyperinsulinemia, is the initial manifestation of insulin resistance and commonly found in individuals with prediabetes and the early-phase type 2 diabetes [8, 9]. Hyperinsulinemia represents the β-cell compensation to the resistant insulin-target cells by increasing insulin production and secretion [9]. After that, the level of insulin continuously declines over the period of

diabetes progression according to β-cell degeneration and dysfunction. Absolute loss of insulin secretion is eventually found in the late phase of type 2 diabetes [138].

Previous evidences found that the level of FPI is considerably varied among studies. The concentration of FPI in high-risk populations for type 2 diabetes was reported. Choi *et al.* [142] and Gaddam *et al.* [143] showed that the mean FPI in individuals who met the criteria of prediabetes and mild-stage type 2 diabetes was approximately 11.6 ± 5.3 and 11.2 ± 3.7 μIU/mL, respectively. Meanwhile, a study of Chuengsamarn et al. [144], which was conducted in Thai people, similarly found that the value of FPI was 15.84 ± 6.19 μIU/mL in prediabetic persons.

2) Homeostasis model assessment

Another surrogate marker for insulin resistance assessment widely used in research is the homeostasis model assessment of insulin resistance (HOMA-IR) because of its simplicity and being minimally invasive [139]. Previous data also suggested a strong correlation between HOMA-IR and the hyperinsulinemic euglycemic glucose clamp method (correlation coefficient; $r = 0.88$, $p < 0.0001$) [145, 146]. The HOMA-IR is also a predictor for cardiometabolic risks [145]. The value of HOMA-IR positively correlated with BMI, body fat percentage, waist circumference, and TG and inversely correlated with HDL-C [90, 147]. Moreover, individuals with the HOMA-IR values in the highest quartile had the greatest risks of hypertriglyceridemia, low HDL-C, and hypertension [147].

The HOMA refers to a model describing the relationship between glucose and insulin in the basal state, representing the balance between hepatic glucose efflux and pancreatic insulin secretion [140]. A mathematic equation relies on FPG (in mg/dL) and FPI (in μIU/mL) is used to quantify the degree of insulin resistance as follow: HOMA-IR = (FPG x FPI)/450 [140]. The output of the model is calibrated to give normal insulin resistance of 1 [145].

The higher value of HOMA-IR indicates the greater degree of insulin resistance [138, 147]. The HOMA-IR ≥ 2.5 was initially suggested as the cut-off point for determining insulin resistance in general population in the study of Matthews *et al.* [140]. However, differences in the HOMA-IR level among populations were reported.

The HOMA-IR of 3.16 was used for pubertal obese children and adolescents [148], whereas the value of 2.0 or over was used for young women with polycystic ovarian [149]. In addition, there is a large variation in the proposed cut-off values of HOMA-IR among different race and ethics groups. Previous studies showed that the cut-off points of HOMA-IR for diagnosis of insulin resistance were > 3.80 for Hispanic [150], ≥ 2.5 for Japanese [151], and ≥ 2.0 for Chinese [152]. In Thai population, the study of Do *et al.* [147] proposed the HOMA-IR ≥ 1.56 and ≥ 1.64 for men and women for classifying insulin resistance and estimating the prevalence of insulin resistance in Thai adults aged over 35 years. The results additionally reported approximately 25.1% of men and 21.5% of women had insulin resistance [147]. In prediabetic persons and early, non-treated type 2 diabetic patients, the approximate HOMA-IR were in the range of 2.70-4.03 [142-144].

2.4 Determination of protein expression using proteomic analysis

2.4.1 Overview of proteomic analysis

The term “proteomics” is a combination of “proteome” and “omics”. Proteome is the total set of proteins produced and regulated by living organisms, whereas omics refers to an analysis of large amounts of data, specifically in biological field [153]. Proteomics is a high-throughput bioinformatics technology for large-scale analysis of proteins [154, 155]. The objective of proteomics is to determine characteristics of the entire set of proteins, in terms of identity, structure, expression, function, and protein-protein interaction network [153, 154]. Rationale of proteomics is that protein is the endpoint component responsible for cell phenotypes and biological functions [156]; therefore, expression of proteins reflects the ongoing processes at the cellular level in response to the circumstances that the cell experiences [156, 157]. Furthermore, the technique is capable of determining the large number of proteins at the same time [155]. In other words, proteomics reveals the comprehensive view of protein profile in a specific condition at the specific time point.

Regarding the purposes of investigation, proteomics can be categorized into (a) structural proteomics, (b) expression proteomics, and (c) functional proteomics [153]. The latter two fields are considerably important in medical and pharmaceutical areas. Expression proteomics distinguishes the protein expression profile in relation to the different conditions, for example, disease *vs.* non-disease and before treatment *vs.* after treatment. In addition to the patterns of protein dysregulation (over- and down-expression), the analysis reveals the expression level of proteins as quantitative data [158]. Meanwhile, functional proteomics explores the activities of proteins in specific pathways. The findings expose a relationship between proteins and disease mechanism [154, 158]. As a result, proteomics is useful in clinical implementation to help understanding pathophysiology of the disease of interest at the molecular level and to uncover disease-associated proteins for screening and predicting the risk of disease [153, 154]. Moreover, the protein markers may have potential for developing drug targets and evaluating response to the assigned treatment [159].

The main procedures of proteomic analysis include sample collection and preparation, protein separation and digestion, protein identification, and quantitative determination of protein expression level. First, protein samples are harvested from cells, tissues, and biological fluids. Selection of the sample source is normally performed based on relevance and specificity to the disease pathophysiology [159]. Next, due to complexity of biological samples, it is necessary to separate individual proteins from the complex mixture before the identification process [158]. For the procedure of protein separation, gel-based techniques (i.e. one- and two-dimensional gel electrophoresis) were widely used in the early era of proteomics; however, many limitations have been noted in previous data. Subsequently, the gel-free techniques (i.e. liquid chromatography (LC) and high-performance liquid chromatography (HPLC)) have been applied in the recent studies [158, 160]. After separation, tryptic digestion is performed to digest proteins to peptides. The unique proteins are then subjected to mass spectrometer (MS). To identify the selected molecules, the mass to charge ratios (m/z) of the peptides are matched to the known proteins existing in the database. Then, quantification of protein abundance or expression level is further investigated [158].

2.4.2 Rationales of proteomic analysis in diabetic research

With respect to the ability of reflecting the global view of proteome, proteomics has been implemented in biomedical research, especially in the complex disease with multiple etiologies including cancer, Alzheimer's disease, and diabetes [154, 158]. As described, the development of insulin resistance and type 2 diabetes is complicated involving multiple organ dysfunction, primarily in adipose tissue, liver, skeletal muscle, and pancreatic β-cells. A huge diversity of proteins therefore plays the distinct roles in the molecular mechanisms underlying these conditions [159]. Disease-associated markers are the cornerstone of diabetes care [135]. In general, the measurement of FPG, PPG, and A1C are performed as the standard tools for diagnosis and management of type 2 diabetes due to accessibility, simplicity, and cost-effectiveness [135]. Nonetheless, the significant shortcoming of the glucose-based parameters should be addressed. It is important to note that metabolic dysregulation develops in the specific cells over time although the blood glucose concentration remains in the normal range because of the silent progression of prediabetes and the early-stage type 2 diabetes [9].

For these reasons, there have been the great interests in molecular biology approaches, including genomics, transcriptomics, and proteomics, in order to gain the insight knowledge of cellular modification [155]. Of these, proteomics seems superior to the others. Protein is the final modified product passing through multiple cellular processes; hence, it reflects the real-time status of the disease better than its encoding gene and upstream molecules [157, 161].

2.4.3 Implementation of proteomics in clinical research of type 2 diabetes

Proteomics has been implemented in numerous studies among type 2 diabetic patients as well as the high-risk populations. Characteristics of the studies that included in this review are demonstrated in Table 4. The majority of existing studies aimed to explore protein profiles among the specific population of interest, whereas few studies investigated changes in protein expression in response to the given treatment.

Table 4 Characteristics of clinical studies of type 2 diabetes using proteomic analysis

Purposes of study	Design	Participants (N)	Samples	References
To characterize and identify differentially expressed proteins	Cross-sectional study	- Healthy controls (12) - Individuals with type 2 diabetes with FPG 11.6 ± 1.9 mM (8)	Erythrocyte membrane	Jiang M, *et al.*, 2003 [162]
To characterize and identify differentially expressed proteins	Cross-sectional study	- Individuals with NGT (3) - Individuals with type 2 diabetes based on OGTT (the WHO criteria) (3)	Serum	Sundsten T, *et al.*, 2006 [163]
To characterize and identify differentially expressed proteins	Cross-sectional study	- Healthy controls (50) - Individuals with type 2 diabetes based on the ADA criteria (125)	Serum	Riaz S, *et al.*, 2010 [164]
To characterize and identify differentially expressed proteins	Cross-sectional study	- Non-diabetic controls (mean age 67.6 ± 1.67 years) - Individuals with type 2 diabetes (mean age 67 ± 1.71 years)	Serum	Li RX, *et al.*, 2008 [165]

Table 4 Characteristics of clinical studies of type 2 diabetes using proteomic analysis (cont.)

Purposes of study	Design	Participants (N)	Samples	References
To characterize and identify differentially expressed proteins To determine influences of genetics on protein expression	Cross-sectional study	- Individuals with NGT (20): - NGT with no family history of diabetes (10) - NGT with family history of diabetes (10) - Individuals with IGT (20) - Individuals with type 2 diabetes (20): - type 2 diabetes with no family history of diabetes (10) - type 2 diabetes with family history of diabetes (10)	Serum	Sundsten Y, *et al.*, 2008 [166]
To characterize and identify differentially expressed proteins To investigate variations of protein expression regarding the severity of β-cell dysfunction	Cross-sectional study	- Individuals with NGT (10) - Individuals with type 2 diabetes with high EIR (10) - Individuals with type 2 diabetes with low EIR (10)	Plasma	Sundsten Y, *et al.*, 2008 [167]

Table 4 Characteristics of clinical studies of type 2 diabetes using proteomic analysis (cont.)

Purposes of study	Design	Participants (N)	Samples	References
To characterize and identify differentially expressed proteins	Cross-sectional study	- Healthy controls (29) - Individuals with type 2 diabetes (28)	Urine	Chu L, *et al.*, 2013 [168]
To characterize and identify differentially expressed proteins	Cross-sectional study	- Clinically healthy controls (10) - Individuals with IGT (10) - Individuals with IFG + IGT (10) - Individuals with type 2 diabetes based on the ADA criteria (10)	Saliva	Rao PV, *et al.*, 2009 [169]
To characterize and identify differentially expressed proteins	Cross-sectional study	- Pre-obese, individuals with NGT (8) - Pre-obese, individuals with type 2 diabetes (8)	Visceral adipose tissue	Murri M, *et al.*, 2013 [170]
To investigate changes in protein abundance in insulin resistant individuals	Cross-sectional study	- Lean, non-diabetic controls (8) - Obese, non-diabetic controls (8) - Individuals with type 2 diabetes (8)	Skeletal muscle	Hwang H, *et al.*, 2010 [171]

Table 4 Characteristics of clinical studies of type 2 diabetes using proteomic analysis (cont.)

Purposes of study	Design	Participants (N)	Samples	References
To characterize and identify differentially expressed proteins	Cross-sectional study	- Lean, non-diabetic controls (27) - Obese, non-diabetic controls (27) - Obese, type 2 diabetes patients who were on insulin therapy (27)	Serum	Sleddering MA, *et al*., 2014 [172]
To investigate changes in protein markers after receiving the given intervention	Open-label, RCT (16 weeks)	- Obese, type 2 diabetes patients who were on insulin therapy - VLCD only (14) - VLCD + exercise (13)	Serum	Sleddering MA, *et al*., 2014 [172]

NGT: normal glucose tolerance; FPG: fasting plasma glucose; PPG: postprandial glucose; IFG: impaired fasting glucose; IGT; impaired glucose tolerance; OGTT: oral glucose tolerance test; WHO: World Health Organization; ADA: American Diabetes Association; RCT: randomized controlled trial; VLCD: very low caloric diet

The earliest study of Jiang *et al.* [162] found the different pattern of protein expression in erythrocyte of type 2 diabetic patients (n = 8) when compared with non-diabetic controls (n = 12) ($p < 0.05$). The investigation was on the basis of structural alteration in GLUT-1 aligned in erythrocyte membrane is associated with pathophysiology of diabetes. Forty-two proteins: 27 over-expressed and 15 down-expressed proteins were found in erythrocyte membrane of diabetic patients. Of these, three proteins were identified as flotillin-1, arginase, and syntaxin 1C in this study [162].

A total of 15 differentially expressed proteins were detected in serum of type 2 diabetic patients (n = 3) aged between 54 to 65 years old when compared with persons with normal glucose tolerance (NGT) at the same age (n = 3) ($p < 0.05$). Two over-expressed proteins were identified as apolipoprotein C3 and transthyretin, whereas two down-expressed proteins were albumin and transferrin in diabetic serum [163]. Due to the small number of study participants, the results could not represent much information of diabetic serum proteome.

A study of Riaz *et al*. [164] was further conducted with the larger sample size of patients with type 2 diabetes (n = 125) and healthy controls (n = 50). Identification and quantitative determination of serum proteins were performed. The expressions of apolipoprotein E, CRP, and leptin in serum of diabetic patients were higher than that of the controls, suggesting the up-regulation of these proteins. By contrast, apolipoprotein A-I was identified as the only down-expressed protein in diabetic serum [164].

Investigation of serum proteome in type 2 diabetes was also conducted in elderly. Persons aged older than 65 years with and without history of diabetes were recruited into the study of Li *et al*. [173]. A total of 1,377 serum proteins were identified, including 266 and 223 proteins that uniquely present in persons with type 2 diabetes and non-diabetic ones, respectively, whereas the rest were concurrently found in the both groups. Furthermore, there were 142 serum proteins in diabetic patients that expressed differentially when compared with the controls ($p < 0.01$). The functional analysis also suggested that the proteins were involved in complement

system, peroxisome proliferator-activated receptors (PPARs) system, and cell communication [173].

In addition, the different protein profile was observed in serum of type 2 diabetic patients who have the different genetic background as defined by the family history of diabetes [166]. In this study, a total of 13 differentially expressed proteins were reported. Among these, alteration of three proteins were relevant to genetics, including apolipoprotein C3 and albumin, whereas the other remained unknown. The finding confirmed the relationship between genetics and diabetic risk.

Blood-derived specimens have been extensively used in proteomic studies. Moreover, other biological samples, for example, urine, saliva, and adipose tissues, have been investigated to demonstrate the comprehensive view of protein profile in type 2 diabetes [154]. Chu *et al*. [168] examined urinary proteome among individuals with type 2 diabetes (n = 28) and healthy persons (n = 29). The results showed that the expressions of histidine triad nucleotide-binding protein 1 (HINT1), bifunctional aminoacyl-tRNA synthetase (EPRS), and clusterin precursor protein (CLU) were down-regulated in urine samples of diabetic persons.

Furthermore, proteomic analysis has been implemented in the studies of persons at high risk of type 2 diabetes, including prediabetic- and obese individuals. The study of Rao *et al.* [169] was conducted to distinguish the pattern of salivary proteome in type 2 diabetes from prediabetic- and non-diabetic conditions. A total of 487 proteins were identified in this work. The significant differences in protein profile were observed in diabetic saliva when compared with the other groups. In terms of function, the identified proteins were associated with several pathways, including metabolism regulation, immune response, signal transduction, development, cell organization and biogenesis, and cell motility [169]. Sundsten *et al.* [166] also discovered the differential protein expression in type 2 diabetic patients (n = 20) in comparison with those who were diagnosed with prediabetes (n = 20). The analysis found eight over-expressed and five down-expressed proteins in the diabetic samples.

In addition, protein expression in adipocytes of obese adults with and without type 2 diabetes were investigated. The study revealed the difference in adipocyte

proteins between the two groups. The 19 dysregulated proteins are associated with oxidative stress, inflammation, and metabolic process [170]. Hwang *et al.* [171] found that obese subjects (n = 8) had more impaired insulin sensitivity than lean subjects (n = 8). In accordance with the insulin sensitivity profile, protein expression in insulin-resistant skeletal muscle significantly differed from the controls

Association between obesity and type 2 diabetes was further elucidated in serum proteome in the recent study of Sleddering *et al*. [172]. The study participants consisted of obese patients with type 2 diabetes who were on insulin treatment, obese non-diabetic controls, and lean non-diabetic controls. Thirteen targeted serum proteins were identified and measured their expression level. An overexpression of complement C3 was observed in the both obese groups regardless of diabetic status, indicating that complement C3 could be an obesity-associated marker. Furthermore, apolipoprotein A-IV, apolipoprotein B-100, and fibrinogens (α, β, and γ chains) overexpressed in diabetic patients when compared to controls. Meanwhile, transthyretin expression was in the similar trend as apolipoproteins and fibrinogens; however, the protein underexpressed in type 2 diabetic group. Apolipoprotein A-IV, apolipoprotein B-100, fibrinogens, and transthyretin were proposed as diabetes-associated markers. This study also revealed another application of proteomics in clinical research. Patients with type 2 diabetes were further recruited into the 16-week prospective study and randomly assigned into two groups: (a) very low caloric diet (VLCD) only and (b) VLCD together with exercise program. Longitudinal changes in expression level of the candidate proteins were analyzed to evaluate effects of the given interventions on protein markers. At the end of study, the significant changes in expression of several proteins were observed, for example, apolipoproteins and complement C3 when compared to the baseline level ($p < 0.05$). Nonetheless, there was no difference in the intervention effects between the groups [172].

The review suggests that proteomics plays the promising roles to uncover the proteins associated with insulin resistance and type 2 diabetes. A large number of the differentially expressed proteins and their functions were discovered in previous researches. The findings are useful for advanced understanding the pathophysiology and the underlying mechanism of the disease.

2.4.4 Limitations of proteomics

Many difficulties of implementation of proteomic analysis in clinical studies have been noted.

1) Disease-based limitations

A critical challenge of proteomics is that there is a large variation in protein profiles towards disease stages and interindividual and intraindividual differences [158]. In addition, consideration of sample sources is based on the involvement in the disease pathophysiology [159]. Type 2 diabetes is a multifactorial disease associated with the dysfunction of many organs: pancreas, liver, skeletal muscle, and adipose tissue. To obtain the samples, the tissue biopsy method is required [163]; however, the procedure is relatively invasive with enhanced risks for infection and bleeding in the donors. Investigations of protein profiling and human proteome in these relevant tissues are still limited to few clinical studies.

2) Experiment-based limitations

Experiment-based limitations of proteomics are that the methods are time-consuming and expensive. Also, the high-throughput technologies, including instruments, software, and database, are not easily accessible. Lack of the standardized protocol, resulting in the low reproducibility across laboratories, remains problematic for the implementation of proteomics in general settings. Furthermore, biological samples are considerably vulnerable. Procedures for harvesting, handling, and storage of specimens are the critical step of the investigation [160]. The complexities of biological samples are evident as another major limitation of proteomics analysis. Although serum and plasma are commonly used for proteomics analysis in human studies, a large diversity of proteins in blood-derived samples is significantly problematic in general experiments. The challenge in developing the analytic tools and techniques used in proteomics is the capacity for separating individual proteins and to remove interferences, including binding proteins and lipids, from the complex mixture. Moreover, the concentrations of circulating proteins vary widely from picograms to milligrams [160]. The limit of detection of the tools is a significant concern for accurate findings.

2.5 Evidence-based medicinal plants in the management of prediabetes and type 2 diabetes

A large number of medicinal plants with antidiabetic effects have been used in traditional medicine for many decades. The plants have been also received much attention in scientific researches in order to explore novel agents with antidiabetic potentials at present. It was proposed that medicinal plants could be effective for the management of type 2 diabetes via several pathways, including lowering blood glucose, improving insulin resistance, restoration of β-cell function, and delay disease progression.

2.5.1 Efficacy of medicinal plants with antidiabetic effects in clinical studies

1) Cinnamon (Cinnamomum spp.)

The recent meta-analysis of 10 RCTs showed that daily supplementation with 120 to 6,000 mg of cinnamon extract for 4 - 18 weeks significantly decreased FPG by -24.59 mg/dL (95% CI: -40.52 to -8.67 mg/dL) [174]. A 12-week supplementation of 1,000 mg of cinnamon was also effective in diabetic patients with poorly glycemic control. FPG significantly reduced by -17.4% ($p < 0.001$) compared with the baseline level and placebo [175]. In addition, a single-dose administration of 1,000 mg of cinnamon extract ameliorated postprandial hyperglycemia. The $AUC_{0\text{-}60\ min}$ of glucose significantly decreased by -21.2% ($p < 0.05$) after receiving intervention [176]. Preclinical studies reported that antidiabetic effects of cinnamon were the result of polyphenol compounds that activated PPARs expression [177] and enhanced GLUT-4 translocation [178]. Furthermore, Beejmohun *et al.* [176] showed that cinnamon had the inhibitory effect on α-glucosidase and amylase enzymes in gastrointestinal tract.

2) Aloe (Aloe barbadensis)

A total of nine RCTs involving 283 diabetic patients were recruited in the recent meta-analysis of Dick *et al.* [179]. The authors found a wide range of daily dose were used and aloe products were prepared in the form of dried powder, leaf juice, and latex resin. Huge reductions in FPG and A1C by -46.6 mg/dL ($p < 0.001$)

and -1.05% (p = 0.004), respectively, in relative to the control group by aloe consumption. A greater benefit was observed in patients with FPG higher than 200 mg/dL. Similarly, these effects were reported in individuals with prediabetes and type 2 diabetes who had mild elevation of FPG. Weighed mean difference (WMD) of FPG was -30.05 mg/dL (95% CI: -54.87 to -5.23 mg/dL) and A1C was -0.41% (95% CI: -0.55 to -0.27%) in the aloe-treated group when compared with the placebo-control group [180]. The HOMA-IR significantly reduced (p = 0.047) after the eight weeks of aloe consumption when compared with placebo [142]. High molecular weight polysaccharides in aloe could diminish the activities of intestinal α-glucosidase enzymes [181]. In addition, it was hypothesized that aloe could enhance insulin secretion, improve insulin sensitivity [181], inhibit gluconeogenesis [182], and modify expressions of genes involved in glucose and fat metabolism [183].

3) Garlic (Allium sativum)

Recently, a meta-analysis of seven RCTs revealed that garlic products, including raw, powder, oil, and aged extract, in the daily dose varied from 600 to 1500 mg were supplemented for 4-24 weeks. The level of FPG significantly decreased standardized mean differences (SMD) by -1.67 (95% CI: -2.80 to -0.55; p = 0.004) at the end of study [184]. Few studies suggested significant reductions in A1C ($p < 0.005$) and PPG ($p < 0.01$) among the group receiving garlic in combination with conventional drugs [185, 186]. Magnitude of glucose-lowering effect of garlic depended on baseline glucose level, daily dose intake, and treatment duration [184]. Garlic products could be an alternative supplement for diabetic patients with abnormally high cholesterol level because blood lipid profiles simultaneously improved by the long-term administration of garlic [187]. Organosulfur compounds are mainly responsible for antidiabetic effect of garlic. Proposed mechanisms of action include promoting insulin secretion and improving insulin sensitivity [188].

4) Fenugreek (Trigonella foenum-graecum)

Improvement in glycemic control among patients with type 2 diabetes treated with fenugreek seed was found in a meta-analysis of 10 RCTs. Fenugreek seed powder and seed extract were prepared in the form of capsule or as an active ingredient in bread. In comparison with placebo, FPG and A1C significantly reduced

by -0.96 mmol/L (95% CI: -1.52 to -0.4 mmol/L) and 0.85% (95% CI: -1.49 to -0.22%), respectively. Two-hour PPG following 75-g OGTT significantly decreased by -2.19 mmol/L (95% CI: -3.19 to -1.19 mmol/L) [189]. A 3-year RCT additionally found that fenugreek ingestion was inversely associated with development of diabetes in prediabetic subjects. Preventive action was linked to reversion of insulin resistance [143]. Favorable effects on glucose homeostasis of fenugreek seed and its extracts could be from various compounds, including soluble fiber, saponins, trigonelline, and 4-hydroxyisoleucine [189]. Moreover, Zhou *et al.* and Hannan *et al.* [190, 191] suggested that fenugreek inhibited intestinal glucose absorption, stimulated insulin secretion, and modulated insulin sensitivity.

5) Ginseng (Panax spp.)

A meta-analysis of eight studies revealed the significant effects of ginseng supplementation on FPG, postprandial insulin, and insulin resistance in the treatment group when compared with the non-treated group. The SMDs were -0.306 (95% CI: -0.539 to -0.074; p = 0.01) for FPG, -2.132 (95% CI: -3.706 to -0.558; p = 0.008) for postprandial insulin, and -0.397 (95% CI: -0.679 to -0.115; p = 0.006) for HOMA-IR [192]. Meanwhile, the other meta-analysis involving 16 RCTs did not found the improvement in insulin resistance although FPG significantly reduced by -0.31 mmol/L (95% CI: -0.59 to -0.03 mmol/L; p = 0.03) [193]. The large differences in participant characteristics might be attributed to the inconsistent findings. Persons with IGT and type 2 diabetes were recruited into the former study, whereas both healthy and diabetic subjects were included to the latter one. There was no alteration in A1C from baseline observed in the both meta-analyses. Over 30 ginsenosides, which are saponin derivatives, are related to antihyperglycemic effect of ginseng [192]. Possible mechanisms of action of ginseng include promoting insulin production and secretion, blocking intestinal glucose absorption, and increasing glucose uptake via up-regulating GLUT-4 translocation [194].

6) Tea (Camellia sinensis)

A meta-analysis of 12 cohort studies found that daily consumption of more than three cups of tea appeared to reduce the risk for diabetes; nonetheless, the association was not statistically significant [195]. The conflicting results were

reported in the other meta-analysis of 17 RCTs involving 1,133 participants. Tea products were administered in a wide variation of catechins doses from 208-1,207 mg/day over the period of 2 - 24 weeks. Green tea consumption was associated with the significant decreases in FPG and A1C by -0.09 mmol/L (95% CI: -0.15 to -0.03 mmol/L; $p < 0.01$) and -0.30% (95% CI: -0.37 to -0.22%; $p < 0.01$), respectively, in comparison with the control. However, there was no effect of tea consumption on PPG. The effects were found only in those at high risk for metabolic syndromes, but not in healthy subjects [196]. The major constituents accounting for health-promoting effect of tea are flavonol compounds called catechins and its derivatives [195, 197]. A study of Ortsater *et al*. [198] showed that glucose-lowering effect of catechins and its extracts involved enhancing insulin secretion and inhibiting gluconeogenesis enzymes in animal model.

7) Milk thistle (Silybum marianum)

Seed extract of milk thistle, a well-known natural product among Western countries, contains a bioactive constituent named silymarin. Silymarin exerted favorable effects on glycemic profiles although its mechanism of lowering blood glucose remains unclear [199, 200]. A recent meta-analysis of five RCTs and 270 patients with type 2 diabetes reported that daily administered dose of silymarin was in the range from 200-600 mg for 45 days to six months. In comparison with placebo, FPG and A1C were significantly affected by silymarin (mean difference; MD of FPG = -26.86 mg/dL; 95% CI: -35.42 to -18.30 mg/dL and MD of A1C = -1.07%; 95% CI: -1.73 to -0.40%). The results also suggested therapeutic efficacy on diabetic nephropathy in the silymarin-treated group [200].

8) Bitter melon (Momordica charantia)

A total of 95 patients with type 2 diabetes who had no history of taking oral antidiabetic drug were randomly assigned to receive 2,000 and 4,000 mg of bitter melon powder in capsule, or 5 mg of glibenclamide daily for 10 weeks. In comparison with baseline levels, treatment of 2,000 and 4,000 mg of bitter melon significantly decreased the levels of A1C ($p < 0.05$ and $p < 0.02$, respectively) and FPG ($p < 0.05$ and $p < 0.04$, respectively). There was however no difference among the three groups at the end of study [201]. A significant reduction in fructosamine at week 4 was found

in the groups receiving 1,000 mg/day of metformin and 2000 mg/day of bitter melon by -16.8 µmol/L (95% CI: -31.2 to -2.4 µmol/L) and 10.2 µmol/L (95% CI: -19.1 to -1.3 µmol/L), respectively. The lower doses of bitter melon (500 and 1,000 mg/day) seemed slightly effective [202]. No change in 2-hour PPG following the standard OGTT, serum lipids, and anthropometric profiles was observed in previous studies [201, 202]. Benefits of bitter melon on glucose homeostasis could be from several mechanisms, including enhance glucose utilization of muscle cells [203], decrease intestinal glucose absorption [204], and inhibit hepatic gluconeogenesis [205]. Bitter melon did not exhibit only blood glucose-lowering effect, but it also preserved β-cell mass and function in animal studies [206].

9) Ivy gourd (Coccinia cordifolia)

A total of 60 patients with early diagnosed type 2 diabetes were included in a RCT to treat with 1,000 mg alcoholic extract of ivy gourd over a period of 12 weeks. The results suggested significant decreases in FPG (-20.6 mg/dL), A1C, and PPG (-34 mg/dL) levels at the end of study were associated with the ivy gourd supplementation and lifestyle intervention [207]. The findings were consistent with a prior study, showing the significant decreases in FPG and PPG by 1,800 mg of freeze-dried leaf tablet daily in 16 patients with type 2 diabetes [208]. It was hypothesized that antidiabetic effects of ivy gourd are caused by active compounds that mimicking insulin action. Previous animal studies also found the reduction in activities of enzymes related to gluconeogenesis by ivy gourd extract [209].

10) Turmeric (Curcuma longa)

A single administration of six grams of turmeric extract following the 75-g OGTT significantly increased postprandial insulin level, but without the suppression on glucose level [210]. A large RCT aimed to investigate preventive effect of turmeric on development of type 2 diabetes in 240 prediabetic subjects. After daily consumption of 1,500 mg of turmeric extract concurrently with lifestyle modification for nine months, none of participant in the treatment group developed type 2 diabetes. In contrast, there was 16.4% of the control group diagnosed with diabetes in this study [210]. Furthermore, β-cells function and insulin resistance as assessed by the HOMA method significantly improved by turmeric intervention throughout the study period.

A significant elevation of adiponectin was additionally observed among the turmeric-treated group [144]. Promising activities of turmeric extract were also confirmed in a 4-week within-subject study. Although a slight reduction in FPG was seen, progression of diabetic nephropathy was markedly improved at the end of study [211]. Rhizome of turmeric contains curcumin exerting antidiabetic properties. Reversing insulin resistance [212], improving β -cell function, and preventing β-cells apoptosis [213] were proposed as mechanisms of action of turmeric. In addition, antioxidative- and anti-inflammatory effects of curcumin were postulated to be effective for delaying the onset of diabetic complications [211, 214].

11) Gymnema sylvestre and Gymnema inodorum

Gymnema plants are the genus of vegetable commonly found in Southeast Asian countries. Leaf of *Gymnema* exhibits antihyperglycemic effect via various mechanisms. Animal studies proposed inhibitory effect on mammalian α-glucosidase enzymes of *Gymnema* [215]. Gymnemic acids isolated from the leaf is associated with pancreatic β-cells recovery, leading to improving in insulin secretion [216]. Limited numbers of human studies indicated efficacy of *Gymnema* on glycemic outcomes. Effect of a single administration of *G. inodorum* tea following carbohydrate meals on PPG was examined among 73 healthy volunteers. Results showed that the peak glucose level significantly decreased regardless of carbohydrate sources. However, favorable changes in FPG and insulin secretion were not found throughout a 28-day treatment period [217].

2.5.2 Safety of medicinal plants

Table 5 demonstrates adverse effects and toxicity of medicinal plants that have been suggested in previous evidences in preclinical and clinical studies.

Table 5 Adverse effects and toxicity of medicinal plants

Medicinal plants	Adverse effects/toxicity	Experiment models
Cinnamon	Hepatotoxicity resulting from isolated coumarin	*In vivo* [218]
	Platelet count drop	*In vivo* [219]
	Allergic contact dermatitis caused by the exposure of cinnamon products	Human (case report) [220]
	Non-immunologic and immunologic allergic reactions (i.e. stomatitis, gingivitis, and lip edema) after cinnamon ingestion	Human (case report) [220]
Aloe	Genotoxic caused by anthraquinone glycosides	*In vitro* [221]
	Diarrhea after chronic consumption, leading to water loss and electrolyte imbalance	Human (case report) [222]
Garlic	Unpleasant odor and gastrointestinal symptoms (i.e. nausea and diarrhea)	Human (meta-analysis) [184, 188]
Fenugreek	Hypersensitivity	Human (case report) [223]
	Specific sweat and urine smell	Human (meta-analysis) [189]
	Abdominal distention, dyspepsia, and nausea	Human (meta-analysis) [223]
Ginseng	Hypersensitivity	Human (case report) [223]
	Weakness, gastrointestinal discomfort, and upper respiratory infection	Human (RCT) [224]
Tea	Significant elevation of transaminase levels and acute reversible hepatitis	Human (case report) [223]

Table 5 Adverse effects and toxicity of medicinal plants (cont.)

Medicinal plants	Adverse effects/toxicity	Experiment models
Soy	Mild gastrointestinal symptoms including nausea, bloating, and constipation	Human (case report) [223]
	Increasing risk of estrogen-dependent tumors	*In vivo* and human [222]
Milk thistle	Gastrointestinal disturbances and headache	Human (RCT) [200]
Bitter melon	Favism in individuals with G6PD deficiency	*In vitro* and human [223]
	Heartburn, loss of appetite, and headache	Human (RCT) [201]
Turmeric	Anaphylaxis	Human (case report) [223]
	Kidney stone due to high level of oxalate	*In vitro* [223]
Gymneman spp.	Taste alteration	Human (case report) [223]
	Hepatotoxicity	Human (case report) [223]

RCT: randomized controlled trial; G6PD: glucose-6-phosphate dehydrogenase

2.6 Mulberry leaves

Mulberry (*Morus* spp.; family Moraceae) is a multi-functional plant widespread in tropical and subtropical areas throughout the world, particularly in Asia [225]. In agriculture field, leaves of mulberry serve as the food of silkworm and dairy cattle [226]. In addition, mulberry leaves have been traditionally used in folk remedies for treating several conditions such as fever, cough, hypertension, hypercholesterolemia, and hyperglycemia [227]. Moreover, many functional foods and dietary supplements were made from mulberry leaves and commercially available at present [228].

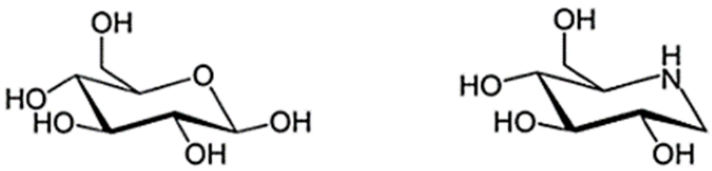
2.6.1 Phytochemicals

There are plenty of macronutrients, vitamins, and minerals such as ascorbic acid (160-280 mg/100g), β-carotene (10,000.00-14,688.00 µg/100g), iron (19.00-35.72 mg/100g), zinc (0.72-3.65 mg/100g), and calcium (786.66-2,266.66 mg/100g)in

mulberry leaves in relative to other green leafy vegetables [229]. Moreover, mulberry leaves are known as an excellent source of secondary metabolites exhibiting various biological activities. Mulberry leaves are rich in alkaloids [30]. A huge numbers of polyhydroxylated alkaloids with inhibitory effect against α-glucosidase enzymes in mulberry leaves were isolated and identified, for example, 1-deoxynojirimycin (DNJ), fagomine, isofagomine, and 2-*O*-α-D-Gal-DNJ [230, 231]. In addition, mulberry leaves contain various antioxidative compounds, including phenolic acids (i.e. chlorogenic acid, gallic acid, and caffeic acid) and flavonoids (i.e. rutin, kaempferol, quercetin, and isoquercitrin) [232-236]. Of these, DNJ is established as the most remarkable secondary metabolite responsible for antihyperglycemic property of mulberry leaves. Chemistry, determination, occurrence, and pharmacokinetics of DNJ are hereby described below.

1) Chemistry of DNJ

DNJ (chemical formula: $C_6H_{13}NO_4$, molecular mass: 163.17 g/mol, IUPAC name: (2R,3R,4R,5S)-2-(hydroxymethyl)piperidine-3,4,5-triol, synonym: moranoline) is a naturally occurring polyhydroxylated piperidine alkaloid (also called as iminosugar) [30]. As shown in Figure 5, DNJ is a highly polar molecule containing multiple hydroxyl groups. Its chemical structure is closely similar to glucose, but the pyranose ring contains nitrogen atom instead of oxygen atom [230].



Glucose DNJ

Figure 5 Chemical structure of DNJ in comparison with glucose

2) Determination and occurrence of DNJ in mulberry leaves

DNJ is the most abundant compound accounting for over 50% of alkaloids in mulberry leaves [237]. Regarding the different species, varieties, and cultivated areas of mulberry leaves, a large variation in DNJ concentration in the leaves samples was

reported in prior studies. Vichasilp *et al.* [238] found the concentration of DNJ in Thai mulberry leaves varieties ranged from 0.3 to 1.7 mg/g. Song *et al.* [236] and Hao *et al.*[239] revealed that DNJ constituted 1.389 to 3.483 mg/g and 0.401 to 5.309 mg/g, respectively in dried mulberry leaves harvested in China. Meanwhile, Bajpai *et al.* [240] showed that the content of DNJ varied in the range of 0.68 to 2.72 mg/g of Indian dried mulberry leaves. In addition, seasons of harvesting and leaf positions also affected the concentration of DNJ in mulberry leaves [32]. Young leaves collected from the top part of branches provided the highest amount of DNJ when compared with mature leaves [32, 238]. Moreover, differences in drying methods, extraction techniques, and extracted solvents are the factors influencing the content of DNJ in mulberry leaves [238, 241].

3) Pharmacokinetics of DNJ

Oral pharmacokinetic profiles of DNJ in the form of extracts and purified DNJ was determined in preclinical models [242-245]. Around 1% of administered DNJ was incorporated into plasma in the intact form, suggesting a slight absorption of DNJ into bloodstream [243]. After ingestion of 110 mg/kg of purified DNJ (purity > 95%) from aqueous mulberry leaves extract, DNJ reached the maximum plasma level of 15 μg/mL at 30 min [243]. Prior pharmacokinetic studies reported that plasma concentration at 30 min of DNJ was higher than other phytochemicals, including fagomine, 1,4-dideoxy-1,4-imino-d-arabinitol, anthocyanin, and catechin [245-247]. Furthermore, gastrointestinal absorption of DNJ was observed in a dose-dependent manner when the leaves extracts containing 1.1, 11, and 110 mg/kg of DNJ were ingested [243]. The studies further found that concentration of DNJ rapidly declined over few hours after absorption [242, 243].

Since DNJ is a high-polar compound, the small amount of DNJ was distributed into tissues [243]. Nonetheless, the highest concentration of DNJ was detected in stomach and duodenum at 0.25 hour and in the high blood supply organs, including kidney and liver, within 0.5 hour after ingestion 40 mg/kg of the extract [244]. It was also proposed that a decrease in hepatic glucose production could be attributed to hepatic distribution of DNJ [244]. DNJ was unchanged throughout metabolic pathways in the body [242, 243]. No metabolite and degradation product of

DNJ was found in blood circulation and tissues [243]. The intact form of DNJ was majorly excreted in feces (7%) and the relatively low amount of DNJ was also found in urine (2%) [242, 243]. With respect to the rapid absorption and excretion, it was suggested that DNJ has the relatively low half-life of 0.71 ± 0.02 hours [245].

Furthermore, DNJ from the whole mulberry leaves extract underwent the similar metabolic processes to purified DNJ in *in vitro* and *in vivo* models [244, 245]. However, the higher absorption and bioavailability of DNJ was observed after ingestion of purified DNJ in relative to the whole extract [242]. It was postulated that soluble fiber and flavonoids may interfere absorption of DNJ in mulberry leaves extract [242]. Conversely, the higher amount of DNJ was excreted after ingestion of mulberry leaves extract than the purified form [242].

2.6.2 Glucose-lowering effect

In the early era of research and discovery, a marked reduction in blood glucose in streptozotocin (STZ)-induced diabetic mice fed with mulberry leaves was found promptly with the discovery of many iminosugar alkaloids including DNJ in mulberry leaves extract [230]. Mulberry DNJ is a potent α-glucosidase inhibitor, principally responsible for antihyperglycemic effect of mulberry leaves. According to the sugar-mimicking structure, DNJ acts as a competitive inhibitor of dietary sugar by binding to the active sites of α-glucosidases in mammalian small intestine [230]. These enzymes play the key roles in disaccharides hydrolysis and monosaccharide absorption through intestinal brush border membrane. Inhibition on α-glucosidases results in the delay of carbohydrate digestion and absorption, and the decrease in blood glucose, particularly in postprandial state [227, 230].

1) Preclinical studies

The greater content of DNJ in the concentrated fractions of mulberry leaves extract exhibited the relatively stronger inhibitory action on α-glucosidase enzymes than crude extract [248]. This finding was supported by the robust correlation between the content of DNJ and the magnitude of inhibition on α-glucosidases with $r = 0.84$ [238] and $r = 0.90$, $p < 0.001$ [249].

Mulberry DNJ possessed the potent effect comparable to conventional α-glucosidase inhibitor in *in vitro* studies [248, 250]. The 50% inhibitory concentration (IC_{50}) value of mulberry leaves extract containing 0.11% DNJ was 41.0 μg/mL, whereas the IC_{50} of acarbose (positive control) was 19.0 μg/mL against rat α-glucosidase [250]. The most dominant inhibitory effect of mulberry DNJ was found for sucrase followed by isomaltase and maltase, respectively [248, 251, 252]. In contrast, it slightly inhibited the activities of trehalase, lactase, and α-amylase [248, 250, 251]. Hot water extract of mulberry leaves suppressed the digestive activities of sucrase and maltase bound on Caco-2 cell lines. This cell culture experiment found the decrease in glucose liberation in the apical chamber and the delay of glucose transportation to basal chamber through the Caco-2 cell monolayer [252].

Effects of mulberry leaves on blood glucose level among various animal models are present in Table 6. The results suggested that mulberry leaves did not only reduce blood glucose after carbohydrate load, but it was also effective for glycemic outcomes in the long-term feeding studies. Moreover, mulberry leaves ameliorated insulin resistance in multiple pathways. The studies of Hamdy *et al.* [37] and Liu *et al.* [38] showed modulation of proteins regulating hepatic glucose metabolism including the suppressive effect on gluconeogenic enzymes: glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) by mulberry leaves water extract. Mulberry leaves also activated glucose uptake in skeletal muscle and adipose tissue by regulating phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) and glycogen synthase kinase-3β (GSK-3β) signaling pathways, leading to GLUT-4 translocation to the cell membrane [36, 39]. In addition, mulberry leaves could preserve β-cell morphology and function. Histological examination by Mohammadi *et al.* [40] and Saenthaweesuk *et al.* [41] showed the restoration of β-cells to be closely to the normal condition in diabetic rats after the daily feeding of mulberry leaves extract.

Table 6 Animal studies investigating antihyperglycemic effects of mulberry leaves

Models	Interventions	Experiments	Results	References
Non-diabetic Wistar rats	- Water extract of mulberry leaves (0.11% DNJ) - Dose: 1,000 mg of extract/kg body weight	Single-dose feeding with carbohydrate load	- The $AUC_{0\text{-}3\ hours}$ of blood glucose and the peak glucose level after carbohydrate load in the mulberry-treatment group were lower than the non-treated group ($p < 0.001$ and $p < 0.01$, respectively) regardless of carbohydrate sources.	Kim *et al.*, 2011 [36]
Non-diabetic Wistar rats	- Water-ethanolic extract of mulberry leaves (1.10% DNJ) - Dose: 20, 100, and 500 mg of extract/kg body weight	Single-dose feeding with carbohydrate load	- The level of 2-hour PPG was suppressed when 100 and 500 mg of the extract were simultaneously fed with sucrose and starch ($p < 0.05$). - Time observed the significant suppression were 0.5 to 1 hour and 1 to 2 hours after sucrose and starch load, respectively.	Miyahara *et al.*, 2004 [248]

Table 6 Animal studies investigating antihyperglycemic effects of mulberry leaves (cont.)

Models	Interventions	Experiments	Results	References
Non-diabetic Wistar rats and non-obese, spontaneous diabetic Goto-Kakizaki rats	- Water extract of mulberry leaves (0.16 g of DNJ/100 g) - Dose: 3.75 g of extract/kg body weight (6 mg of DNJ/kg body weight)	Single-dose feeding with carbohydrate load	- Mulberry leaves feeding was associated with the decreases in PPG and AUC in after maltose and glucose load in non-diabetic rats and diabetic rats. - The stronger suppressive effect of the extract on PPG was observed in normal rats than diabetic rats. - The peak level of PPG in the treatment groups significantly decreased by 62% and 28% in normal rats and diabetic rats, respectively.	Park *et al*., 2009 [253]
Non-diabetic Wistar rats and non-obese, spontaneous diabetic Goto-Kakizaki rats	- Crude extract of mulberry leaves (0.05 g of DNJ/100 g powder) - Dose: 3.15 ± 0.11 mg of DNJ/kg body weight/day	Daily feeding for 8 weeks	- Glucose-lowering effect was found in diabetic rats, but not in non-diabetic rats. - FPG significantly decreased in diabetic rats fed with mulberry leaves ($p < 0.05$). - The levels of plasma insulin and HOMA-IR in diabetic rats tended to reduce by 12% and 18%, respectively, at the end of study.	Park *et al*., 2009 [253]

Table 6 Animal studies investigating antihyperglycemic effects of mulberry leaves (cont.)

Models	Interventions	Experiments	Results	References
STZ-induced diabetic Wistar rats	- Ethanolic extract of mulberry leaves - Dose: 400 and 600 mg/kg body weight /day	Daily feeding for 5 weeks	- FPG and A1C significantly decreased ($p < 0.05$) after daily feeding of mulberry leaves extract in the mulberry-treatment group.	Mohammadi *et al.*, 2008 [40]
STZ-induced diabetic Sprague Dawley rats	- Ethanolic extract of mulberry leaves - Dose: 250, 500, and 1,000 mg/kg body weight /day	Daily feeding for 6 weeks	- Daily treatment of 500 and 1,000 mg/kg of mulberry leave extract decreased FPG at the end of study. - Mulberry leaves exerted antiglycation effect *in vitro*, resulting in the decline in glycation product including A1C.	Naowaboot *et al.*, 2009 [254]
Alloxan-induced diabetic Albino rats	- Water extract of mulberry leaves - Dose: 600 mg/kg body weight /day	Daily feeding for 28 days	- The reductions in FPG were observed throughout the treatment period when compared with the baseline level. - Antihyperglycemic effect of mulberry leave was comparable to the treatment of 0.5 mg/kg glibenclamide and 10 mg/kg metformin.	Kumar, 2012 [186]

Table 6 Animal studies investigating antihyperglycemic effects of mulberry leaves (cont.)

Models	**Interventions**	**Experiments**	**Results**	**References**
STZ-induced diabetic Sprague Dawley rats	- Water extract of mulberry leaves - Dose: 150, 300, and 600 mg/kg body weight /day	Daily feeding for 12 days	- Mulberry leaves had no effect on glycemic outcome in non-diabetic control rats. In contrast, the extract at the dose of 300, and 600 mg/kg significantly decreased the level of FPG in diabetic-treated rats.	Saenthaweesuk, *et al*., 2009 [41]
High-fat and high-sucrose diet induced overweight Sprague Dawley rats	- Water extract of mulberry leaves (0.365 g of DNJ/100 g) - Dose: 5% of daily diets	Daily feeding for 6 weeks	- Compared with the baseline levels, mulberry leave extract significantly reduced plasma insulin, HOMA-IR, and plasma free fatty acid ($p < 0.05$).	Kim *et al*., 2011 [36]
High-fat diet and Alloxan-induced diabetic Sprague Dawley rats	- Water extract of mulberry leaves (5% DNJ) - Dose: 75 mg/kg body weight /day	Daily feeding for 3 weeks	- Mulberry leave extract reduced FPG in the mulberry-treatment group ($p < 0.05$ and < 0.001) when compared with the non-treated group.	Liu *et al*., 2016 [38]

DNJ: 1-deoxynojirimycin; STZ: streptozotocin; FPG: fasting plasma glucose; PPG: postprandial glucose; IFG: impaired fasting glucose; IGT; impaired glucose tolerance; OGTT: oral glucose tolerance test; AUC: area under the curve; HOMA-IR: homeostasis model assessment of insulin resistance

2) Clinical trials

Glucose-lowering efficacy of mulberry leaves was extensively demonstrated in clinical studies [31-34, 255-259]. Experiments of carbohydrate challenge test concurrently with a single-dose administration of mulberry leaves aim to determine effect of mulberry leaves on the elevation of blood glucose after carbohydrate load. Investigation of the effective doses of mulberry DNJ was performed simultaneously; however, it was determined in few studies [31, 32, 34, 259]. Characteristics of the studies and the main findings are summarized in Table 7. The results revealed the significant reduction in PPG in the mulberry leaves-treated group when compared with the control group regardless of participant characteristics (non-diabetic-, prediabetic-, and type 2 diabetic patients) and carbohydrate sources (disaccharides and complex carbohydrate). These indicated efficacy of mulberry leaves on postprandial hyperglycemia. Furthermore, the study of Chung *et al.* [31] also found that order of administration of mulberry leaves (30-min before and co-administered with carbohydrate) did not affect glucose-lowering efficacy of mulberry leaves.

With respect to the favorable effect on the elevated PPG, previous studies investigated efficacy of mulberry leaves on other glycemic indices including FPG and A1C in order to determine effects of daily supplementation of mulberry leaves on long term glycemic control [32, 34, 35, 260, 261]. The studies details are described in Table 8.

Table 7 Clinical studies investigating effect of a single-dose administration of mulberry leaves concurrently with carbohydrate tolerance test

Design	Participants (N)	Interventions	Outcomes	Results	References
A double-blinded, randomized, placebo-controlled, 4-group parallel trial	Healthy persons aged 25.3 ± 0.7 years (24)	- DNJ-enriched mulberry leaves powder (1.5% DNJ) or placebo dissolved in hot solution of sucrose - Test doses of DNJ: 6, 12, and 18 mg - Carbohydrate: 50 g of sucrose	- Pre- and postprandial glucose (at 30, 60, 90, and 120 min)	- Mulberry leaves containing 12 and 18 mg of DNJ effectively reduced PPG in response to sucrose load - Time observed the significant suppression on plasma glucose was 60 min after receiving intervention	Kimura *et al.*, 2007 [32]
A double-blinded, randomized, placebo-controlled, cross-over trial	1) Healthy persons aged 24 - 61 years (10) 2) Patients with type 2 diabetes (A1C 7.1 ± 0.9%) treated with oral antidiabetic drugs and aged 59 -75 years (10)	- 1 g of mulberry leaves extract or placebo plus sucrose in hot water - Test doses of DNJ: not indicated - Carbohydrate: 75 g of sucrose	- Pre- and postprandial glucose (at 30, 60, 90, and 120 min) - Breath hydrogen concentration	- The significant reductions in PPG were observed in both healthy persons and patients with type 2 diabetes who taking mulberry leaves when compared with control - Breath hydrogen concentration in the treatment group increased comparing with the control group, indicating the undigested carbohydrate remained in the digestive tract	Mudra *et al.*, 2007 [33]

Table 7 Clinical studies investigating effect of a single-dose administration of mulberry leaves concurrently with carbohydrate tolerance test (cont.)

Design	**Participants (N)**	- **Interventions**	- **Outcomes**	- **Results**	**References**
A within-person, repeated measures trial	Healthy females with no history of type 2 diabetes aged 22.3 years (10)	- 1.2 g and 3.0 g of dried powder of mulberry leaves ethanolic extract (0.77% DNJ) mixed with sucrose solution - Test doses of DNJ: not indicated - Carbohydrate: 30 g of sucrose	- Pre- and postprandial glucose (at 30, 60, 90, 120, 150, and 180 min)	- Both 1.2 g and 3.0 g of dried powder of mulberry leaves significantly lowered the level of PPG at 30 min when compared with control in the dose-dependent manner	Nakamura *et al.*, 2009 [255]
A within-person, repeated measures trial	Healthy females with no history of type 2 diabetes aged 22.3 years (10)	- Confections (mizu-yokan, daifuku-mochi, and chiffon cake) added with mulberry leaves extract in the ratio of sucrose and mulberry leaves = 10:1 - Test doses of DNJ: not indicated	- Pre- and postprandial glucose and insulin (at 30, 60, 90, 120, 150, and 180 min) - $AUC_{0\text{-}3\ hour}$ of postprandial glucose and insulin	- The suppressive effects of mulberry leaves on glucose and insulin were observed at 30 and 60 min after daifuku-mochi ingestion, and at 30 min after mizu-yokan and chiffon cake ingestion - Glucose-lowering efficacy of mulberry leaves seemed to be affected by the complication of food composition (i.e. oil, baking powder, egg, and milk)	Nakamura *et al.*, 2009 [255]

Table 7 Clinical studies investigating effect of a single-dose administration of mulberry leaves concurrently with carbohydrate tolerance test (cont.)

Design	Participants (N)	- Interventions	- Outcomes	- Results	References
A double-blinded, randomized, placebo-controlled, cross-over trial	Persons who had FPG between 100 and 140 mg/dL aged 50.0 ± 10.6 years (12)	- Dried powder of mulberry leaves extract (1.5% DNJ) or placebo in capsules, followed by boiled white rice with seasoning at the next 15 min - Test doses of DNJ: 3, 6, and 9 mg - Carbohydrate: 200 g of boiled white rice with 2 g of dry seasoning	- Pre- and postprandial glucose and insulin (at 30, 60, 90, and 120 min)	- The effective doses of mulberry DNJ for suppressing the elevated PPG were 6 and 9 mg - Time observed the significant suppression on plasma glucose and insulin was 30 min after receiving intervention	Asai *et al.*, 2011 [34]
A single-blinded, randomized placebo-controlled, 4-group parallel trial	1) Healthy persons aged 23.6 ± 2.1 years (10) 2) Patients with type 2 diabetes aged 62.4 ± 12.5 years who treated with or without sulfonylurea (10)	- Jelly containing 3.3 g of mulberry leaves extract (254 μg of DNJ) or placebo jelly - Test doses of DNJ: not indicated - Carbohydrate: sucrose added in jelly (a ratio of sucrose to mulberry leaves extracts of 10:1)	- Pre- and postprandial glucose (at 30, 60, 90, and 120 min) - $AUC_{0\text{-}2\ hour}$ of postprandial glucose	- The $AUC_{0\text{-}2\ hour}$ of glucose excursion in the treatment group was lower than the control group - Time observed the significant suppression on PPG were 30 min for healthy persons and 60 min for patients with type 2 diabetes after receiving intervention	Nakamura *et al.*, 2011 [256]

Table 7 Clinical studies investigating effect of a single-dose administration of mulberry leaves concurrently with carbohydrate tolerance test (cont.)

Design	Participants (N)	Interventions	- Outcomes	- Results	References
A double-blinded, randomized, placebo-controlled, 5-group parallel trial	Healthy persons aged 22.7 ± 0.4 years who had FPG below 125 mg/dL (50)	1) Dried powder of mulberry leaves aqueous extract (0.36% DNJ) or placebo dissolved in water mixed with maltose 2) Dried powder of mulberry leaves aqueous extract (0.36% DNJ) dissolved in water, followed by maltose solution at the next 30 min - Test doses of DNJ: 4.5, 9, and 18 mg - Carbohydrate: 75 g of maltose	- Pre- and postprandial glucose (at 30, 60, 90, and 120 min) - AUC $_{0\text{-}2\ hour}$ of postprandial glucose	- Mulberry DNJ at the doses of 9 and 18 mg were effective to suppress the elevated PPG resulting from maltose load. - Time observed the significant suppression on PPG were 30 and 60 min after receiving intervention	Chung *et al.*, 2013 [31]
A placebo-controlled, 2-group parallel trial	Patients with type 2 diabetes who treated with oral antidiabetic drugs and aged 53.80 ± 11.15 years (48)	- 70 mL of mulberry leaves tea (DNJ content was not indicated) in hot water mixed with 1 teaspoon of sugar - Carbohydrate: 2 idlis with coconut paste	- Pre- and postprandial glucose (at 90 min)	- The level of PPG at 90 min in the mulberry leaves-treated group was lower than the control group	Banu *et al.*, 2015 [257]

Table 7 Clinical studies investigating effect of a single-dose administration of mulberry leaves concurrently with carbohydrate tolerance test (cont.)

Design	**Participants (N)**	- **Interventions**	- **Outcomes**	- **Results**	**References**
A placebo-controlled, randomized, crossover trial	Healthy persons aged 51.21 ± 9.45 years who had FPG below 126 mg/dL (14)	- 2 g of mulberry leaves tea powder mixed with boiling water, followed by sucrose solution at the next 30 min - Test doses of DNJ: not indicated - Carbohydrate: 75 g of sucrose	- Pre- and postprandial glucose and insulin (at 30, 60, 90, 120, and 150 min) - AUC of glucose and insulin	- When compared with control, the treatment group had the decreases in PPG at 30 min and the AUC of glucose and insulin	Bumrungpert *et al.*, 2016 [258]
A double-blinded, randomized, placebo-controlled, cross-over trial	Normoglycemic adults aged 19 – 59 years (37)	- Capsules containing 125 mg of mulberry leaves aqueous extract (5% DNJ) co-administered with maltodextrin solution - Test doses of DNJ: 6.75, 12.5, and 25 mg - Carbohydrate: 50 g of maltodextrin	- Pre- and postprandial glucose and insulin (at 15, 30, 45, 60, 90, and 120 min) - $AUC_{0\text{-}2\ hour}$ of postprandial glucose and insulin	- The $AUC_{0\text{-}2\ hour}$ of glucose and insulin excursion in the treatment group were lower than the control group - The effective doses were 12.5 and 25 mg of DNJ - Time observed the significant suppression on plasma glucose and insulin was 30 min after receiving intervention	Lown *et al.*, 2017 [259]

DNJ: 1-deoxynojirimycin; FPG: fasting plasma glucose; PPG: postprandial glucose; IFG: impaired fasting glucose; IGT; impaired glucose tolerance; OGTT: oral glucose tolerance test; AUC: area under the curve; HOMA-IR: homeostasis model assessment of insulin resistance

Table 8 Clinical studies investigating effects of daily supplementation of mulberry leaves on glycemic outcomes

Design (duration)	Participants (N)	Interventions	Outcomes	Results	References
A double-blinded, randomized, placebo-controlled, 2-group parallel trial (38 days)	Healthy persons (12) - Age: 24.7 ± 1.0 years - BMI: 21.3 ± 0.6 kg/m^2	- DNJ-enriched mulberry leave powder (1.5% DNJ) or placebo dissolved in hot water - Test dose: 18 mg of DNJ (1.2 g of powder) thrice daily before meals - Daily dose: 54 mg of DNJ (3.6 g of powder)	FPG and lipids (day 0, 24, and 38)	No significant change in the levels of FPG and lipids in the treatment group when compared to the control and baseline levels throughout the treatment period.	Kimura *et al.*, 2007 [32]
A double-blinded, randomized, placebo-controlled, 2-group parallel trial (12 weeks of treatment period and 4 weeks of post-treatment observation)	Persons who had FPG between 110 and 140 mg/dL (65) - Age: 53.7 ± 6.7 years	- Capsule of mulberry leaves extract (1.5% DNJ) or placebo - Test doses: 6 mg of DNJ (3 capsules) thrice daily before meals - Daily dose: 18 mg of DNJ (9 capsules)	FPG, A1C, fructosamine, GA,1,5-AG, and FPI (week 0, 4, 8, 12, and 16)	No reduction in FPG and FPI was observed in both groups throughout the study. However, mulberry leaves treatment was associated with the significant increase in 1,5-AG level, whereas the level of A1C and GA tended to be improved during supplementation period.	Asai et al., 2011 [34]

Table 8 Clinical studies investigating effects of daily supplementation of mulberry leaves on glycemic outcomes (cont.)

Design (duration)	Participants (N)	Interventions	Outcomes	Results	References
A within-person trial (8 weeks)	Patients with dyslipidemia (46)	- Mulberry leaves tea - Daily dose: 2 g of mulberry leaves tea thrice daily before meals	FPG	The level of FPG significantly decreased from 97.65 ± 10.09 to 85.43 ± 7.76 mg/dL at the end of study	Banchobphutsa, 2012 [260]
A placebo-controlled, 2-group parallel trial (2 weeks of run-in period and 4 weeks of treatment period)	Patients who had FPG between 100 and 125 mg/dL (42) - Age: 53.0 ± 7.2 years - BMI: 24.7 ± 2.2 kg/m^2	- Tablet of mulberry leaves extract (0.36% DNJ) or placebo - Test dose: 6 mg of DNJ (6 tablets) thrice daily before meals - Daily dose: 18 mg of DNJ (18 tablets)	2-hour postprandial glucose, insulin, and C-peptide (week 0 and 4)	The level of 2-hour PPG was significant suppressed by mulberry leaves. Moreover, improvement in postprandial insulin response was observed at the end of study.	Kim et al., 2014 [35]
A double-blinded, randomized, placebo-controlled, 2-group parallel trial (2 weeks of run-in and 12 weeks of treatment period)	Patients with type 2 diabetes who had A1C 7.0 – 8.0%, but not treated with insulin (17)	- Capsule containing 500 mg of standardized mulberry leaves extract (DNJ content was not indicated) or placebo - Test dose: 1,000 mg of extract (2 capsules) thrice daily before meals - Daily dose: 6 capsules	Postprandial SMBG and A1C (week 0 and 12)	At the end of study, postprandial self-monitoring blood glucose in the treatment group was significantly reduced when compared to the control group and baseline levels. In addition, A1C tended to decrease from baseline; however, no difference between the groups was found.	Riche et al., 2017 [261]

DNJ: 1-deoxynojirimycin; FPG: fasting plasma glucose; PPG: postprandial glucose; A1C: glycated hemoglobin; FPI: fasting plasma insulin; 1,5-AG: 1,5-anhydroglucitol; IFG: impaired fasting glucose; IGT; impaired glucose tolerance; OGTT: oral glucose tolerance test; AUC: area under the curve; HOMA-IR: homeostasis model assessment of insulin resistance

2.6.3 Lipid-lowering effect

In this section, lipid-lowering effect includes anti-obesity and antihyperlipidemic properties. Anti-obesity refers to the effects of mulberry leaves on modulating lipid accumulation in adipocytes. Meanwhile, antihyperlipidemia is the effects of mulberry leaves on reducing blood lipids including cholesterols and TG.

1) Preclinical studies

Lipid-lowering effect of mulberry leaves were demonstrated in numerous *in vitro* and *in vivo* studies. Mulberry leaves exerted several effects on lipid metabolism including inhibiting lipogenesis and promoting lipolysis through multiple mechanisms of action.

Being the α-glucosidase inhibitor was postulated as a plausible explanation of lipid-lowering effect of mulberry leaves. In hepatic cells, excess circulating glucose is converted to fatty acid, which is the substrate of TG. DNJ could lower glucose hepatic influx because it inhibits carbohydrate digestion and absorption, resulting in the decreased blood glucose [251].

An enzyme activity analysis of Tsuduki *et al.* [262] showed that mulberry leaves extract enriched with DNJ promoted the activities of PPAR-α in the transcription level by upregulating mRNA expressions of the PPAR-α target genes: carnitine palmitoyltranferase (CPT) and acyl-CoA oxidase (ACO). This leads to an increased lipolysis. Results in animal model consistently found a significant reduction in hepatic TG. Polyphenols also possessed the agonistic effect on PPAR-α. Kobayashi et al. [263, 264] suggested that polyphenol-rich extract of mulberry leaves upregulated the transcription of *PPAR-α* gene. In addition, the extract lowered mRNA expressions of β-hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting step of endogenous cholesterol synthesis.

Ethanolic mulberry leaves extract containing quercetin, kaemferol, caffeic acid, and hydroxyflavin regulated expressions of several genes and proteins involved in lipid metabolism including sterol regulatory element-binding proteins (SREBPs), HMG-CoA reductase, fatty acid synthase (FAS), glycerol-3-phosphate acyltransferase

(GPAT), and liver X receptor (LXR) in hepatic cells and adipocytes [265-267]. Histological examination confirmed the decrease in lipid accumulation in the cells [265].

Inhibition on adipogenesis was also stated as the major anti-obesity mechanism of mulberry leaves. Ethanolic and water extracts of mulberry leaves suppressed expressions of SREBP-1 and PPARs, which are the transcriptional factors of adipocyte differentiation and maturation [265, 266, 268]. Mulberry leaves also promoted apoptosis in adipocytes [266]. These findings could explain the decreases in the numbers of mature and functional adipocytes [269, 270]. In addition, mulberry leaves containing the high content of DNJ stimulated mRNA expression level of adiponectin [262], a significant adipokine regulating metabolic pathways of glucose and fatty acid.

Furthermore, the mixture of mulberry polyphenols and fiber showed efficacy on body weight and plasma lipids reduction in the recent study of Li *et al.* [271]. It was proposed that soluble fiber might potentiate lipid-lowering activity of mulberry leaves. Soluble fiber interferes dietary cholesterol absorption by binding with bile acid and delays gastric emptying time [272].

These mechanisms were the explanations of the induction of β-oxidation of FFA and the decrease in lipid accumulation in peripheral tissues. Mulberry leaves retarded body weight gain in high-fat diet-induced obese rats [265, 266, 273]. The final body weight of the mulberry leaves-treated group was 7 to 10% lower than the control group [265]. Mulberry leaves feeding was also able to decrease fat to body ratio in obese mice induced by high-fat diet, reflecting the reduction in body fat mass [265]. This was in line with the finding from microscopic analyses. There were significant reductions in the amount of adipocytes as well as size and numbers of lipid droplets in the cells after mulberry leaves interventions [262, 265, 266, 268]. In addition to antiobesity effect, mulberry leaves exhibited favorable effect on plasma lipids. The marked reductions in TC, LDL-C, and TG and the increase in HDL-C in blood circulation were observed in hypercholesterolemic animals fed with mulberry leaves [263, 265, 266, 269, 274].

2) Clinical studies

Many clinical studies consistently reported potentials of mulberry leaves for improving lipid profiles in persons with known history of dyslipidemia. Patients with early-stage dyslipidemia (N = 23) defined by the baseline LDL-C concentration in the range of 140 – 189 mg/dL were recruited to the study of Aramwit *et al*. [275]. The study intervention was mulberry leaves tablets containing 0.367 mg of DNJ/tablet. All participants were assigned to take three tablets thrice daily before meals consecutively for 12 weeks. Results suggested that mulberry leaves significantly decreased TC (-4.9%, $p < 0.05$), LDL-C (-5.6%, $p < 0.05$), and TG (-14.1%, $p < 0.05$), and increased HDL-C (19.7%, $p < 0.05$) compared with the baseline levels. The authors further suggested that lipid-lowering efficacy of mulberry leaves was superior to lifestyle modification alone.

Banchobphutsa *et al*. [260] consistently found that mulberry leaves brewed tea was effective for lowering blood lipids in patients with dyslipidemia (N = 46) defined by TC ≥ 200, LDL-C 101 – 190, or TG ≥ 150 mg/dL. Daily consumption of 3 g mulberry leaves tea three times a day before meals was capable of reducing the concentrations of TC (-9.8%), TG (-14.9%), and LDL-C (-2.02%) after the 8-week intervention period in comparison with the baseline levels.

The other 12-week within-person study of Kojima *et al*. [276] was conducted to determine efficacy of mulberry leaves capsules corresponding to 36 mg DNJ/day among patients with hypertriglyceridemia defined by the level of TG ≥ 200 mg/dL (N = 10). The authors found the moderate effect of mulberry leaves on plasma TG, which decreased from 312 ± 90 mg/dL at baseline to 252 ± 78 mg/dL at week 12 ($p = 0.058$); however, no improvement in the other lipid outcomes was observed. Moreover, this study showed that mulberry leaves lowered very small, dense LDL particles, which are highly atherogenic.

In addition, Andallu *et al.* [277] reported that mulberry leaves were effective for improving lipid profiles in patients with type 2 diabetes who had abnormal lipids levels (N = 24). Participants were randomly divided into two groups to take mulberry leaves capsules (500 mg/capsule) and 5 mg glibenclamide. There were the significant decreases in TC (-12%, $p < 0.01$), TG (-16%, $p < 0.01$), LDL-C (-23%, $p < 0.01$),

very low-density lipoprotein cholesterol (VLDL-C) (-17%, $p < 0.01$), and plasma FFA (-12%, $p < 0.01$) from baseline after the 4-week treatment period. The lipid-lowering effect of mulberry leaves was comparable to glibenclamide. The authors also showed that mulberry leaves effectively reduced lipids on erythrocyte membrane, which are strongly associated with lipid peroxidation, vascular membrane rigidity, and atherosclerotic plaque formation.

2.6.4 Antihypertensive effect

Abnormally high systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate were normalized by mulberry leaves [278-280]. Investigation of vascular reactivity showed that mulberry leaves extract improved the responses of blood vessels to exogenous vasodilators and vasoconstrictors. Diminished dilatation and increased constriction of blood vessels significantly restored after long term mulberry leaves administration [279].

It was proposed that mulberry leaves reduced blood pressure as well as heart rate of the animals treated with mulberry leaves extract via the inhibition of angiotensin-converting enzyme (ACE) . *In vitro* studies observed the lower ACE activity after the cells were treated with mulberry leaf extract with the IC_{50} of 29.8 mg/mL [278]. Additionally, the decline contraction in response to phenylephrine indicated the blockade of calcium channel by mulberry leaves extract [280]. Antihypertensive effect of mulberry leaves could be from γ-aminobutyric acid (GABA) content in the extract. Results showed that the mean blood pressure in the mulberry leaves treatment group reduced in the similar trend as the group receiving pure GABA in a single administration study [278]. Moreover, published studies documented that antioxidative potentials of mulberry phenolics attenuated vascular dysfunction in hypertension [280].

2.6.5 Antioxidative and anti-inflammatory effects

Mulberry leaves were responsible for the significant increase in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity; however, the effect was weaker than ascorbic acid and butylated hydroxytoluene (BHT) that used as positive controls

[254, 281]. Determination of peroxides formation observed the lower level of lipid peroxidation end-products by mulberry leaves treatment [254, 280, 282]. The thiobarbituric acid (TBA) assay showed the lower level of malondialdehyde (MDA) in the dose-dependent fashion [279, 281]. The findings indicated potentials of mulberry leaves against free radical formation and oxidative stress damage. Activities of enzymes involved in antioxidant defense system– including glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and superoxide dismutase (SOD)–in diabetic rats fed with mulberry leaves extract were significantly elevated [283].

Phenolic compounds are well established as the excellent antioxidants [233, 235]. Methanolic and ethanolic mulberry leaves extracts have the potent ability against oxidative stress because the extracts contain the great amount of phenolics. Quercetin, isoquercitrin, rutin, and chlorogenic acid were isolated and identified as antioxidants of mulberry leaves [281].

Mulberry leaves were associated with the suppression of inflammatory process through signaling pathways of NF-κB, a crucial physiological part of macrophage activation-induced inflammation [284, 285]. Result found that the effects were due to the down-regulation of NF-κB transcription factors, resulting from the decreases in proinflammatory cytokines including iNOS, cyclooxygenase-2 (COX-2), TNF-α, IL-1β, and IL-6 [284]. In addition, endothelial cell adhesion of monocyte induced by TNF-α significantly reduced by the treatment of mulberry leaves extract [285]. The results consistently revealed that anti-inflammatory effect of mulberry leaves was in a dose-response relationship [284, 285].

Antioxidative and anti-inflammatory effects of mulberry leaves have been investigated in human by measuring various surrogate markers. A previous clinical trial suggested the significant reduction in 8-isoprostane, a surrogate marker of oxidative injury, in patients with mild dyslipidemia who supplemented with mulberry leaves tablet containing 0.367 mg of DNJ/tablet for the duration of 12 weeks. The improvement in GPx activities in erythrocytes was also observed. Moreover, the results showed that mulberry leaves treatment ameliorated inflammatory process. The mean monthly level of CRP had a decreasing tendency in this study. However, no

significant difference between the initial and the final levels of GPx activities and CRP was reported [286]. Lack of statistically significant change might be from the low baseline levels among study population. Also, determination of the end-products of lipid peroxidation found that mulberry leaves consistently reduced the level of peroxides in various biological samples including plasma, erythrocyte, and urine after four weeks of mulberry leaves treatment [277].

2.6.6 Anti-atherosclerosis

Mulberry leaves treatment attenuated the development of atherosclerotic events. Benefits of mulberry leaves were observed both in the early- and the advanced stages of coronary atherosclerosis. Besides the inhibitory effects on oxidative modification of LDL particles, the transfer of LDL through arterial wall and foam cell formation were dose dependently inhibited by mulberry leaves extract in *in vitro* models [287]. Furthermore, the extract was capable of inhibiting vascular smooth muscle cells (VSMC) proliferation and migration, which contributes to the progression of atherosclerosis, in rat aortic smooth muscle cell lines as shown in the growth curve assay [288, 289]. Analysis of intracellular lipid additionally revealed the decreases in TC and TG accumulation in the foam cells [287]. Also, the levels of numerous markers related to endothelial dysfunction including soluble vascular cell adhesion molecule-1 (sVCAM-1), fibrinogen, and nitric oxide were restored to the normal levels [290].

Beneficial effects on atherosclerotic lesions were also demonstrated in animal studies. Pathological analysis showed that the atherosclerotic plaque volume was significantly decreased. Endothelial injury and the proliferation and migration of VSMCs were also inhibited by mulberry leaves extract in a dose-dependent fashion [291].

2.6.7 Cardioprotective effect

Mulberry leaves preserved cardiac structure and function in various experimental models. After the daily treatment of mulberry leaves extract, histological studies showed the amelioration of damaged heart structure in isoproterenol (ISO)-

induced myocardial infarction rats. Areas of myonecrosis and myocarditis were less distributed in the treatment group when compared to the non-treated group [280]. This was similar to hemodynamic and echocardiographic analyses since mulberry leaves extract treatment significantly reversed the defective heart development including left ventricular remodeling [292]. Also, mild infiltration of inflammatory cytokines and low fibrous tissues in myocardial cells were observed [280, 292]. In addition, the depression of abnormally high cardiac marker enzymes induced by ISO [280].

2.6.8 Safety of mulberry leaves

Mulberry leaves are considered a relatively safe medicinal plant regarding the traditional uses. Meanwhile, no report of toxicity and life-threatening adverse events of mulberry leaves was evident. The most common side effects of mulberry leaves are gastrointestinal symptoms including bloating, flatulence, loose stool, and constipation [32, 35]. These effects are explained by the major mechanism of action of mulberry DNJ. Being the α-glucosidase inhibitor results in the production of the large amount of gas and osmotic pressure by intestinal bacterial fermentation of undigested carbohydrate remaining in large intestine [293]. Nonetheless, it was proposed that mulberry leaves may cause fewer abdominal flatulence than acarbose. An *in vitro* study showed that mulberry leaves extract possessed the weak inhibitory effect against pancreatic α-amylase when compared with acarbose [250].

Clinical studies of Lown *et al.* [259] and Li *et al.* [293] found that the incidence rates of gastrointestinal symptoms of mulberry leaves in the early-period of the studies were approximately 50% among participants who receiving mulberry leaves. However, the severity and the numbers of persons suffering from the side effects subsequently reduced over time, suggesting the better tolerance to the side effects. There was no alteration in hematological and biochemical parameters from the baseline levels. The values remained in the normal range after receiving mulberry leaves in the long duration [35, 276].

CHAPTER III
MATERIALS AND METHODS

The present work was divided into three studies as follows;

Study I (Phase I clinical trial): to determine effect of a single administration of mulberry leaves on plasma glucose excursion in response to carbohydrate load among healthy persons and determining the effective dose with minimal adverse effects of mulberry DNJ

Study II (Phase II clinical trial): to determine efficacy and safety of a long-term supplementation of mulberry leaves on glycemic control in persons who had impaired glucose metabolism including obese persons with prediabetes and patients with early-stage type 2 diabetes

Study III (proteomic analysis): to determine effect of the long-term supplementation of mulberry leaves on expressions of plasma proteins in persons who had impaired glucose metabolism including obese persons with prediabetes and patients with early-stage type 2 diabetes

3.1 Study I (Phase I clinical trial)

The study protocol was reviewed and approved by the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (No. 121.1/59) (Appendix A).

3.1.1 Study design : A four-parallel group, randomized controlled trial

3.1.2 Participants : Healthy adults at Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand who met eligibility criteria.

1) Inclusion criteria

a. Women and men aged 18 to 50 years

b. No history of diabetes

c. FPG < 100 mg/dL

d. BMI 18.5 - 22.9 kg/m^2

2) Exclusion criteria

a. Allergic to mulberry leaves

b. Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) > 40 IU/L

c. Serum creatinine (Cr) < 0.6 or > 1.2 mg/dL

d. Blood urea nitrogen (BUN) > 20 mg/dL

e. Had a history of gastrointestinal surgery or had abnormal conditions affecting digestion and intestinal absorption

f. Presences of uncontrolled or life-threatening conditions

g. Had a history of or diagnosed with hematological disorders, thyroid diseases, cardiovascular diseases, stroke, and chronic kidney disease

h. Taking drugs, supplements, or herbs affecting blood glucose level: corticosteroids, second-generation antipsychotics, niacin, thiazide diuretics, and β-blockers, within a month prior to study enrollment

i. Women during pregnancy or lactation

3) Sample size calculation

Sample size for comparing means of continuous variables between independent samples was calculated by following equations;

$$\text{n/group} = \frac{2\,(Z_\alpha + Z_\beta)^2\,Q^2}{d^2}$$

Regarding the previous study of Asai *et al.* [34], the results showed a significant reduction in the level of PPG at 30 min (PPG-30) who receiving a single dose of mulberry leaves after sucrose load when compared to control group. The values of PPG-30 were 121 ± 19 mg/dL and 145 ± 28 mg/dL in the treatment group (n = 10) and the control group (n = 10), respectively ($p < 0.05$).

$Z_{\alpha\ (0.05,\ 2\text{-tailed})} = 1.96$

$Z_{\beta\ (0.20,\ 2\text{-tailed})} = 0.84$

$$Q^2 = \frac{(n_1 - 1)\ S_1^2 + (n_2 - 1)\ S_2^2}{n_1 + n_2 - 2}$$

$$= \frac{(10 - 1)(19^2) + (10 - 1)(28^2)}{10 + 10 - 2} = 572.5$$

d = A critical difference of PPG-30 between groups

= 121 – 145 = -24 mg/dL

$$\text{n/group} = \frac{2\ (1.96 + 0.84)^2\ (572.5)}{(-24)^2} \approx 16$$

Assuming a 20% dropout rate; $\text{n/group} = \frac{16}{1 - 0.2} = 20$

Therefore, this study required total participants at least; N = 4 (20) = 80

3.1.3 Documents and interventions

1) Documents

a. Clinical record forms (Appendix B)

- General information
- Clinical outcomes
- Adverse events and Naranjo's algorithm

b. Participants information sheet

c. Informed consent form

2) Mulberry leaves powder

Mulberry leaves product used in this study was in the form of dried powder with no solvent extraction. Fresh leaves of white mulberry (*M. alba* L.; variety Sakonnakhon) were from Chiang Mai, Thailand. Young leaves (aged 50 to 70 days) from the top shoot to two feet below were harvested.

Foreign substances were removed, and the leaves were gently cleaned with tap water. Next, raw mulberry leaves were soaked in cold water (4ºC) for 30 min. The leaves were then dried by microwave oven at 600 Watts until completely dry and ground to fine powder by blender. In addition, aluminum sieve (size No.70) was used to grade the powder size at 200 μm and smaller and to remove undesirable contaminations. The dried powder was sterilized by gamma radiation with the standard radiation dose of 25 kGray for 30 min thereafter. Mulberry leaves powder was kept avoiding from light, heat, and moisture throughout the preparation processes. A single-meal ration of mulberry leaves powder at the weight equivalent to the desired dose of DNJ was packed in light- and moisture-protection sachets and stored at room temperature until dispensing. Figure 6 shows the mulberry leaves products used as the intervention in this study.

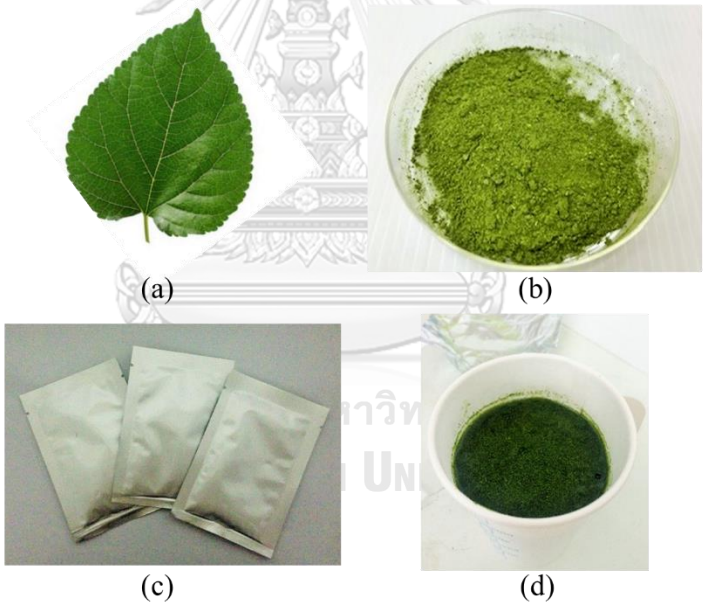


Figure 6 Mulberry leaves intervention: (a) fresh mulberry leaves; (b) dried mulberry powder; (c) a single-meal ration of mulberry leaves powder in a light- and moisture-protection sachet; and (d) mulberry leaves powder in warm water

Phytochemicals in mulberry leaves powder were determined using liquid chromatography coupled to mass spectrometry (LC/MS) and LC-system coupled with a Maxis Impact Ultra High-Resolution Time-of-flight mass spectrometry (TOF-MS) (Appendix C). The content of DNJ in the samples was 2.6 mg/g (0.26% DNJ).

Furthermore, stability of DNJ in mulberry leaves powder were also analyzed. The content of DNJ in our mulberry leaves product was stable after the 10-hour exposure of heat (80^{o}C) and light (220 Volt; 50 Hz). The most optimal condition providing the highest DNJ content in the products was room temperature.

3.1.4 Randomization

Each participant was assigned a participant number based on the chronological order of study enrollment in order to identify their corresponding sequences for the study interventions and to protect their personal identity. The method of block randomization (block size of four) was used for random allocation to receive one of the following test beverages containing different doses of mulberry DNJ as described below:

Group 1: 50 g of sucrose in 150 ml of warm water (sucrose solution)

Group 2: Sucrose solution mixed with 2.3 g of mulberry leaves powder (equivalent to DNJ 6 mg)

Group 3: Sucrose solution mixed with 4.6 g of mulberry leaves powder (equivalent to DNJ 12 mg)

Group 4: Sucrose solution mixed with 6.9 g of mulberry leaves powder (equivalent to DNJ 18 mg)

3.1.5 Study protocol

Printed advertisements were used to announce a research recruitment. Purposes and methods of the study were informed before enrollment by the researcher. Individuals who met eligibility criteria were then asked to sign inform consent voluntarily.

Baseline characteristics of study participants were collected a week before experiment day. First, participants were asked to complete general information record forms for demographic data, health status, and current medications by themselves. Weight, height, BMI, blood pressure, and heart rate were measured. In addition, 12-hour fasting blood sampling was performed for biochemical tests including FPG,

A1C, AST, ALT, BUN, and Cr by registered nurse. Participants were requested to maintain regular diets as well as physical activity and avoid taking medications, supplements, or herbs affecting blood glucose level until the experiment day.

At the experiment visit, sucrose loading test was held at 7.00 AM. Participants were asked to take a whole glass of the assigned beverage within a five-minute period. After that, postprandial glucose excursion was measured by collecting capillary blood glucose. Blood samples were drawn five min before and at 30, 60, 90, 120, and 180 min after taking the test beverages. The brief protocol of the experiment is shown in Figure 7. Any foods and beverages, smoking, and heavy physical activities were prohibited throughout the three-hour experiment. Adverse events according to mulberry leaves ingestion and the study protocol were closely monitored over the session. Those who cannot tolerate the adverse events or did not well comply with the study protocol were withdrawn from the study. Moreover, participants in group 2, 3, and 4 were asked to self-monitor and report side effects of mulberry leaves for seven days after the experiment.

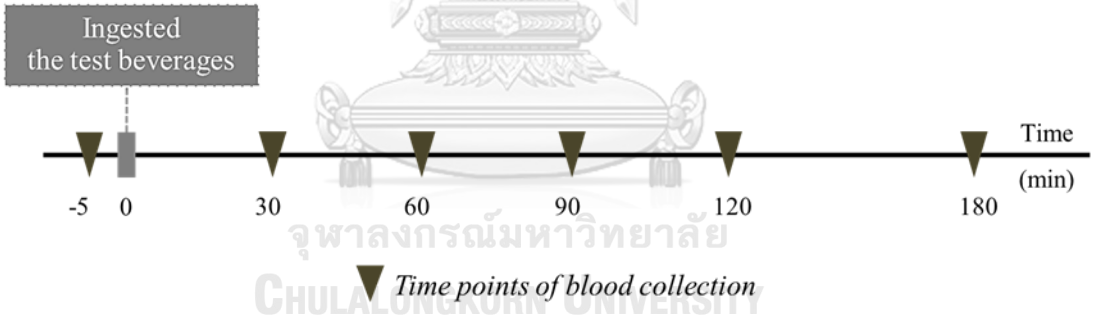


Figure 7 Experimental protocol of study I

3.1.6 Outcome measurement

1) Anthropometric data

Participants were asked to remove outer clothing, accessories, things in pockets, and shoes. Weight (in kg) was measured by digital-scale equipment and height (in cm) was measured by height meter. The measures of weight and height were to the nearest of 0.1 kg and 0.1 cm, respectively. BMI (in kg/m^2) was then calculated based on body weight in kg divided by the square of height in m.

2) Vital signs

Systolic and diastolic blood pressure and heart rate were measured by digital sphygmomanometer after participants took a rest and relaxed for at least 10 - 15 min.

3) Biochemical parameters

Six mL of 12-hour fasting blood were obtained from the median cubital vein by registered nurse for the tests of FPG, A1C, AST, ALT, BUN, and Cr. The concentration of FPG was determined using glucose oxidize method, whereas the percentage of A1C was analyzed via high-performance liquid chromatography (HPLC). AST, ALT, BUN, and Cr were measured by colorimetric method using the Hitachi Model 7170 analyzer (Hitachi Ltd., Tokyo, Japan).

Meanwhile, capillary blood glucose was consecutively measured over three hours using blood glucometer (Accu-Chek® Performa: Roche Diagnostics, Manheim, Germany). Participants' fingers were sterilized by alcohol before collecting 300 μL of the blood with disposable needles.

4) Adverse events

Participants were asked to self-monitor and report adverse events, including abdominal pain, diarrhea, constipation, allergic reaction, etc. during seven days after the experiment by completing a questionnaire. Probabilities of adverse reactions was evaluated by Naranjo's algorithm.

3.1.7 Statistical analysis

Per-protocol analysis was performed. The Kolmogorov-Smirnov test was used for normality test. To compare baseline characteristics of the four groups of participants, one-way analysis of variance (ANOVA) followed with Bonferroni post-hoc test was used for continuous variables and chi-square test or Fisher's exact test for categorical variables.

Also, one-way ANOVA with Bonferroni post-hoc test was performed to compare the values of PPG at individual time point and the $AUC_{0\text{-}180\ min}$ of glucose following carbohydrate load among the four groups. Repeated measures ANOVA was also used for analysis the alteration in PPG over time within the same group.

A value of $p < 0.05$ was considered significant difference. All of statistical analyses were run on the Statistical Package for Social Scientists (SPSS) version 22.0 (SPSS. Co., Ltd, Bangkok Thailand).

3.2 Study II (Phase II clinical trial)

The study protocol was reviewed and approved by Institutional Review Boards of the Royal Thai Army Medical Department, Phramongkutklao College of Medicine (No. Q038h/60) (Appendix E).

3.2.1 Study design : A 12-week, two-parallel group, randomized controlled trial

3.2.2 Participants

1) Inclusion criteria

a. Women and men aged 20 to 65 years

b. Had one of the two characteristics as follows;

- Obese (defined by BMI ≥ 25 kg/m^2) and had prediabetes (defined by FPG 100 to 125 mg/dL and/or 2-hour PPG 140 to 199 mg/dL)

- Newly diagnosed with type 2 diabetes by physicians within six months before study enrollment and had no history of taking oral antidiabetic drugs and insulin

c. Well communicate in the Thai language

2) Exclusion criteria

a. Allergic to mulberry leaves

b. Had indications for antidiabetic drugs and/or insulin (defined by FPG ≥ 180 mg/dL and/or A1C ≥ 8.0%)

c. Presences of significant diabetic complications

d. Elevated AST and/or ALT ≥ three times the upper limit of normal

e. Cr ≥ 2.0 mg/dL

f. Had a history of gastrointestinal surgery or had abnormal conditions affecting digestion and intestinal absorption

g. Presences of uncontrolled or life-threatening conditions

h. Diagnosed with hematological disorders, thyroid diseases, cardiovascular diseases, stroke, and chronic kidney disease

i. Taking drugs, supplements, or herbs affecting blood glucose level: corticosteroids, second-generation antipsychotics, niacin, thiazide diuretics, and β-blockers, within one month prior to study enrollment

j. Women during pregnancy or lactation

3) Sample size calculation

Sample size was calculated by the following equations to compare means of continuous variables between the two groups of independent samples.

$$\text{n/group} = \frac{2\,(Z_\alpha + Z_\beta)^2\,Q^2}{d^2}$$

The study of Kim *et al.* [35] reported that the $AUC_{0\text{-}2\ hour}$ of PPG of the mulberry leaves-treated group was significantly lower than the control group after a 4-

week supplementation period. The values of AUC $_{0\text{-}2\ hour}$ were 269.80 ± 38.17 mg/dL.hour and 309.50 ± 45.79 mg/dL.hour in treatment group (n = 19) and control group (n = 19), respectively ($p < 0.05$).

$$Z_{\alpha\ (0.05,\ 2\text{-tailed})} = 1.96$$

$$Z_{\beta\ (0.20,\ 2\text{-tailed})} = 0.84$$

$$Q^2 = \frac{(n_1 - 1)\ S_1^2 + (n_2 - 1)\ S_2^2}{n_1 + n_2 - 2}$$

$$= \frac{(19 - 1)(38.17)^2 + (19 - 1)(45.79)^2}{19 + 19 - 2} = 1{,}776.84$$

d = A critical difference of AUC $_{0\text{-}2\ hour}$ of PPG between groups

= 309.50 – 269.80 = 39.70 mg/dL

$$\text{n/group} = \frac{2\ (1.96 + 0.84)^2\ (1{,}776.84)}{(39.70)^2} \approx 18$$

Assuming a 30% dropout rate; $\text{n/group} = \frac{18}{1 - 0.3} = 26$

Therefore, this study required total participants at least; N = 2(26) = 52

3.2.3 Documents and interventions

1) Documents

a. Clinical record forms (Appendix F)

- General information
- Clinical outcomes
- Food diary
- Compliance
- Self-report questionnaire for monitoring adverse events

b. Participants information sheet

c. Informed consent form

2) Mulberry leaves powder

Mulberry leaves powder was prepared by the methods as described previously. According to the results of study I, 12 mg of mulberry DNJ was the effective dose for suppressing the elevated PPG after carbohydrate load with minimal adverse effects; hence, the powder at the weight equivalent to 12 mg of DNJ was used as the study intervention.

The powder was packed in light- and moisture-protection sachets in a single-meal ration. Participants in the treatment group were instructed to mix one sachet of the powder with 120 mL of warm water and then take thrice daily immediately before meals. They were also asked to rinse the glass with water and drink to ensure that the whole given powder was taken. Participants were also informed the proper condition for storage of the products to maintain the stability of DNJ in the products throughout the study period. The adequate amount of the test product was dispended monthly to participants and they were asked to return the remaining packets to assess % compliance every visit.

3) Diet control program

Diet control program was mainly conducted by a licensed dietitian. The program aimed at educating participants how to improve eating habit, which is the fundamental approach for the target population of the present study.

First, face-to-face diet counseling including review and discussion of usual eating habit and introduction to the food exchange method was performed to develop an individualized eating pattern. All participants were asked to maintain the modified eating pattern throughout the study period to minimize interfering effects of foods on clinical outcomes of interest.

In addition, participants were instructed how to record a food diary. They were assigned to record all items, portions, and cooking methods of foods, snacks, and drinks that they consumed in a represent day. The portion sizes were estimated using the standard household measuring cups and spoons. Individual total caloric intake and percentage of carbohydrate, protein, and fat consumed were calculated by Thai

Nutrisurvey® software (developed by Faculty of Tropical Medicines, Mahidol University). Individual interview was also performed at every visit to ensure the compliance of the participants.

3.2.4 Randomization

Similar to study I, the recruited participants were assigned participants' number based on the chronological order of study enrollment in order to identify their corresponding sequences for the study interventions and to protect their personal identity. Participants were randomly assigned to one of the two groups including treatment group (mulberry leaves ingestion and diet control) and control group (diet control alone) by the method of block randomization (block size of four).

3.2.5 Study protocol

This study was divided into three periods: screening and recruitment, run-in period, and experimental period. Schematic flow of the study was shown in Figure 8.

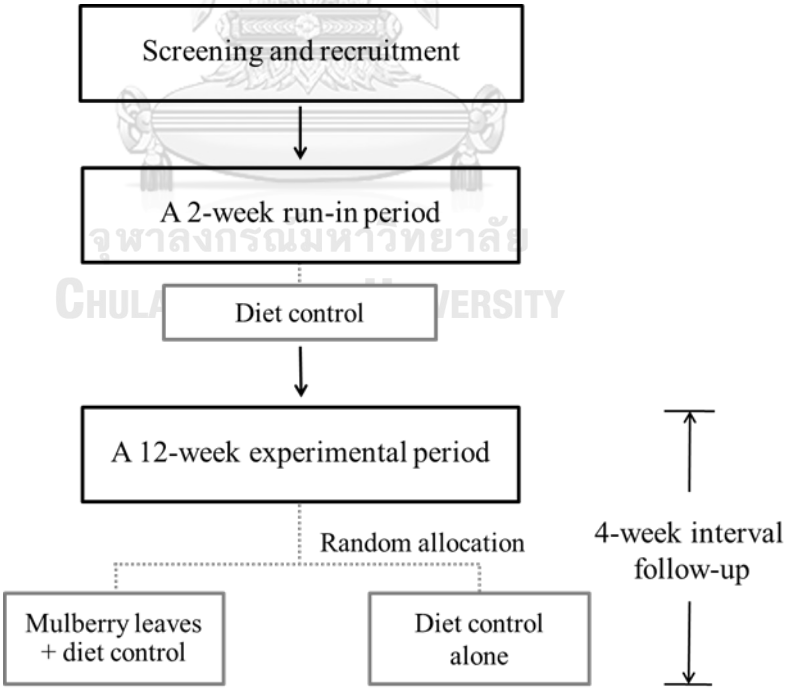


Figure 8 Schematic flow of study II

1) Screening and recruitment

Printed advertisements were used to announce a research recruitment. Medical records of outpatients who went to the Department of Family Medicine and the Department of Medicine, Phramongkutklao Hospital were also reviewed for screening. Individuals who met eligibility criteria were informed purposes and research methods. After making voluntary decision, they were asked to sign inform consent and participated in the two consecutive study periods: a 2-week run-in period followed by a 12-week experimental period.

2) Run-in period *(week -2 to week 0)*

Aims of the run-in period were to allow participants become acquainted with the diet control program and lower the dropout rate. On the first day, participants were asked to complete general information record form and then attend diet counseling. They were asked to maintain the modified eating habit and the usual physical activity over the period. Non-prescription drugs, supplements, or herbs that affecting clinical outcomes were prohibited.

Compliance to the diet control program was evaluated based on the food diary and individual interview at the end of this period. Those who well comply with the study protocol as assessed by dietitian were allowed to participate in the further period.

3) Experimental period *(week 0 to week 12)*

Baseline levels of anthropometric outcomes and vital signs were measured on the first day. Fasting blood was drawn for the analysis of blood glucose (FPG and A1C), insulin (FPI), lipids (TC, TG, HDL-C, and LDL-C), AST, ALT, and Cr. After that, OGTT was carried out to determine the ability in glucose clearance of insulin target tissues. Participants were assigned to ingest a 75-g glucose solution. The levels of PPG were then measured at 30, 60, 90, and 120 min thereafter. All of the outcomes were measured and collected by the same procedures at the end of study.

Appointments were held every four weeks during the period, in which fasting blood was collected to monitor blood glucose level. Mulberry leaves were dispensed to participants in the treatment group. In addition, adverse events and compliance were also evaluated. A monthly review of treatment regimens and concomitant medications of participants was done using online medical records to avoid confounding effects. Scheduled activities for each follow-up visit are summarized in Table 9. Like the run-in period, all participants were assigned to maintain the intensity of physical activity and avoid taking non-prescription drugs, supplements, or herbs.

Table 9 Scheduled activities for each follow-up visit

Visit	**1**	**2**	**3**	**4**	**5**
Week	**-2**	**0**	**4**	**8**	**12**
Signed inform consent	●				
Completed record forms for demographic data, health status, current medications, eating habits, and physical activity	●				
Face-to-face diet counseling	●				
Dispensing of mulberry leaves (treatment group)		●	●	●	
Measurements					
Weight, height, BMI, and waist circumference		●			●
Blood pressure and heart rate		●			●
PPG following 75-g OGTT		●			●
FPG		●	●	●	●
A1C		●			●
FPI		●			●
HOMA-IR		●			●
Lipids (TC, LDL-C, HDL-C, and TG)		●			●
AST, ALT, and Cr		●			●
Adverse events monitoring (treatment group)			●	●	●
% Compliance (treatment group)			●	●	●
Total caloric intake		●			●
% Carbohydrate, % protein, and % fat consumed		●			●
Protein expression		●			●
Review of treatment regimen and concomitant medications		●	●	●	●

BMI: body mass index FPG: fasting plasma glucose; PPG: postprandial glucose; OGTT: oral glucose tolerance test; A1C: glycated hemoglobin; FPI: fasting plasma insulin; HOMA-IR: homeostasis model assessment of insulin resistance; TC: total cholesterol;

LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; TG: triglycerides; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Cr: creatinine

3.2.6 Outcome measurement

1) Anthropometric data

Participants were asked to remove outer clothing, accessories, things in pockets, and shoes. Weight (in kg) was measured using digital-scale equipment and the nearest 0.1 kg was recorded. Height (in cm) was measured using height meter and reported to the nearest 0.1 cm. BMI (in kg/m^2) was then calculated based on body weight in kg divided by the square of height in m. Waist circumference (in cm) was measured using measuring tape. The tape was placed parallel to the floor at the midline between the lowest rib and the top of the hip bone. The measure was done to the nearest of 0.1 cm.

2) Vital signs

Systolic and diastolic blood pressure, and heart rate were measured using standard autonomic device after at least 10 min relax.

3) Biochemical parameters

Participants were asked for 12-hour fasting overnight before appointments. Blood samples were collected from the median cubital vein by registered nurse. At week 0 and week 12, there were two experimental stages: fasting blood collection and followed by a 2-hour, 75-g OGTT. The total of nine mL of fasting blood was firstly obtained for analyzing FPG, A1C, FPI, TC, LDL-C, HDL-C, TG, AST, ALT, and Cr. A catheter with normal saline lock was inserted into the vein and then still placed for the next blood sampling until a 2-hour OGTT was completed. Participants were subsequently instructed to drink a solution of 75 g of glucose. To determine the concentration of 2-hour PPG, blood samples were collected every 30 min thereafter. Foods and heavy activities were not allowed during the test. Meanwhile, two mL of fasting blood was obtained to analyze FPG at the follow-up visits at week 4 and week 8.

An enzymatic UV test (hexokinase method) using the Olympus AU 400 Analyzer was employed to analyze the values of FPG and PPG. The percentage of A1C was measured by using HPLC. The concentration of insulin was determined by radioimmunoassay technique. Then, the HOMA-IR was calculated from FPG (in mg/dL) multiplied by FPI (μIU/mL), and divided by 405. Lipids, concentration were measured by enzymatic method. AST, ALT, and Cr were determined by colorimetric analysis using the Hitachi Model 7170 analyzer (Hitachi Ltd., Tokyo, Japan).

4) Adverse events

A 5-scale questionnaire was used to evaluate adverse events of interest from mulberry leaves ingestion including abdominal pain, flatulence, loose stool, constipation, and allergic reactions. Participants in the treatment group were asked to rate the events by severity (0 = none to 5 = worst). If any, other possible adverse events were welcome to be recorded in the document.

5) Compliance

Compliance of mulberry leaves ingestion was assessed on the same basis as pill count method. Numbers of the remaining sachets were counted and the percentage of compliance (% compliance) was calculated by the following equation.

$$\% \text{ Compliance} = \frac{(\text{No. of dispensed sachets} - \text{No. of remaining sachets}) \times 100}{\text{No. of dispensed sachets}}$$

6) Total caloric intake and percentage of carbohydrate, protein, and fat consumed

All participants were requested to complete the food diary and returned to dietitian at the first week of experimental period (baseline) and at week 12. Individual total caloric intake and % carbohydrate, % protein, and % fat consumed were calculated by the Thai Nutrisurvey® program.

3.2.7 Statistical analysis

Per-protocol analysis was performed. The Kolmogorov-Smirnov test was used for normality test. One-way ANOVA and the chi-square or the Fisher's exact test were performed to compare the differences in baseline characteristics of participants

in the treatment group and the control group for continuous variables and categorical variables, respectively.

For the analyses of clinical outcomes, both between-group and within-group comparisons were performed to evaluate the effects of daily administration of mulberry leaves. One-way ANOVA was carried out for between-group comparison, whereas paired t-test was used for within-group comparison of clinical outcomes. In addition, repeated measures ANOVA was performed to analyze the change in adherence to the study intervention in each group throughout the study. A value of $p < 0.05$ was considered significant difference. All of statistical analyses were run on the SPSS version 22.0 (SPSS. Co., Ltd, Bangkok Thailand).

3.3 Study III (Proteomic analysis)

A semiquantitative proteomic analysis was performed as part of our phase II study. The study protocol was approved by Institutional Review Boards of the Royal Thai Army Medical Department, Phramongkutklao College of Medicine (No. Q038h/60) (Appendix E).

3.3.1 Study design : A prospective, single-group, experimental study

3.3.2 Participants : The participants who enrolled the clinical study II were considered as the selected cases for the determination of protein expression using proteomic analysis. The selection criteria are described below. Table 10 shows characteristics of the selected participants whose plasma protein expression were analyzed.

1) Participants who were assigned to the treatment group (mulberry leaves + diet control)
2) The top 12 persons who had the greatest reduction in A1C at the end of study

Table 10 Characteristics of the selected participants for proteomic analysis (N = 12)

	Participant ID	A1C (%)		
		Before [a]	After [b]	Δ A1C (after-before)
1	*37*	6.0	5.1	-0.9
2	*35*	6.9	6.5	-0.4
3	*46*	6.2	5.8	-0.4
4	*49*	6.2	5.9	-0.3
5	*39*	5.9	5.6	-0.3
6	*33*	6.0	5.8	-0.2
7	*17*	5.8	5.6	-0.2
8	*7*	5.6	5.4	-0.2
9	*11*	5.6	5.4	-0.2
10	*22*	5.5	5.3	-0.2
11	*34*	6.7	6.6	-0.1
12	*1*	6.0	5.9	-0.1

3.3.3 Sample collection and preparation

At the time of blood collection at week 0 and week 12 of the study II, three mL of the blood specimen was obtained from each participant into an anticoagulant vacutainer (the lavender-top tube containing ethylenediaminetetraacetic acid; EDTA) and kept refrigerated intermediately. Then, the whole blood was centrifuged and the plasma fraction was carefully collected and stored frozen at -80^{o}C until the analysis.

Plasma samples obtained from the 12 selected cases were divided into three groups (n = 4/group) and the samples were pooled in order to lower the individual variance of proteins within the study. Each of the pooled samples were considered as independent replicate and analyzed separately.

3.3.4 Wet-lab experiments

1) Protein separation by gel electrophoresis

Concentration of total proteins in the pooled samples was determined using Bradford protein assay (Bio-Rad®, USA). Protein separation was conducted in one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (1D SDS-PAGE) consisting of 4% stacking and 12% separating parts in the gel electrophoresis

unit (Bio-Rad®, USA). Each pooled sample were subjected to the gel in the individually parallel lanes before running the gel with the constant voltage at 120 Volt for 80 min. The protein gel was stained with Coomassie Blue-R for 10 min and then incubated in destaining solution containing 30% methanol and 10% acetic acid for three hours. Then, the gel was washed and incubated in distilled water for 15 min thereafter. Images of the gel were taken by the gel documentary (Bio-Rad®, USA). As shown in Figure 9, each excised section was cut and divided into 12 pieces before storing separately in -20°C until the further processes.

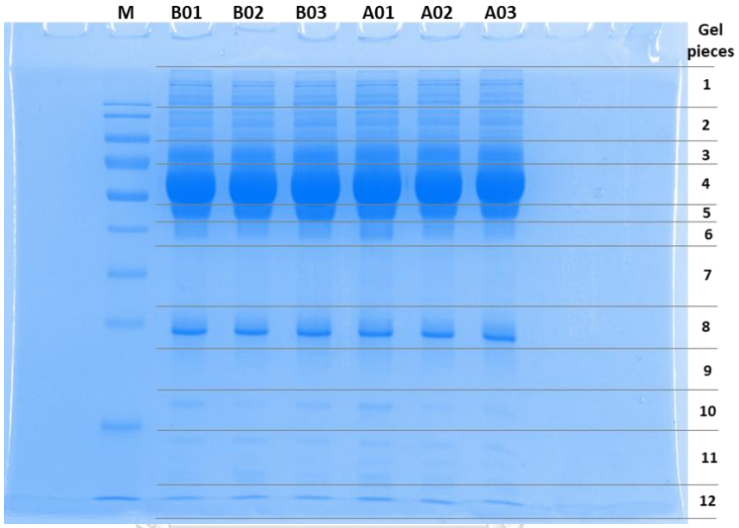


Figure 9 Protein separation using 1D SDS-PAGE and the excised sections for protein identification (M: markers; B01-B03: before-treatment pooled samples 1-3; A01-A03: after-treatment pooled samples 1-3)

2) In-gel tryptic digestion

The gel pieces were first dehydrated in 50% acetonitrile (Merck®, USA) in water (HPLC grade, Merck®, USA). The proteins were reduced in 7 mM DTT in 50 mM ammonium bicarbonate (NH_4HCO_3) for 15 min at 60°C and alkylated in 250 mM iodoacetamide for 30 min at room temperature under the light-protection condition. Next, 7 mM DTT in 50 mM NH_4HCO_3 were added for quenching the alkylation reaction. Then, the solutions were removed before gel dehydration using 100% acetonitrile and drying at room temperature for one hour. In order to reduce the complexity of the proteins to peptides, the complex molecules of the samples were digested by trypsin in 50 mM NH_4HCO_3 at 37°C for 16 hours. The supernatants

containing the digested peptides were subsequently collected and centrifuged at 10,000 X g at room temperature for 15 min before drying by CentriVap Vacuum Concentrators (Labconco, USA) at 40°C until completely dried.

3) Label-free proteomic analysis for protein identification

Identification of the peptide sequences (protein fractions) was performed using MS analysis. The dried samples were dissolved in 0.1% formic acid. The label-free proteomic analysis was performed using the UltiMate® 3000 Nano-LC systems (Dionex, UK) with column Acclaim PepMap RSLC C18 75 μm x 15 cm (Thermo Scientific, USA) as stationary phase. The pump flow rate was 300 nl/min. Mobile phase A was 0.1% formic acid and mobile phase B was 80% acetonitrile in 0.1% formic acid. The initial mobile phase was maintained at 4% solution B for 5 min. After that, the peptide mixtures were eluted using gradient condition from 4% to 50% solution B for 30 min and held for 5 min. Subsequently, the condition was followed to the initial step for 10 min. The eluates containing peptides were further subjected into the positive electrospray ionization system couple with microTOF-Q II (Bruker, Germany) to identify peptide spectra. The MS and MS/MS spectra were set covering the range of m/z 400-2000 and m/z 50-1500, respectively.

3.3.5 Computational analysis

First, the Data Analysis software (version 3.4) was used for conversion of the acquired MS/MS spectra to the mascot generic files. Second, identification of the peptide sequences was performed on November 2, 2020 using the Mascot Daemon program version 2.3.02 (Matrix Science, London, UK) and the SwissProt database specific to *Homo sapiens* species. Methionine oxidation and carbamidometylation of cysteines were considered as fixed modification and variable modification, respectively. Third, semiquantitative determination of protein expression level was determined by Exponentially Modified Protein Abundance Index (emPAI) [301], which is the parameter for estimation of absolute protein abundance in the samples based on the number of sequenced peptides per protein. Furthermore, the fold change in expression of individual protein was determined by the ratio of the protein

abundance of the two samples following the equation: fold change = $emPAI_{after}$ /$emPAI_{before}$.

Regarding scope of the present study, the proteins that expressed differentially in response to the assigned treatment were included in the result interpretation. Specifically, the differentially expressed proteins refer to the ones which their expression levels changed after receiving the intervention (at week 12) in comparison with the baseline profile (at week 0). The differential expressions of proteins are classified as up-expression (↑) and down-expression (↓). Definitions of the up-expressed and down-expressed proteins in this work are described below.

Up-expressed proteins	(i) Proteins with fold change ≥ 1.50 or (ii) Proteins that uniquely present in the after-treatment sample
Down-expressed proteins	(i) Proteins with fold change ≤ 0.67 or (ii) Proteins that uniquely present in sample the before-treatment sample

Fourth, UniProt database (www.uniprot.org) searching against gene ontology (GO), including molecular function, biological process, and cellular component, was performed in December 2020 to obtain biological information of the differentially expressed proteins (n = 523 entities). The GO enrichment analysis was subsequently conducted using the following searching keywords to specifically focus on the proteins that closely linked to pathophysiology and pathogenesis of insulin resistance and type 2 diabetes.

- Glucose/carbohydrate
- Lipid/fat/cholesterol/triglycerides
- Immune
- Inflammation
- Tumor necrosis factor
- NF-κB
- Microparticle
- Ceramides/diacylglycerol
- Surface protein
- Extracellular matrix
- Focal adhesion
- Chemoattractant
- Insulin
- Apoptosis/apoptotic bodies
- Complement system
- Acute phase reactant
- Cytokine/interleukin/adipokine
- Unfolded/misfolded protein
- Exosome
- Endothelial dysfunction
- Collagen/hyaluronan/laminin/ fibronectin/integrin
- Extracellular matrix remodeling
- Intermediate filament
- Inflammasome

In this regard, 311 proteins were excluded, whereas the other 212 proteins were submitted to the further analysis. Fifth, to explore the functional relationships among the proteins of interest, the functional enrichment analysis was performed using STRING database (www.string-db.org) specific to *Homo sapiens* species. Next, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was carried out (n = 212 entities) at the level of biological pathways. Finally, the candidate KEGG pathways and the matching proteins belonging to each pathway were considered for the evaluation of molecular effects of the given intervention on plasma protein expression. The summary of procedures of proteomic analysis are shown in Figure 10.

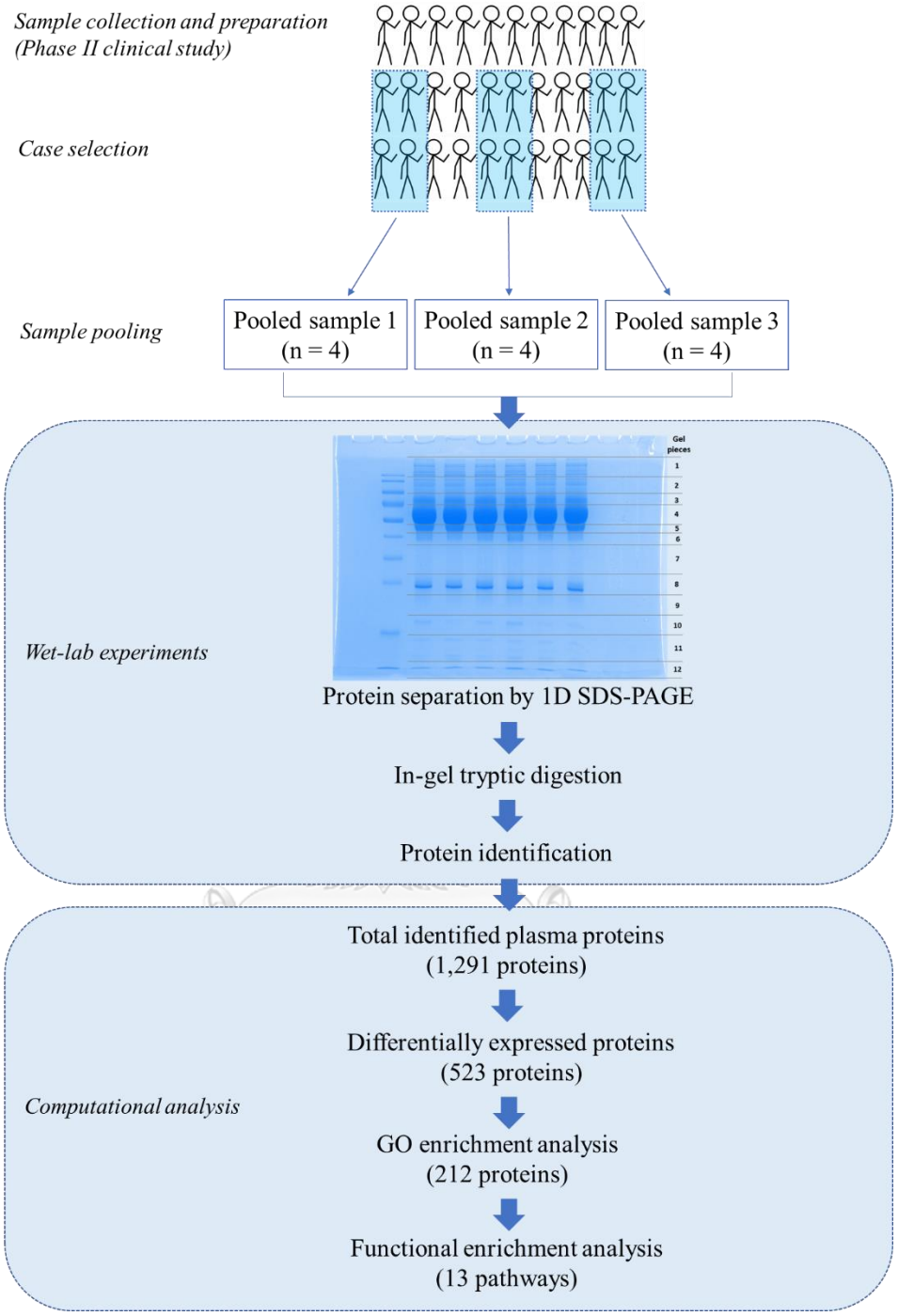


Figure 10 Procedures of proteomic analysis

CHAPTER IV
RESULTS

4.1 Study I (Phase I clinical trial): To determine effect of a single administration of mulberry leaves on plasma glucose excursion in response to carbohydrate load among healthy non-diabetic persons and determining the effective dose with minimal adverse effects of mulberry DNJ

Study I was carried out from September 2016 to April 2017 at the Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Description of algorithm for study enrollment and data collection is shown in Figure 11. Ninety volunteers were enrolled to this study. Four persons were excluded at the screening visit due to abnormal laboratory tests, whereas another was not able to comply with the study protocol. Therefore, 85 healthy adults were recruited into randomization, and then allocated into group 1 (n = 21), group 2 (n = 21), group 3 (n = 21), and group 4 (n = 22) for analysis.

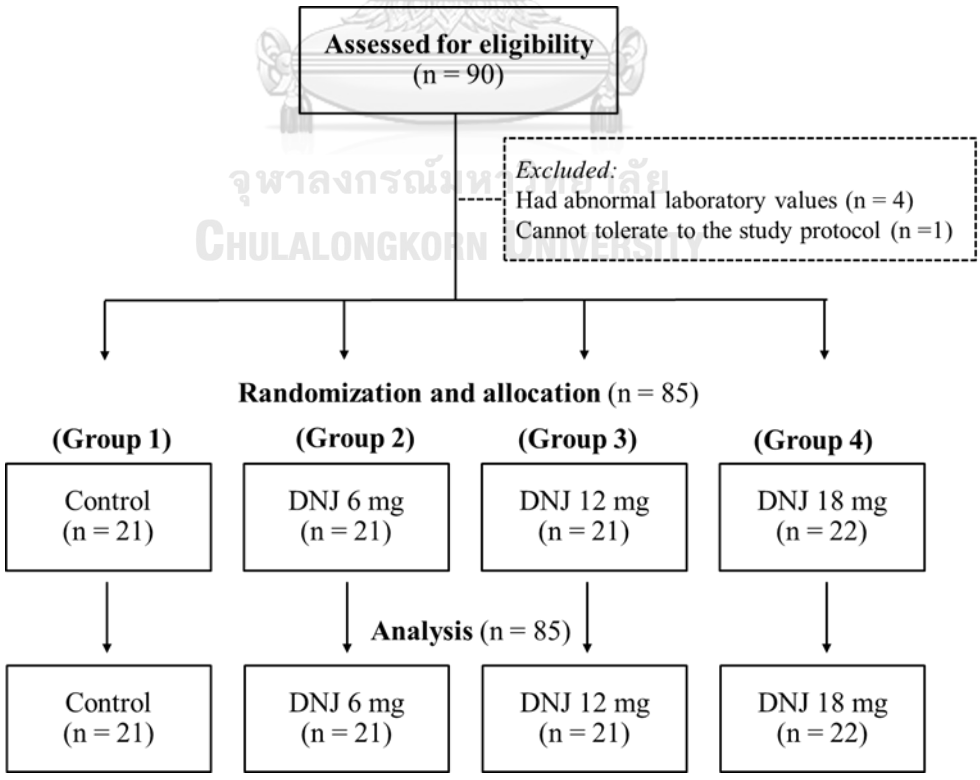


Figure 11 Algorithm for study enrollment and data collection of study I

4.1.1 Participants characteristics

Of 85 participants, 68 persons (80.0%) were female. University students were the majority (85.9%) of study population. Overall, the mean age of the enrolled participants was 23.31 ± 6.94 years. The mean body weight and BMI were 54.29 ± 6.89 kg and 20.36 ± 1.40 kg/m^2, respectively. Blood glucose concentration of participants were averaged at 83.06 ± 5.92 mg/dL for FPG and 5.1 ± 0.3% for A1C. In addition, the values of hepatic enzymes, BUN, and Cr were in the normal ranges, indicating the absence of hepatic and renal dysfunction in the included participants. There was no difference in demographic data and clinical outcomes at baseline among the four groups as shown in Table 11.

Table 11 Baseline characteristics of participants of study I (N = 85)

	Group 1 (Control)	**Group 2** (DNJ 6 mg)	**Group 3** (DNJ 12 mg)	**Group 4** (DNJ 18 mg)	***P values***
Number of participants	21	21	21	22	
Demographic data					
Age (year)	21.81 ± 5.72 (18 - 45)	25.81 ± 9.29 (18 - 48)	22.33 ± 5.57 (18 - 41)	23.27 ± 6.33 (18 - 42)	*0.250*
Female	17 (81.0)	17 (81.0)	17 (81.0)	17 (77.3)	*0.987*
Occupation					*0.086*
– University students	20 (95.2)	15 (71.4)	19 (90.5)	19 (86.4)	
– Government officers	0 (0)	3 (14.3)	0 (0)	0 (0)	
– Private employees	1 (4.8)	3 (14.3)	2 (9.5)	3 (13.6)	
Current smoking	1 (4.8)	1 (4.8)	0 (0)	0 (0)	*0.553*
Current drinking alcohol	2 (9.5)	5 (23.8)	8 (38.1)	3 (13.6)	*0.105*
Concomitant diseases					*0.764*
– Allergic rhinitis	3 (14.3)	2 (9.5)	3 (14.3)	2 (9.1)	
– Hypertension	0 (0)	0 (0)	0 (0)	1 (4.5)	

Table 11 Baseline characteristics of participants of study I (N = 85) (cont.)

	Group 1 (Control)	**Group 2** (DNJ 6 mg)	**Group 3** (DNJ 12 mg)	**Group 4** (DNJ 18 mg)	***P values***
Clinical outcomes					
Weight (kg)	54. 37 ± 5.40 (47 – 66)	54.55 ± 5.80 (45 – 65)	53.76 ± 8.78 (43 – 78)	54.45 ± 7.48 (41 – 69)	*0.983*
BMI (kg/m^2)	20.34 ± 1.41 (17.42 – 23.56)	20.57 ± 1.28 (18.57 – 22.64)	20.01 ± 1.47 (18.37 – 22.34)	20.52 ± 1.46 (18.22 – 23.44)	*0.578*
SBP (mmHg)	104.71 ± 9.97 (89 – 131)	107.19 ± 10.93 (92 – 128)	104.95 ± 12.40 (86 – 127)	105.77 ± 12.06 (83 – 133)	*0.890*
DBP (mmHg)	68.57 ± 4.74 (61 – 78)	67.67 ± 10.11 (48 – 86)	68.29 ± 8.30 (55 – 84)	66.86 ± 10.86 (41 – 84)	*0.925*
HR (bpm)	74.71 ± 10.33 (60 – 97)	74.95 ± 12.99 (58 – 101)	78.67 ± 12.02 (53 – 100)	75.45 ± 9.41 (61 – 95)	*0.642*
FPG (mg/dL)	82.71 ± 5.36 (74 – 99)	83.00 ± 6.99 (72 – 100)	81.43 ± 5.55 (63 – 88)	85.00 ± 5.51 (75 – 101)	*0.261*
A1C (%)	5.0 ± 0.3 (4.3 – 5.4)	5.0 ± 0.4 (4.3 – 5.7)	5.1 ± 0.3 (4.6 – 5.6)	5.1 ± 0.3 (4.5 – 5.7)	*0.541*

Table 11 Baseline characteristics of participants of study I (N = 85) (cont.)

	Group 1 (Control)	**Group 2** (DNJ 6 mg)	**Group 3** (DNJ 12 mg)	**Group 4** (DNJ 18 mg)	***P values***
Clinical outcomes					
AST (U/L)	22.52 ± 6.99 (15 – 37)	21.52 ± 6.02 (12 – 36)	20.48 ± 6.59 (13 – 36)	22.18 ± 5.60 (13 – 35)	*0.732*
ALT (U/L)	11.95 ± 4.18 (5 – 21)	12.76 ± 5.70 (5 – 26)	15.14 ± 9.17 (6 – 39)	13.09 ± 5.58 (6 – 30)	*0.425*
BUN (mg/dL)	10.76 ± 2.17 (7 – 15)	10.81 ± 2.40 (7 – 18)	11.38 ± 2.92 (7 – 19)	11.05 ± 2.89 (6 – 17)	*0.864*
Cr (mg/dL)	0.76 ± 0.13 (0.6 – 1.0)	0.75 ± 0.12 (0.6 – 1.0)	0.74 ± 0.17 (0.5 – 1.1)	0.73 ± 0.14 (0.5 – 1.0)	*0.909*

Data are reported as number (%) and mean ± S.D. with range (min – max) for categorical and continuous variables, respectively
P values for between-group comparison analyzed by Chi-square (or Fisher's Exact test) and one-way ANOVA for categorical and continuous variables, respectively
BMI: body mass index; kg: kilogram; kg/m^2: kilogram per square meter; SBP: systolic blood pressure; DBP: diastolic blood pressure; mmHg: millimeter of mercury; HR: heart rate; bpm: beat per minute; FPG: fasting plasma glucose; mg/dL: milligram per decilite r; A1C: glycated hemoglobin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; U/L: unit per liter: BUN: blood urea nitrogen; Cr: creatinine

4.1.2 Efficacy of mulberry leaves in lowering postprandial hyperglycemia in response to carbohydrate load

Table 12 shows the mean concentrations of PPG at individual time points and AUC $_{0\text{-}180\ min}$ of glucose after receiving the test beverages in the four groups of participants. As seen in the control group, ingestion of 50-g sucrose resulted in an elevation of blood glucose and the level of PPG sharply increased in the early period ($p < 0.001$ at 30 min, $p < 0.001$ at 60 min, and $p = 0.003$ at 90 min *vs.* FPG). Time observed the peak level of blood glucose was at 30 min. After that, the concentration of PPG continuously declined and returned to the baseline level within 120 min ($p = 0.287$ *vs.* FPG). There was no episode of hypoglycemia throughout 180 min of the experimental period. These findings represent blood glucose excursion after carbohydrate load without mulberry leaves administration in healthy non-diabetic adults.

Co-administration of sucrose and mulberry leaves caused the declines in PPG at specific time points. The significant differences in PPG levels among the groups were found at 30 min ($p < 0.001$) and 60 min ($p < 0.001$) as analyzed by one-way ANOVA. In addition, post-hoc analysis found effects of the different doses of mulberry DNJ on postprandial glucose excursion as demonstrated in Figure 12. When compared with the control group, all of the three doses of DNJ effectively suppressed the levels of PPG-30 ($p < 0.05$ for DNJ 6 mg, $p < 0.001$ for DNJ 12 mg, and $p < 0.001$ for DNJ 18 mg). Moreover, at the same time point, the glucose-lowering effects of DNJ 12 mg and DNJ 18 mg also differed from DNJ 6 mg ($p < 0.05$ and $p < 0.001$, respectively).

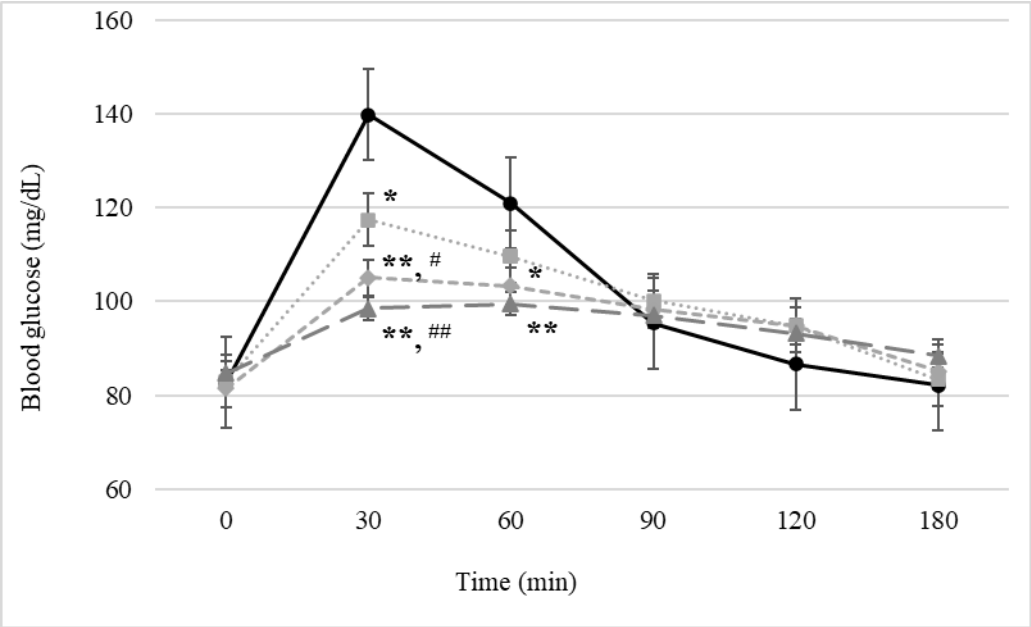


Figure 12 Postprandial glucose excursion over 180 min following co-administration of 50-g sucrose and the different doses of mulberry DNJ: control (●), DNJ 6 mg (■), DNJ 12 mg (♦), and DNJ 18 mg (▲). (* $p < 0.05$, ** $p < 0.001$ *vs.* control and $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.001$ *vs.* DNJ 6 mg, analyzed by one-way ANOVA and followed with Bonferroni post-hoc test)

Table 12 The mean concentrations of PPG at individual time points and AUC $_{0\text{-}180\ min}$ of glucose after receiving the test beverages

Outcomes	Group 1 (Control)	Group 2 (DNJ 6 mg)	Group 3 (DNJ 12 mg)	Group 4 (DNJ 18 mg)	*P values*
FPG (mg/dL)	80.25 ± 6.44 (79 – 100)	82.09 ± 4.19 (75 – 95)	85.20 ± 6.38 (72 – 101)	83.11 ± 4.42 (78 – 95)	*0.335*
PPG-30 (mg/dL)	139.76 ± 15.36 (99 – 157)	117.43 ± 14.93 (99 – 165)	105.05 ± 13.00 (82 – 130)	98.50 ± 8.93 (83 – 116)	***< 0.001***
PPG-60 (mg/dL)	120.95 ± 20.65 (89 – 178)	109.48 ± 18.06 (90 – 174)	103.33 ± 14.38 (84 – 146)	99.00 ± 12.00 (79 – 125)	***< 0.001***
PPG-90 (mg/dL)	95.43 ± 15.24 (77 – 139)	100.24 ± 11.19 (83 – 131)	98.33 ± 11.22 (83 – 134)	97.00 ± 8.35 (83 – 117)	*0.593*
PPG-120 (mg/dL)	86.67 ± 16.83 (66 – 139)	94.90 ± 8.21 (82 – 111)	94.81 ± 9.36 (78 – 121)	93.50 ± 9.88 (75 – 119)	*0.072*
PPG-180 (mg/dL)	82.24 ± 9.08 (70 – 100)	83.38 ± 6.77 (72 – 96)	85.24 ± 10.49 (61 – 106)	88.41 ± 8.77 (76 – 104)	*0.471*
AUC $_{0\text{-}180\ min}$ (mg/dL.min)	262.64 ± 20.85 (232.25 – 303.00)	252.62 ± 20.21 (230.50 – 329.25)	242.43 ± 19.77 (212.25 – 305.00)	237.94 ± 15.78 (215.00 – 280.75)	***< 0.001***

Data are reported as mean ± S.D. with range (min – max)

P values for between-group comparison analyzed by one-way ANOVA

FPG: fasting plasma glucose; PPG; postprandial plasma glucose; AUC: area under the curve

There were the significant suppressive effects on PPG-60 by DNJ 12 mg ($p < 0.05$) and DNJ 18 mg ($p < 0.001$) in comparison with the control group. The results suggested that mulberry leaves effectively reduced PPG at 30 min and 60 min after carbohydrate ingestion in the dose-dependent fashion. Meanwhile, no effect of the three doses of mulberry DNJ was observed on PPG-90, PPG-120, and PPG-180.

In addition to the suppressive effect of mulberry leaves on PPG at individual time points, mulberry leaves containing DNJ 12 mg and DNJ 18 mg significantly decreased the AUC $_{0\text{-}180\ min}$ of blood glucose ($p < 0.05$ and $p < 0.001$, respectively) when compared with control as shown in Figure 13.

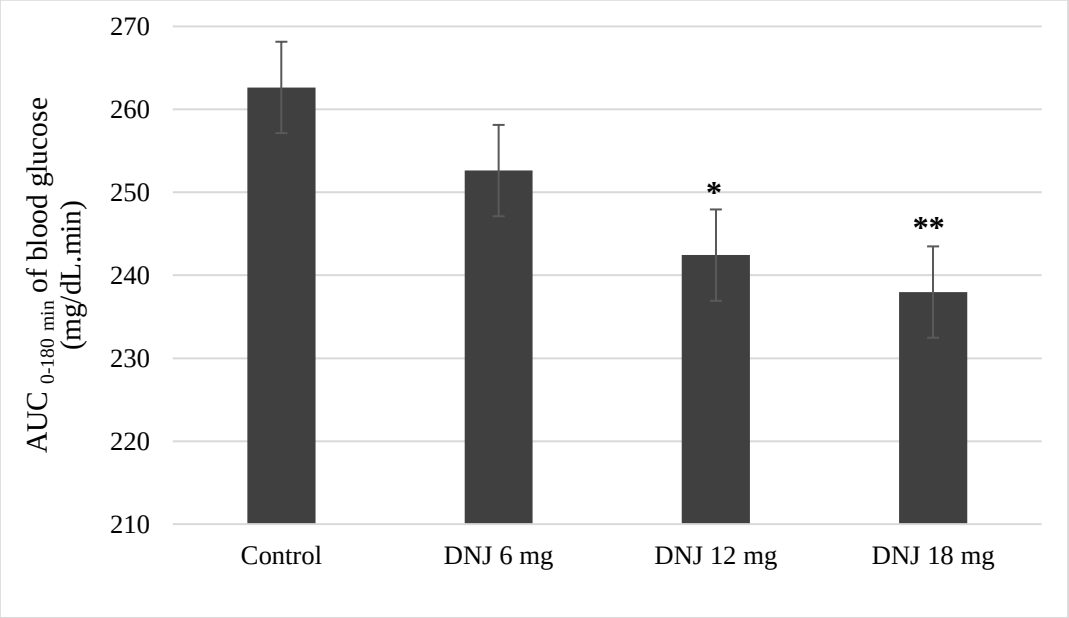


Figure 13 The AUC $_{0\text{-}180\ min}$ of blood glucose following co-administration of 50-g sucrose and the different doses of mulberry DNJ (* $p < 0.05$, ** $p < 0.001$ *vs.* control)

4.1.3 Adverse events of a single administration of mulberry leaves containing 6, 12, and 18 mg of DNJ

Regarding the assessment by Naranjo's algorithm, probable adverse events of a single administration of mulberry leaves containing the three doses of DNJ are summarized in Table 13. There were significant differences in the numbers of participants experiencing bloating and flatulence among the groups ($p = 0.002$).

Furthermore, persons who received mulberry leaves containing DNJ 18 mg were more likely to develop bloating and flatulence when compared with those who ingested mulberry leaves containing DNJ 12 mg (p = 0.064). On the other hand, no difference in frequencies of loose stool (p = 1.000) and nausea (p = 0.304) caused by the study intervention among the four groups was observed in this study. Nonetheless, participants well tolerated to the events. The symptoms gradually resolved with no need for any treatment within few days. In addition, no serious adverse effect was reported during the experiment and seven days thereafter.

Table 13 Adverse events of the different doses of mulberry leaves

Adverse events	**Numbers of participants (%)**			
	Group 1 (Control)	**Group 2** (DNJ 6 mg)	**Group 3** (DNJ 12 mg)	**Group 4** (DNJ 18 mg)
Bloating and flatulence	0 (0)	0 (0)	1 (4.8)	7 (31.8)
Loose stool	0 (0)	0 (0)	0 (0)	1 (4.5)
Nausea	1 (4.8)	0 (0)	0 (0)	2 (9.1)

Data are reported as numbers (%)

4.1.4 The effective dose with minimal adverse effects of mulberry DNJ

The effective dose of mulberry DNJ with minimal adverse effects was determined in study I. As described in the section 4.1.2, the strongest effective dose suppressing postprandial hyperglycemia in response to carbohydrate load was 18 mg and followed by 12 mg of DNJ. There were small differences in the significance levels between the two effective doses. In the meantime, in terms of safety, administration of DNJ 18 mg caused the high incidence of bloating and flatulence and the rate tended to be higher than DNJ 12 mg (p = 0.064). This however indicated the clinical meaning of the findings. Also, it could be possible to observe the statistical difference between the groups when the large sample size is included to the further study. As a result, DNJ 12 mg was considered the effective dose with minimal adverse effects of mulberry DNJ and was used in the study II.

4.2 Study II (Phase II clinical trial): To determine efficacy and safety of a long-term supplementation of mulberry leaves on glycemic control in persons who had impaired glucose metabolism including obese persons with prediabetes and patients with early-stage type 2 diabetes

The study was conducted from February 2018 to February 2019 at the Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand. The first participant was recruited on February 23, 2018. The last participant was followed up and the study completed on February 14, 2019.

Algorithm for study enrollment and data collection of study II is described in Figure 14. In the screening and recruitment period, 143 outpatients who had regular appointments at the Department of Family Medicine were informed the purposes and the methods of this study. Sixty persons declined to participate and 24 persons failed to meet the inclusion criteria. Therefore, a total of 59 persons were subsequently recruited into the run-in period and randomly allocated into the two groups: treatment group (n = 29) and control group (n = 30).

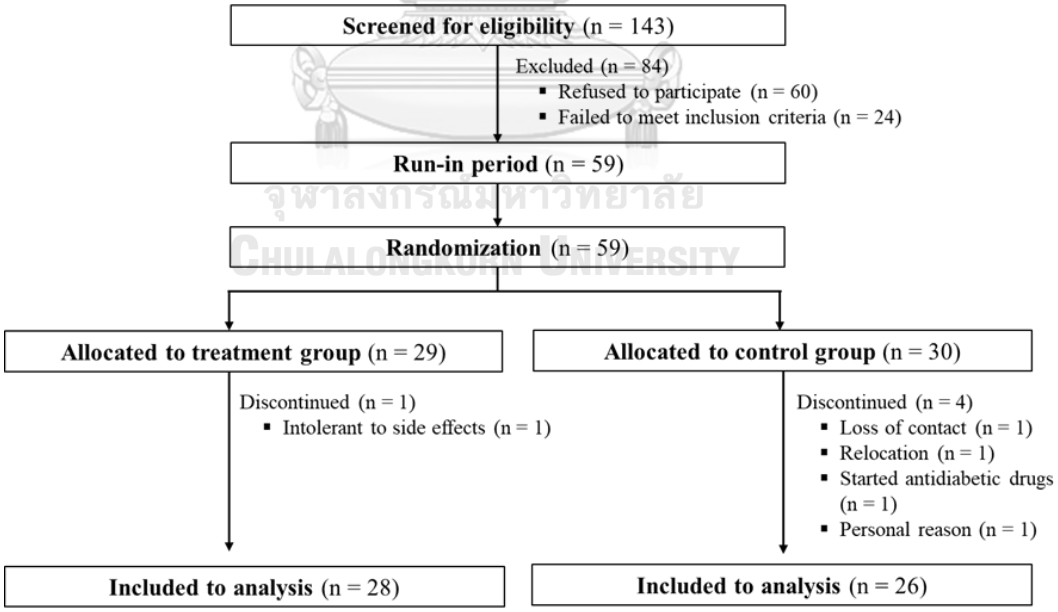


Figure 14 Algorithm for study enrollment and data collection of study II

One person in the treatment group terminated research participation because he was not able to tolerate to side effects of mulberry leaves, including bloating and frequent belching and fart. Four participants in the control group withdrawn from the study according to loss of contact, relocation, changing treatment regimen for diabetes, and personal reason. Finally, 28 and 26 participants in the treatment group and the control group, respectively, were included to the per-protocol analysis.

4.2.1 Participants characteristics

Table 14 summarizes numbers of the study participants in each group regarding the categories of impaired in glucose metabolism. Most of participants (92.6%) were categorized as prediabetes including 26 persons (92.9%) in the treatment group and 24 persons (92.3%) in the control group. Meanwhile, two persons (7.1% in the treatment group and 7.7% in the control group) were newly diagnosed patients with type 2 diabetes.

Table 14 Categories of impaired in glucose metabolism

Categories	**Treatment group (n = 28)**	**Control group (n = 26)**	***P values***
Prediabetes	26 (92.9)	24 (92.3)	*0.939*
IFG	21 (75.0)	21 (80.8)	*0.610*
IGT	14 (50.0)	16 (61.5)	*0.394*
Type 2 diabetes	2 (7.1)	2 (7.7)	*0.939*

Data are reported as numbers (%)
P values for between-group comparison analyzed by Chi-square (or Fisher's Exact test)
IFG: impaired fasting glucose; IGT: impaired glucose tolerance

Thirty-nine (72.2%) of all participants were female. The mean age of participants was 52.59 ± 6.89 years. BMI was averaged at 30.81 ± 5.02 kg/m^2. Table 15 presents demographic data and the baseline levels of clinical outcomes of participants. Results showed that the baseline characteristics were comparable between the two groups.

Table 15 Baseline characteristics of participants of study II (N = 54)

	Treatment group (n = 28)	**Control group (n = 26)**	***P values***
Demographic data			
Age (year)	53.14 ± 5.48 (40 - 63)	52.00 ± 8.22 (38 – 65)	*0.548*
Gender			*0.457*
Female	19 (67.9)	20 (76.9)	
Male	9 (32.1)	6 (23.1)	
Marital status			*0.107*
Single	8 (28.6)	7 (26.9)	
Married	12 (42.9)	17 (65.4)	
Divorce and widow	8 (28.6)	2 (7.7)	
Education			*0.535*
Under Bachelor's degree	16 (57.1)	17 (65.4)	
Bachelor's degree or over	12 (42.9)	9 (34.6)	
Occupation			*0.482*
Government and state enterprise officers	16 (57.1)	14 (53.8)	
Private employees	4 (14.3)	6 (23.1)	
Businesspersons	2 (7.1)	0 (0.0)	
Others	6 (21.4)	6 (23.1)	
Income			*0.070*
None to 10,000 Baht	3 (10.7)	6 (23.1)	
10,001 to 20,000 Baht	6 (21.4)	10 (38.5)	
20,001 to 30,000 Baht	15 (53.6)	5 (19.2)	
Over 30,000 Baht	4 (14.3)	5 (19.2)	
Medical welfare			*0.877*
Government and state enterprise officer	19 (67.9)	15 (57.7)	
Universal coverage (30-Baht) scheme	2 (7.1)	2 (7.7)	
Social security scheme	6 (21.4)	8 (30.8)	
Own payment	1 (3.6)	1 (3.8)	
Health-related data			
Current smoking	2 (7.1)	0 (0.0)	*0.491*
Current drinking alcohol	17 (60.7)	11 (42.3)	*0.186*
Concomitant diseases			
Hypertension	15 (53.6)	13 (50.0)	*0.793*
Dyslipidemia	23 (82.1)	18 (69.2)	*0.599*
First-degree relatives to diabetes	14 (50.0)	16 (61.5)	*0.394*

Table 15 Baseline characteristics of participants of study II (N = 54) (cont.)

	Treatment group (n = 28)	**Control group (n = 26)**	***P values***
Clinical outcomes			
Anthropometric data			
Weight (kg)	78.06 ± 13.98 (61.0 – 115.0)	82.93 ± 20.79 (57.8 – 165.5)	*0.314*
BMI (kg/m^2)	30.06 ± 4.06 (27.25 – 38.18)	31.61 ± 5.85 (25.35 – 54.02)	*0.261*
Waist circumference (cm)	100.52 ± 11.21 (84.0 – 131.0)	102.92 ± 15.67 (85.0 – 165.0)	*0.517*
Vital signs			
SBP (mmHg)	127.89 ± 11.97 (101 – 148)	130.77 ± 17.29 (94 – 161)	*0.478*
DBP (mmHg)	77.68 ± 10.54 (54 – 95)	77.00 ± 13.14 (54 – 109)	*0.834*
HR (bpm)	79.82 ± 9.40 (65 – 95)	77.00 ± 10.01 (59 – 97)	*0.290*
Blood glucose			
FPG (mg/dL)	107.04 ± 10.28 (81 – 127)	107.50 ± 9.09 (78 – 119)	*0.864*
PPG-30 (mg/dL)	173.89 ± 17.52 (133 – 208)	172.04 ± 21.48 (121 – 216)	*0.729*
PPG-60 (mg/dL)	186.61 ± 29.95 (112 – 235)	191.46 ± 31.96 (131 – 271)	*0.567*
PPG-90 (mg/dL)	171.21 ± 33.19 (99 – 252)	177.46 ± 40.25 (120 – 282)	*0.535*
PPG-120 (mg/dL)	141.14 ± 32.97 (71 – 220)	155.12 ± 37.53 (96 – 264)	*0.151*
AUC $_{0\text{-}120\ min}$ (mg/dL.min)	331.83 ± 40.15 (247 – 423)	336.08 ± 52.28 (250 – 458)	*0.738*
A1C (%)	5.8 ± 0.4 (5 – 7)	5.7 ± 0.3 (5 – 7)	*0.180*
Insulin			
FPI (μIU/mL)	13.55 ± 7.85 (3.9 – 36.8)	14.39 ± 6.02 (6.2 – 28.5)	*0.668*
HOMA-IR	3.60 ± 2.18 (1.1 – 9.5)	3.84 ± 1.72 (1.7 – 8.1)	*0.667*

Table 15 Baseline characteristics of participants of study II (N = 54) (cont.)

	Treatment group (n = 28)	**Control group (n = 26)**	***P values***
Lipids			
TC (mg/dL)	194.99 ± 35.53 (125.4 – 270.9)	200.66 ± 37.93 (138.3 – 268.4)	*0.573*
TG (mg/dL)	131.63 ± 45.22 (52.5 – 248.5)	145.03 ± 59.99 (76.8 – 314.5)	*0.356*
HDL-C (mg/dL)	54.89 ± 14.35 (28.0 – 86.6)	53.56 ± 9.82 (36.9 – 69.1)	*0.694*
LDL-C (mg/dL)	139.06 ± 33.80 (72.1 – 196.2)	137.46 ± 34.25 (73.0 – 193.3)	*0.864*
Hepatic and renal function			
AST (U/L)	21.93 ± 4.84 (12.7 – 33.8)	19.94 ± 5.19 (12.7 – 33.2)	*0.151*
ALT (U/L)	23.22 ± 19.24 (11.0 – 39.3)	19.24 ± 7.05 (7.2 – 34.5)	*0.055*
Cr (mg/dL)	0.81 ± 0.18 (0.53 – 1.24)	0.72 ± 0.19 (0.48 – 1.42)	*0.078*

Data are reported as numbers (%) and mean ± S.D. with range (min – max) for categorical and continuous variables, respectively

P values for between-group comparison analyzed by Chi-square (or Fisher's Exact test) and one-way ANOVA for categorical and continuous variables, respectively

kg: kilogram; BMI: body mass index; kg/m^2: kilogram per square meter; SBP: systolic blood pressure; DBP: diastolic blood pressure; mmHg: millimeter of mercury; HR: heart rate; bpm: beat per minute; FPG: fasting plasma glucose; mg/dL: milligram per deciliter; PPG: postprandial plasma glucose; AUC: area under the curve; A1C: glycated hemoglobin; FPI: fasting plasma insulin; μIU/mL: micro international unit per milliliter; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; U/L: unit per liter: Cr: creatinine

4.2.2 Effects of mulberry leaves on glycemic control

Effects of mulberry leaves on blood glucose and insulin resistance were determined and reported as the mean concentrations and the mean changes of glycemic outcomes at baseline and week 12 in Table 16 and Table 17, respectively.

1) PPG following 75-g OGTT

At the end of study, postprandial glucose excursion following the standard OGTT did not differ between the groups. A significant reduction in PPG-30 by -10.75 ± 22.64 mg/dL ($p = 0.018$) when compared with the initial level was however observed in the treatment group at week 12. In addition, the $AUC_{0\text{-}120\ min}$ of glucose in the mulberry leaves-treated group tended to be lower than baseline by -8.81 ± 35.90 mg/dL.min ($p = 0.073$).

2) A1C

The levels of A1C of the treatment group and the control group was not different at week 12. There was a significant improvement in A1C among participants in the treatment group after receiving mulberry leaves. The mean A1C significantly reduced by -0.11 ± 0.22% ($p = 0.011$) in the treatment group, whereas no change in A1C was observed in the control group.

3) FPG

At baseline, week 4, week 8, and week 12, the mean values of FPG were 107.04 ± 10.28, 102.75 ± 10.01, 101.43 ± 9.69, and 103.18 ± 9.45 mg/dL in the treatment group and were 107.50 ± 9.09, 106.73 ± 7.79, 107.73 ± 8.92, and 106.96 ± 10.31 mg/dL in the control group, respectively. There was no difference in FPG between the groups at every time points of measurements, except at week 8. The mean FPG at week 8 of the treatment group significantly lower than the control group ($p = 0.016$).

Figure 15 shows the significant alterations in FPG along the 12-week duration in the mulberry leaves-treated group. When compared with the baseline level, FPG of the treatment group significantly reduced by -4.29 ± 5.69 mg/dL ($p < 0.001$) at week 4, -5.61 ± 8.47 mg/dL at week 8 ($p = 0.002$), and -3.86 ± 5.99 mg/dL ($p = 0.002$) at

week 12. On the other hand, no change in FPG was detected in the control group throughout the study.

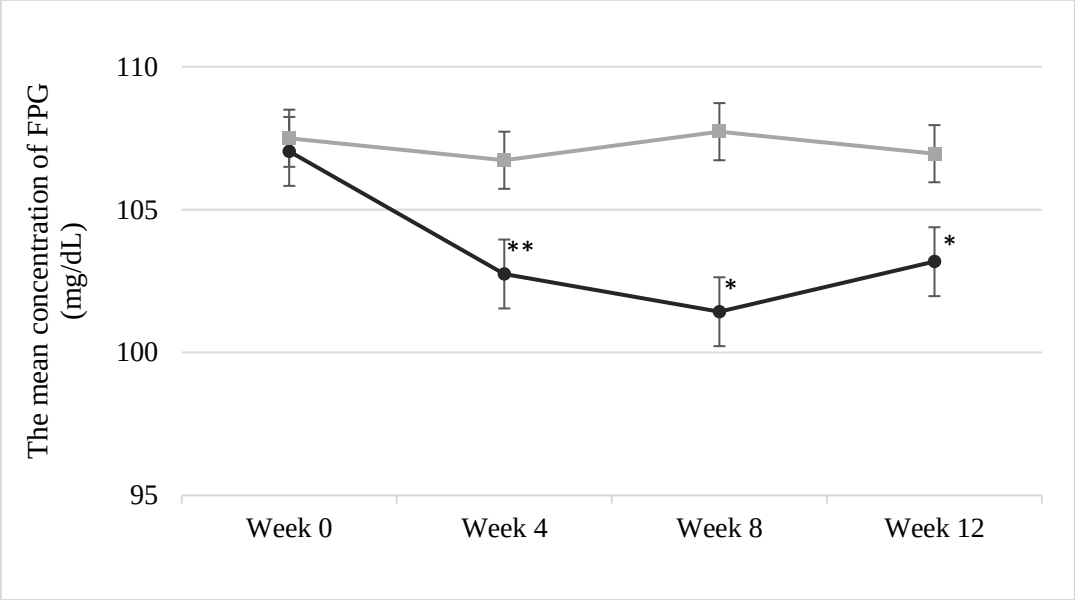


Figure 15 The alterations in FPG of the treatment group (●) and the control group (■) throughout the 12-week study period. (* $p < 0.05$, ** $p < 0.001$ vs. the baseline level analyzed by repeated-measures ANOVA)

Table 16 The mean concentrations and the mean changes of glycemic outcomes at baseline and week 12 (N = 54)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
FPG (mg/dL)	Baseline	107.04 ± 10.28 (81 – 127)		107.50 ± 9.09 (78 – 119)		
	Week 4	102.75 ± 10.01 (87 – 116)	***< 0.001***[+]	106.73 ± 7.79 (77 – 115)	*0.542*	*0.111*
	Week 8	101.43 ± 9.69 (80 – 120)	***0.002***[+]	107.73 ± 8.92 (77 – 112)	*0.850*	***0.016***
	Week 12	103.18 ± 9.45 (81 – 125)	***0.002***[+]	106.96 ± 10.31 (76 – 120)	*0.775*	*0.166*
PPG-30 (mg/dL)	Baseline	173.89 ± 17.52 (133 – 208)		172.04 ± 21.48 (121 – 216)		
	Week 12	163.14 ± 20.63 (113 – 211)	***0.018***	166.96 ± 19.78 (122 – 199)	*0.210*	*0.491*

Table 16 The mean concentrations and the mean changes of glycemic outcomes at baseline and week 12 (N = 54) (cont.)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	*P values*[#]	Mean ± S.D. (Min - Max)	*P values*[#]	
PPG-60 (mg/dL)	Baseline	186.61 ± 29.95 (112 – 235)		191.46 ± 31.96 (131 – 271)		
	Week 12	183.14 ± 24.81 (141 – 256)	*0.528*	190.65 ± 34.30 (122 – 245)	*0.865*	*0.358*
PPG-90 (mg/dL)	Baseline	171.21 ± 33.19 (99 – 252)		177.46 ± 40.25 (120 – 282)		
	Week 12	169.29 ± 29.92 (115 – 226)	*0.734*	174.04 ± 45.20 (102 – 263)	*0.482*	*0.648*
PPG-120 (mg/dL)	Baseline	141.14 ± 32.97 (71 – 220)		155.12 ± 37.53 (96 – 264)		
	Week 12	163.14 ± 20.63 (113 – 211)	*0.251*	166.96 ± 19.78 (122 – 199)	*0.626*	*0.173*

Table 16 The mean concentrations and the mean changes of glycemic outcomes at baseline and week 12 (N = 54) (cont.)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
AUC $_{0\text{-}120\ min}$ (mg/dL.min)	Baseline	331.83 ± 40.15 (247 – 423)		336.08 ± 52.28 (250 – 458)		
	Week 12	323.02 ± 35.75 (231.8 – 404.5)	*0.073*	330.52 ± 55.58 (222.5 – 431.0)	*0.387*	*0.327*
A1C (%)	Baseline	5.8 ± 0.4 (5 – 7)		5.7 ± 0.3 (5 – 7)		
	Week 12	5.7 ± 0.3 (5.1 – 6.6)	***0.011***	5.8 ± 0.4 (5.1 – 6.6)	*0.100*	*0.551*

Data are reported as mean ± S.D. with range (min – max)

[*] P values for between-group comparison (*vs.* control) analyzed by one-way ANOVA

[#] P values for within-group comparison (*vs*. baseline) analyzed by paired t-test

[+] P values for within-group comparison (*vs*. baseline) analyzed by repeated-measure ANOVA

FPG: fasting plasma glucose; PPG: postprandial plasma glucose; mg/dL: milligram per deciliter; AUC: area under the curve; A1C: glycated hemoglobin

Table 17 The mean concentrations and the mean FPI and HOMA-IR at baseline and week 12 (N = 53)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
FPI (μIU/mL)	Baseline	13.55 ± 7.85 (3.9 – 36.8)		14.39 ± 6.02 (6.2 – 28.5)		
	Week 12	12.42 ± 6.66 (2.5 – 27.6)	*0.188*	14.05 ± 7.32 (4.4 – 35.7)	*0.643*	*0.399*
HOMA-IR	Baseline	3.60 ± 2.18 (1.1 – 9.5)		3.84 ± 1.72 (0.7 – 7.8)		
	Week 12	3.18 ± 1.78 (1.7 – 8.1)	*0.057*	3.78 ± 2.18 (1.1 – 10.0)	*0.781*	*0.278*

Data are reported as mean ± S.D. with range (min – max)

[*] P values for between-group comparison (*vs.* control) analyzed by one-way ANOVA

[#] P values for within-group comparison (*vs*. baseline) analyzed by paired t-test

FPI: fasting plasma insulin; μIU/mL: micro international unit per milliliter; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance

4) Insulin resistance

There was a specimen hemolysis during the procedure of blood collection in one sample; thus, the sample was excluded to avoid interference on the biochemical analysis. Finally, there were 27 and 26 blood samples remained in the treatment group and the control group, respectively, for the laboratory test of FPI.

At the end of study, the values of FPI and HOMA-IR did not differ between the two groups. However, the mean HOMA-IR of the mulberry leaves-treated group tended to reduce from the initial level ($p = 0.057$) after the 12-week study period.

5) Changes in the categories of impaired glucose metabolism

Four persons in the treatment group and one person in the control group conversed from prediabetes to normal glucose metabolism at the end of study. No participant who diagnosed with prediabetes in the both groups developed type 2 diabetes during the study period. Table 18 shows changes in the categories of impaired glucose metabolism in the treatment group and the control group from baseline.

4.2.3 Effects of mulberry leaves on anthropometric outcomes and vital signs

Table 19 demonstrates anthropometric profiles and vital signs of participants in the treatment group and the control group at week 12 when compared with baseline. In this study, the anthropometric outcomes refer to body weight, BMI, and waist circumference. No difference in these outcomes between the two groups and no change from baseline in these outcomes were found at week 12. However, there was a trend of BMI reduction in both groups ($p = 0.075$ in the treatment group and $p = 0.094$ in the control group *vs.* the baseline levels).

There was no effect of mulberry leaves administration on vital signs as seen in the level of blood pressure and heart rate in the treatment group at week 12. Similarly, no change in vital signs was found in the control group.

Table 18 Changes in the categories of impaired glucose metabolism (N = 54)

Categories	Treatment group (n = 28)		Control group (n = 26)		*P values*
	Baseline	**Week 12**	**Baseline**	**Week 12**	
Normal glucose metabolism	0 (0)	4 (14.3)	0 (0)	1 (3.8)	*0.385*
Prediabetes	26 (92.9)	22 (78.6)	24 (92.3)	23 (88.5)	*0.330*
IFG	21 (75.0)	18 (64.3)	21 (80.8)	20 (76.9)	*0.310*
IGT	14 (50.0)	9 (32.1)	16 (61.5)	15 (57.7)	*0.059*
Type 2 diabetes	2 (7.1)	2 (7.1)	2 (7.7)	2 (7.7)	*0.939*

Data are reported as number (%)

P values for between-group comparison (*vs.* control) at week 12 analyzed by Chi-square (or Fisher's Exact test)

IFG: impaired fasting glucose; IGT: impaired glucose tolerance

Table 19 The mean anthropometric outcomes and vital signs at baseline and week 12 (N = 54)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
Weight (kg)	Baseline	78.06 ± 13.98 (61.0 – 115.0)		81.31 ± 20.79 (57.8 – 165.5)		
	Week 12	77.43 ± 14.30 (58.7 – 115.4)	*0.075*	80.92 ± 14.06 (58.0 – 114.7)	*0.098*	*0.352*
BMI (kg/m^2)	Baseline	30.06 ± 4.06 (27.25 – 38.18)		31.01 ± 5.85 (25.35 – 54.02)		
	Week 12	29.80 ± 4.07 (25.43 – 37.67)	*0.075*	30.82 ± 3.86 (25.34 – 38.58)	*0.094*	*0.272*
WC (cm)	Baseline	100.52 ± 11.21 (84 – 131)		102.92 ± 15.67 (85 – 165)		
	Week 12	100.77 ± 11.32 (84 – 131)	*0.586*	101.98 ± 11.08 (85 – 134)	*0.459*	*0.693*

Table 19 The mean anthropometric outcomes and vital signs at baseline and week 12 (N = 54) (cont.)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
SBP (mmHg)	Baseline	127.89 ± 11.97 (101 – 148)		130.77 ± 17.29 (94 – 161)		
	Week 12	128.64 ± 12.57 (103 – 155)	*0.658*	129.62 ± 14.61 (103 – 151)	*0.638*	*0.794*
DBP (mmHg)	Baseline	77.68 ± 10.54 (54 – 95)		77.00 ± 13.14 (54 – 109)		
	Week 12	77.68 ± 12.43 (50 – 106)	*1.000*	76.92 ± 11.71 (60 – 101)	*0.963*	*0.819*
HR (bpm)	Baseline	79.82 ± 9.40 (65 – 95)		77.00 ± 10.01 (59 – 97)		
	Week 12	77.89 ± 9.62 (57 – 97)	*0.175*	79.19 ± 10.39 (57 – 102)	*0.311*	*0.635*

Data are reported as mean ± S.D. with range (min – max)

[*] P values for between-group comparison (*vs.* control) analyzed by one-way ANOVA

[#] P values for within-group comparison (*vs*. baseline) analyzed by paired t-test

kg: kilogram; BMI: body mass index; kg/m^2: kilogram per square meter; WC: waist circumference; cm: centimeter; SBP: systolic blood pressure; DBP: diastolic blood pressure; mmHg: millimeter of mercury; HR: heart rate; bpm: beat per minute

4.2.4 Effects of mulberry leaves on blood lipids

Table 20 demonstrates effects of mulberry leaves treatment on lipid profiles based on the per-protocol analysis (N = 54). After the 12-week treatment period, the mean TC of the treatment group was significantly lower than the control group by -17.91 ± 36.50 mg/dL (p = 0.034). The value of TC also significant reduced by -13.42 ± 32.77 mg/dL (p = 0.002) when compared with the initial level. However, no effect of mulberry leaves on TG, HDL-C, and LDL-C was found.

Furthermore, to determine the effects of mulberry leaves on blood lipids in persons who had dyslipidemia only, the case selection analysis was additionally performed. In this analysis, participants who were diagnosed with dyslipidemia, met the criteria of dyslipidemia (TC ≥ 200, TG ≥ 150, HDL-C ≤ 60, and/or LDL-C ≥ 100 mg/dL), or treated with drugs for dyslipidemia, including 25 persons in the treatment group and 20 persons in the control group, were included. However, two in the treatment group and two in the control group were subsequently excluded because treatment regimen for dyslipidemia was changed. Thus, 23 and 18 persons in the treatment group and the control group, respectively, left for the analysis.

As shown in Table 21, the treatment group had the lowered TC by -19.52 ± 27.06 mg/dL (p = 0.024) in comparison with the control group at the end of study. No difference in TG, HDL-C, and LDL-C was observed between the two groups. Meanwhile, the results also showed the significant decreases in TC and LDL-C by -17.10 ± 19.93 mg/dL (p < 0.001) and -10.33 ± 22.45 mg/dL (p = 0.038) in the treatment group after the 12-week study period when compared with the baseline levels. In addition, the level of TG of the treatment group tended to reduce by -12.36 ± 30.78 mg/dL when compared with baseline with the borderline significant level (p = 0.067).

Table 20 The mean concentrations of blood lipids at baseline and week 12 (N = 54)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
TC (mg/dL)	Baseline	194.99 ± 35.53 (125.4 – 270.9)		200.66 ± 37.93 (138.3 – 268.4)		
	Week 12	181.58 ± 26.64 (134.0 – 276.3)	***0.002***	199.49 ± 33.76 (146.9 – 263.9)	*0.786*	***0.034***
TG (mg/dL)	Baseline	131.63 ± 45.22 (52.5 – 248.5)		145.03 ± 59.99 (76.8 – 314.5)		
	Week 12	123.52 ± 35.87 (67.7 – 215.3)	*0.148*	138.10 ± 51.38 (66.0 – 241.8)	*0.583*	*0.193*
HDL-C (mg/dL)	Baseline	54.89 ± 14.35 (28.0 – 86.6)		53.56 ± 9.82 (36.9 – 69.1)		
	Week 12	52.48 ± 10.83 (33.6 – 78.3)	*0.098*	54.63 ± 8.66 (40.5 – 66.8)	*0.302*	*0.424*
LDL-C (mg/dL)	Baseline	139.06 ± 33.80 (72.1 – 196.2)		137.46 ± 34.25 (73.0 – 193.3)		
	Week 12	131.66 ± 29.54 (80.1 – 207.4)	*0.090*	137.25 ± 29.81 (82.5 – 199.4)	*0.959*	*0.492*

Data are reported as mean ± S.D. with range (min – max)

[*] P values for between-group comparison (*vs.* control) analyzed by one-way ANOVA

[#] P values for within-group comparison (*vs*. baseline) analyzed by paired t-test

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; mg/dL: milligram per deciliter

Table 21 The mean concentrations of blood lipids in persons with dyslipidemia at baseline and week 12 (N = 41)

Outcomes	Time points of measurement	Treatment group (n = 23)		Control group (n = 18)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
TC (mg/dL)	Baseline	202.45 ± 29.94 (158.3 – 270.9)		212.75 ± 28.53 (175.3 – 268.4)		
	Week 12	185.35 ± 22.57 (144.2 – 276.3)	***< 0.001***	204.87 ± 30.75 (150.8 – 263.9)	*0.099*	***0.024***
TG (mg/dL)	Baseline	135.88 ± 41.13 (52.5 – 248.5)		151.24 ± 66.30 (76.8 – 314.5)		
	Week 12	123.52 ± 35.87 (52.2 – 215.3)	*0.067*	138.10 ± 51.38 (66.0 – 241.8)	*0.390*	*0.291*
HDL-C (mg/dL)	Baseline	56.68 ± 14.12 (35.6 – 86.6)		56.82 ± 9.73 (38.0 – 69.1)		
	Week 12	53.77 ± 9.97 (38.0 – 78.3)	*0.094*	57.64 ± 8.02 (42.6 – 69.8)	*0.553*	*0.187*
LDL-C (mg/dL)	Baseline	145.89 ± 28.63 (84.7 – 196.2)		145.95 ± 30.00 (73.0 – 193.3)		
	Week 12	135.56 ± 27.03 (89.3 – 207.4)	***0.038***	139.68 ± 29.68 (93.5 – 199.4)	*0.158*	*0.645*

Data are reported as mean ± S.D. with range (min – max)

[*] P values for between-group comparison (*vs.* control) analyzed by one-way ANOVA

[#] P values for within-group comparison (*vs*. baseline) analyzed by paired t-test

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; mg/dL: milligram per deciliter

4.2.5 Effects of mulberry leaves on hepatic and renal function

To determine undesirable effects of mulberry leaves on hepatic and renal function, the levels of AST, ALT, and Cr were measured and demonstrated in Table 22. At week 12, no difference in these outcomes between the groups was observed. Moreover, at the end of study, the mean values of AST, ALT, and Cr of the two groups were in the normal ranges similar to the baseline levels.

4.2.6 Self-reported adverse events of mulberry leaves

Table 23 shows the numbers and frequencies of participants who experienced adverse events of mulberry leaves throughout the study. Severities of the events were generally acceptable by the study participants. Nonetheless, one participant could not tolerate to bloating and frequent belching and fart that considerably interrupt his daily life. He therefore terminated research participation at week 8.

4.2.7 Compliance

Compliance to mulberry leaves ingestion of participants in the treatment group was assessed by the same basis as the pill count method. Results suggested no change in compliance throughout the study. At week 4, 8, and 12, the mean compliances were equal to 90.41 ± 8.35, 90.99 ± 7.78, and 91.81 ± 6.66 % (p = 0.239).

Table 22 The mean concentrations of AST, ALT, and Cr at baseline and week 12 (N = 54)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
AST (U/L)	Baseline	21.93 ± 4.84 (12.7 – 33.8)		19.94 ± 5.19 (12.7 – 33.2)		
	Week 12	21.84 ± 6.17 (13.6 – 40.6)	*0.927*	22.27 ± 8.19 (11.6 – 42.0)	*0.113*	*0.825*
ALT (U/L)	Baseline	23.22 ± 19.24 (11.0 – 39.3)		19.24 ± 7.05 (7.2 – 34.5)		
	Week 12	25.24 ± 13.09 (11.0 – 50.9)	*0.345*	23.58 ± 17.49 (9.8 – 44.0)	*0.114*	*0.692*
Cr (mg/dL)	Baseline	0.81 ± 0.18 (0.53 – 1.24)		0.72 ± 0.19 (0.48 – 1.42)		
	Week 12	0.77 ± 0.23 (0.43 – 1.22)	*0.149*	0.70 ± 0.18 (0.45 – 1.20)	*0.191*	*0.244*

AST: aspartate aminotransferase; ALT; alanine aminotransferase; U/L: unit per liter; Cr: creatinine; mg/dL: milligram per deciliter

Table 23 Adverse events of daily administration of mulberry leaves reported by the study participants

Adverse events	Numbers of participants (%)		
	Week 4	Week 8	Week 12
Hypoglycemia	0 (0)	0 (0)	0 (0)
Abdominal pain	0 (0)	2 (7.1)	1 (3.6)
Bloating and flatulence	14 (50.0)	11 (39.3)	8 (28.6)
Loose stools	7 (25.0)	6 (21.4)	2 (7.1)
Constipation	6 (21.4)	4 (14.3)	5 (17.9)

Data are reported as numbers (%)

4.2.8 Total caloric and macronutrients intake

The mean total caloric and macronutrients intake are presented in Table 24. There was no difference between the group at baseline and at the endpoint of study. Moreover, no change in the mean total caloric and macronutrients intake at the end of study when compared with the baseline levels in the both two groups.

Table 24 The mean total calorie and macronutrients intake per day at baseline and week 12 (N = 54)

Outcomes	Time points of measurement	Treatment group (n = 28)		Control group (n = 26)		*P values*[*]
		Mean ± S.D. (Min - Max)	***P values*[#]**	**Mean ± S.D. (Min - Max)**	***P values*[#]**	
Total calorie (kcal/day)	Baseline	1,707.75 ± 144.16 (1,561.6 – 2001.8)		1,648.29 ± 173.75 (1,535.7 – 1920.9)		
	Week 12	1,777.41 ± 141.94 (1,479.7 – 1804.4)	*0.409*	1,626.62 ± 152.42 (1,458.0 – 1780.2)	*0.156*	*0.083*
Carbohydrate (g/day)	Baseline	169.13 ± 56.71 (88.1 – 215.4)		163.44 ± 69.08 (123.7 – 197.8)		
	Week 12	165.94 ± 52.52 (90.4 – 189.0)	*0.696*	156.78 ± 41.39 (110.6 – 192.3)	*0.534*	*0.482*
Fat (g/day)	Baseline	52.33 ± 14.10 (33.8 – 80.2)		57.29 ± 16.27 (39.9 – 101.5)		
	Week 12	55.98 ± 9.27 (30.5 – 90.9)	*0.224*	56.13 ± 8.54 (48.1 – 104.2)	*0.732*	*0.152*
Protein (g/day)	Baseline	64.16 ± 17.66 (47.2 – 88.6)		63.86 ± 14.40 (51.8 – 96.6)		
	Week 12	70.39 ± 12.19 (50.1 – 90.1)	*0.325*	68.34 ± 9.17 (45.6 – 111.4)	*0.077*	*0.748*

Data are reported as mean ± S.D. with range (min – max)

[*] P values for between-group comparison (*vs.* control) analyzed by one-way ANOVA

[#] P values for within-group comparison (*vs.* baseline) analyzed by paired t-test

4.3 Study III (proteomic analysis): to determine effect of the long-term supplementation of mulberry leaves on expressions of plasma proteins in persons who had impaired glucose metabolism, including obese persons with prediabetes and patients with early-stage type 2 diabetes

4.3.1 Participant characteristics

Twelve obese persons with prediabetes and patients with early-stage type 2 diabetes who participating the phase-II clinical study were considered as the selected cases for proteomic analysis. Eight (66.7%) were female participants. Table 25 presents clinical characteristics of the selected ones.

4.3.2 Plasma protein profile

Protein profiles in plasma of the selected cases at the time before (week 0) and after (week 12) receiving mulberry leaves together with diet control, was determined using LC-MS/MS coupled with the label-free proteomic analysis. A total of 1,291 plasma proteins in the triple pooled samples were identified. Figure 16 shows that 309 and 203 proteins were uniquely found in the before-treatment and the after-treatment samples, respectively, whereas 779 proteins were concomitantly observed in the both samples. When compared with the baseline profile, 523 of 1,291 proteins that expressed differentially after receiving the treatment, including 210 up-expressed proteins and 313 down-expressed proteins (Appendix H). According to biological roles, the 523 differentially expressed proteins were classified as the proteins that associated with cellular process, cell interaction and communication, structural constituents, defense system, glucose and lipid metabolism, signaling pathways, protein metabolism, ion transport, blood coagulation, and others; however, the functions of some proteins remain unknown as demonstrated in Figure 17.

Table 25 Baseline characteristics of participants of study III (N = 12)

	At week 0	**At week 12**
Weight (kg)	77.74 ± 10.64 (63.0 – 96.1)	76.80 ± 10.83 (60.1 – 95.1)
BMI (kg/m^2)	30.23 ± 3.99 (27.25 – 38.18)	29.83 ± 3.81 (27.55 – 37.05)
Waist circumference (cm)	99.88 ± 8.58 (89.0 – 122.0)	99.54 ± 8.74 (86.5 – 121.0)
FPG (mg/dL)	108.10 ± 10.58 (95 – 126)	101.75 ± 10.15 (92 – 116)
PPG-120 (mg/dL)	145.25 ± 39.52 (89 – 220)	137.42 ± 38.13 (110 – 195)
A1C (%)	6.0 ± 0.4 (5.5 – 6.9)	5.8 ± 0.5 (5.1 – 6.6)
HOMA-IR	3.35 ± 1.67 (1.6 – 5.6)	3.27 ± 1.57 (1.1 – 4.7)
TC (mg/dL)	181.41 ± 27.97 (125.6 – 221.4)	168.05 ± 13.88 (150.2 – 193.1)
TG (mg/dL)	123.88 ± 36.65 (61.0 – 172.0)	116.74 ± 30.69 (60.8 – 152.1)
HDL-C (mg/dL)	54.71 ± 15.12 (35.6 – 84.0)	53.23 ± 10.39 (38.0 – 77.3)
LDL-C (mg/dL)	126.06 ± 31.46 (74.4 – 187.1)	118.23 ± 16.21 (99.3 – 159.8)

Data are reported as numbers (%) and mean ± S.D. with range (min – max) for categorical and continuous variables, respectively

kg: kilogram; BMI: body mass index; kg/m^2: kilogram per square meter; FPG: fasting plasma glucose; mg/dL: milligram per deciliter; PPG: postprandial plasma glucose; AUC: area under the curve; A1C: glycated hemoglobin; FPI: fasting plasma insulin; μIU/mL: micro international unit per milliliter; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

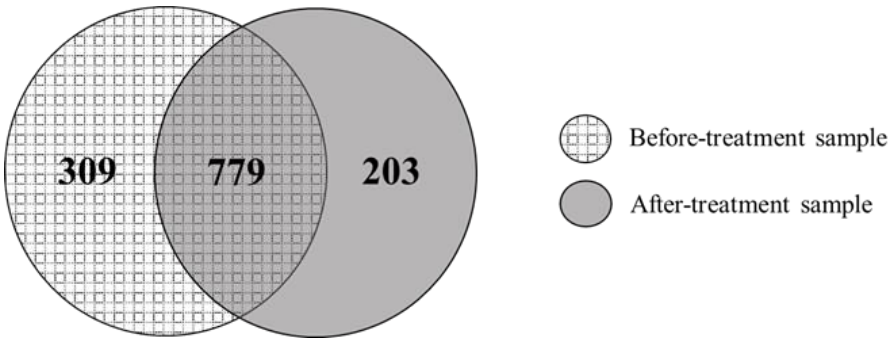


Figure 16 The numbers of identified proteins in the pooled plasma samples before and after receiving the interventions (n = 1,291)

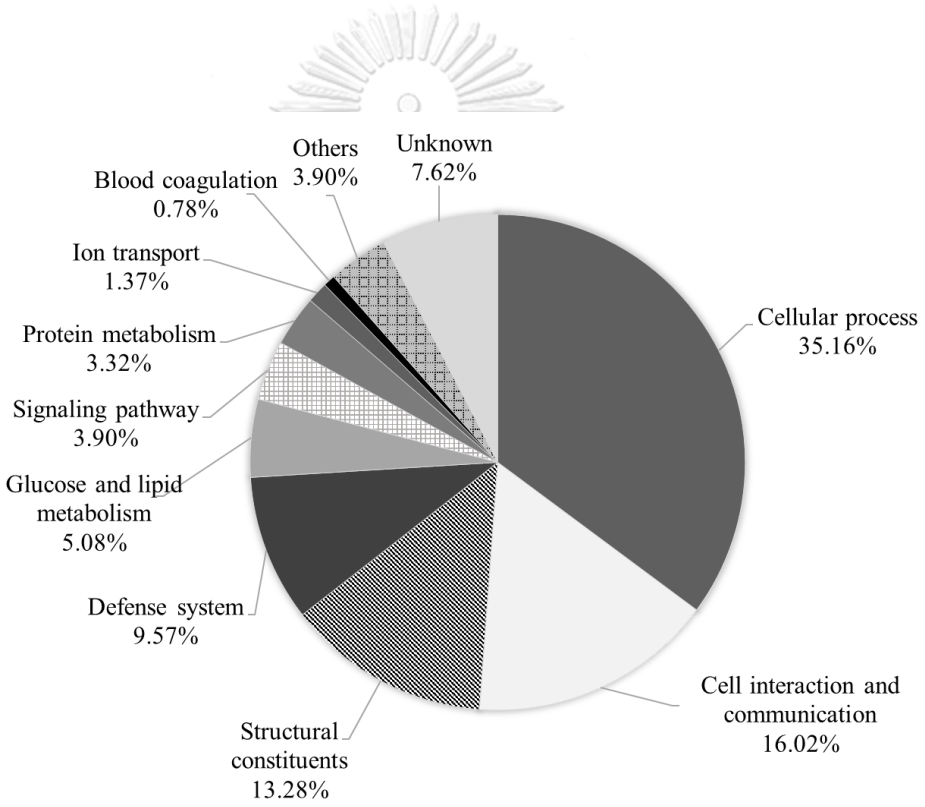


Figure 17 The differential expressed proteins categorized by biological functions (n = 523)

4.3.3 Identification of protein functions and the associated KEGG pathways

Due to the large diversity of the identified proteins in the samples, the GO enrichment analysis was performed. Of the 523 proteins, 212 proteins were considered as the proteins that closely linked to pathophysiology and pathogenesis of insulin resistance and type 2 diabetes. According to the STRING database, the protein-protein interaction networks of 212 proteins were mapped (Appendix I). The protein map revealed the highly complicated relationships among the proteins of interest. As a result, the KEGG pathway analysis and the KEGG database were used in order to better identify the potential associated pathway based on protein function. The analysis showed that the proteins were associated with various biological pathways as described in Table 26. For ease of result interpretation, based on similarities and pathway linkage, the 13 potential pathways were further grouped together into four major categories: (i) metabolic regulation, (ii) ECM constituents and organization, (iii) immune response, and (iv) inflammatory response as demonstrated in Figure 18.

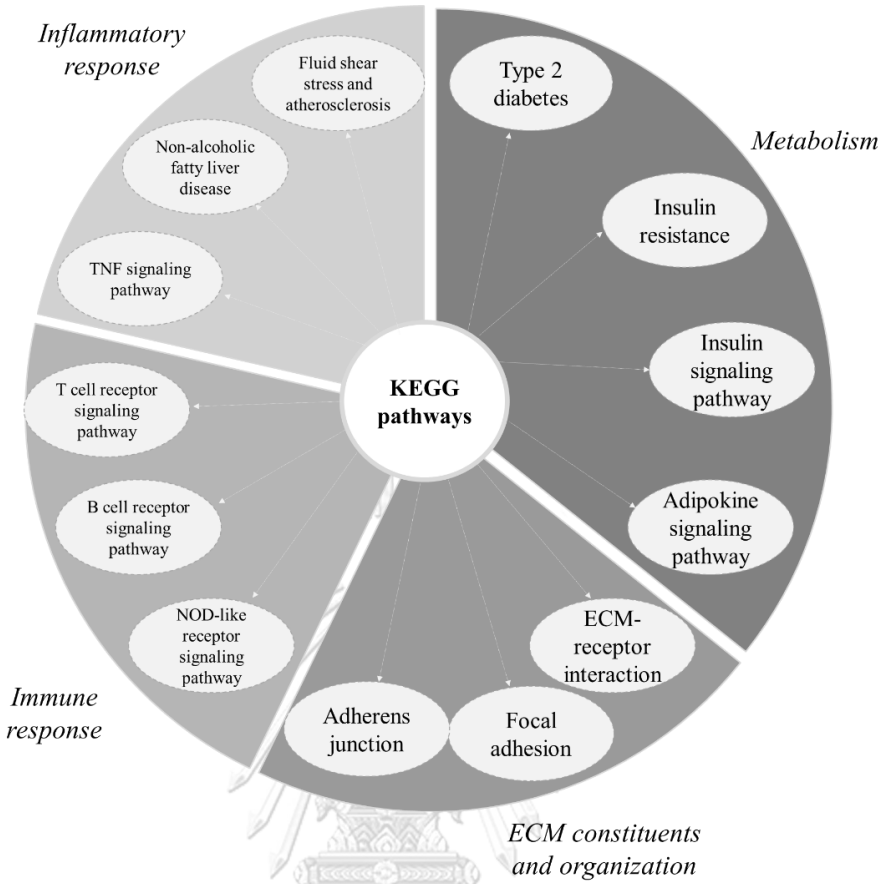


Figure 18 Functional categories of KEGG pathways

Table 26 KEGG pathways and the matching proteins

KEGG pathways	Observed gene count	Encoding gene	Matching proteins
I. Type 2 diabetes	4	*PIK3R2*	Phosphatidylinositol 3-kinase regulatory subunit β
		INSR	Insulin receptor
		IRS2	Insulin receptor substrate 2
		HKDC1	Hexokinase domain-containing protein 1
II. Insulin resistance	5	*PIK3R2*	Phosphatidylinositol 3-kinase regulatory subunit β
		INSR	Insulin receptor
		IRS2	Insulin receptor substrate 2
		NR1H3	Oxysterols receptor LXR-α
		TNFRSF1A	TNF receptor superfamily member 1A
III. Insulin signaling pathway	6	*PIK3R2*	Phosphatidylinositol 3-kinase regulatory subunit β
		INSR	Insulin receptor
		IRS2	Insulin receptor substrate 2
		HKDC1	Hexokinase domain-containing protein 1
		SOS1	Son of sevenless homolog 1
		SOS2	Son of sevenless homolog 2
IV. Adipocytokine signaling pathway	4	*TNFRSF1A*	TNF receptor superfamily member 1A
		CHUK	Inhibitor of NF-κB kinase subunit α
		IKBKG	NF-κB essential modulator
		RBP4	Retinol-binding protein 4

Table 26 KEGG pathways and the matching proteins (cont.)

KEGG pathways	Observed gene count	Encoding gene	Matching proteins
V. ECM-receptor interaction	8	*COL2A1*	Collagen alpha-1(II) chain
		COL4A3	Collagen alpha-3(IV) chain
		COL6A3	Collagen alpha-3(VI) chain
		COMP	Cartilage oligomeric matrix protein
		TRIM46	Tripartite motif-containing protein 46
		EMILIN2	Emilin-2
		ADAMTS12	A disintegrin and metalloproteinase with thrombospondin motifs 12
		ITGB6	Integrin β-6
VI. Focal adhesion	5	*COL2A1*	Collagen alpha-1(II) chain
		COL4A3	Collagen alpha-3(IV) chain
		COL6A3	Collagen alpha-3(VI) chain
		COMP	Cartilage oligomeric matrix protein
		ITGB6	Integrin β-6
VII. Adherens junction	2	*CREBBP*	CREB-binding protein
		TJP1	Tight junction protein 1

Table 26 KEGG pathways and the matching proteins (cont.)

KEGG pathways	Observed gene count	Encoding gene	Matching proteins
VIII. NOD-like receptor signaling pathway	9	*MEFV*	Pyrin
		NOD2	Nucleotide-binding oligomerization domain-containing protein 2
		CHUK	Inhibitor of NF-κB kinase subunit α
		SOS1	Son of sevenless homolog 1
		SOS2	Son of sevenless homolog 2
		DNM1L	Dynamin-1-like protein
		NLRP7	NACHT, LRR and PYD domains-containing protein 7
		FCN3	Ficolin-3
		IKBKG	NF-κB essential modulator
IX. B cell receptor signaling pathway	5	*CHUK*	Inhibitor of NF-κB kinase subunit α
		IKBKG	NF-κB essential modulator
		PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit β
		SOS1	Son of sevenless homolog 1
		SOS2	Son of sevenless homolog 2
X.T cell receptor signaling pathway	6	*CHUK*	Inhibitor of NF-κB kinase subunit α
		IKBKG	NF-κB essential modulator
		PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit β
		SOS1	Son of sevenless homolog 1
		SOS2	Son of sevenless homolog 2
		TEC	Tyrosine-protein kinase

Table 26 KEGG pathways and the matching proteins (cont.)

KEGG pathways	Observed gene count	Encoding gene	Matching proteins
XI.TNF signaling pathway	9	*TNFRSF1A*	TNF receptor superfamily member 1A
		NOD2	Nucleotide-binding oligomerization domain-containing protein 2
		CHUK	Inhibitor of NF-κB kinase subunit α
		MAP2K6	Dual specificity mitogen-activated protein kinase kinase 6
		IKBKG	NF-κB essential modulator
		AGT	Angiotensinogen
		ORM2	Alpha-1-acid glycoprotein 2
		AHSG	Alpha-2-HS-glycoprotein
		HP	Haptoglobin
XII.Non-alcoholic fatty liver disease	3	*CHUK*	Inhibitor of NF-κB kinase subunit α
		MAP2K6	Dual specificity mitogen-activated protein kinase kinase 6
		PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit β
XIII.Fluid shear stress and atherosclerosis	4	*TNFRSF1A*	TNF receptor superfamily member 1A
		CHUK	Inhibitor of NF-κB kinase subunit α
		MAP2K6	Dual specificity mitogen-activated protein kinase kinase 6
		PIK3R2	Phosphatidylinositol 3-kinase regulatory subunit β

ECM: extracellular matrix; TNF: tumor necrosis factor; NOD: nucleotide-binding oligomerization domain; NF-κB: nuclear factor- κB

4.3.4 Expression of the candidate proteins in response to mulberry leaves treatment

Lists of the candidate proteins, biological roles, and expressions in response to the study interventions were described in Table 27.

Table 27 The candidate proteins, biological roles, and expressions in response to the study interventions regarding functional categories

Category	**Proteins (Alternative names)**	**Biological roles**	**Expression**
Metabolic regulation	Phosphatidylinositol 3-kinase regulatory subunit β	Protein kinase: regulation of glucose metabolism and cell growth and proliferation	↓
	Insulin receptor	Receptor of insulin	↓
	Insulin receptor substrate 2	Mediator of insulin action	↑
	Hexokinase domain-containing protein 1	Intermediate of glucose uptake in peripheral tissues	↓
	Oxysterols receptor LXR-α (Liver X receptor-α; LXR-α)	Nuclear receptor: regulation of lipid metabolism	↑
	Retinol-binding protein 4	Adipokine	↓

Table 27 The candidate proteins, biological roles, and expressions in response to the study interventions regarding functional categories (cont.)

Category	Proteins (Alternative names)	Biological roles	Expression
ECM constituents and organization	Collagen alpha-1(II) chain	ECM constituent	↓
	Collagen alpha-3(IV) chain	ECM constituent	↓
	Collagen alpha-3(VI) chain	ECM constituent	↓
	Cartilage oligomeric matrix protein (Thrombospondin-5)	ECM constituent	↓
	Tripartite motif-containing protein 46 (Tripartite, fibronectin type-III and C-terminal SPRY motif protein)	ECM constituent	↓
	Emilin-2 (Elastin microfibril interface-located protein 2; Elastin microfibril interfacer 2)	ECM constituent	↓
	A disintegrin and metalloproteinase with thrombospondin motifs 12 (ADAMTS-12)	ECM constituent	↓
	Integrin β-6	Cell surface protein	↑
	Nucleotide-binding oligomerization domain-containing protein 2	Pathogen recognition protein	↓
	Ficolin-3 (H-ficolin)	Pathogen recognition protein	↓

Table 27 The candidate proteins, biological roles, and expressions in response to the study interventions regarding functional categories (cont.)

Category	Proteins (Alternative names)	Biological roles	Expression
Immune response	Pyrin (Marenostrin)	Inflammasome	↑
	NACHT, LRR and PYD domains-containing protein 7 (Nucleotide-binding oligomerization domain protein 12)	Inflammasome	↑
	Inhibitor of NF-kB kinase subunit α (IKK-α; IKK-1)	Mediator of NF-kB activation	↓
	NF-kB essential modulator (NEMO; IKK-γ)	Mediator of NF-kB activation	↓
Inflammatory response	TNF receptor superfamily member 1A (TNF receptor 1; p55; p60)	Receptor of TNF-α	↓
	Dual specificity mitogen-activated protein kinase kinase 6 (MAP kinase kinase 6; MAPKK-6)	Mediator of MAPK and JNK activation	↓
	Angiotensinogen (Angiotensin-II; Serpin A8)	Positive acute phase reactant	↓
	Alpha-1-acid glycoprotein 2 (AGP-2; Orosomucoid-2)	Positive acute phase reactant	↓
	Alpha-2-HS-glycoprotein (Alpha-2-Z-globulin; Fetuin-A)	Positive acute phase reactant	↓
	Haptoglobin (Zonulin)	Positive acute phase reactant	↓

ECM: extracellular matrix; TNF: tumor necrosis factor; NF-κB: nuclear factor- κB: MAPK: mitogen-activated protein kinase; JNK: Jun N-terminal kinase: ↑: up-expression (*vs.* before treatment): ↓ down-expression (*vs.* before treatment)

CHAPTER V
DISCUSSION AND CONCLUSION

5.1 Mulberry leaves powder

Mulberry (*M. alba* L.) variety Sakonnakorn, one of the notable mulberry varieties distributed in the Northern and Northeastern parts of Thailand, was used as raw material of mulberry leaves product in this study. Evidences indicated that concentrations of phytochemicals in natural-derived product are considerably influenced by several factors such as plant species, planting areas, harvesting seasons, and manufacturing processes [234, 236, 294-296]. Thus, our product was prepared by standardized procedures of leaves collection, drying process, powdering, and storage in order to ensure consistency of chemical constituents in the final product.

Quantitative determination using HPLC-MS/MS showed that DNJ constituted 2.6 mg/g (0.26%) in the dried powder of mulberry leaves. The finding was consistent with prior researches. Vichasilp *et al.* [238], who examining DNJ concentration in young mulberry leaves in Thailand, found DNJ ranged 0.3 to 1.7 mg/g among 35 mulberry varieties. Song *et al.* [236] and Hao *et al.* [239] also showed that Chinese mulberry leaves contained DNJ 1.389 to 3.483 mg/g and 0.401 to 5.309 mg/g dried leaves, respectively, whereas Bajpai *et al.* [240] demonstrated that DNJ constituted 0.68 to 2.72 mg/g in the mulberry leaves samples collected from multiple areas in India. Many polyphenolic constituents: chlorogenic acid, rutin, isoquercitrin, quercetin, kaempferol, kaempferol-7-*O*-glucoside, quercetin-3-*O*-(6-malonyl)-β-D-glucopyranoside, and kaempferol 3-*O*-β-D-(6"-*O*-malonyl)-glucoside, were also found in the dried mulberry leaves powder. This was in agreement with the prior data reporting that mulberry leaves are rich in active phytochemicals, including alkaloids, phenolic acid, flavonoids, and flavonol glycosides, exerting various biological properties [237, 297-299].

We realized that the mulberry leaves powder used in this study was not a purified form of DNJ. As a result, we cannot absolutely confirm that DNJ is the sole compound responsible for antihyperglycemic action. However, it could be postulated

that DNJ is the predominant antihyperglycemic constituent of mulberry leaves regarding the following evidences. First, since DNJ is an α-glucosidases inhibitor, modulation of postprandial hyperglycemia is an expected clinical outcome. The results of carbohydrate loading test in animals and human [31, 32, 34, 257] were directly explained by the mechanism of action of DNJ. Second, Vichasilp *et al.* and Yatsunami *et al.* further reported that the concentration of DNJ in mulberry leaves samples were positively correlated with the magnitude of α-glucosidases inhibition with $r = 0.84$ ($p < 0.05$) [238] and 0.90 ($p < 0.001$) [249]. In contrast, the correlation between other phytochemicals in mulberry leaves and the α-glucosidases inhibitory activity is poor understood at present. Third, few evidences have supported glucose-lowering efficacy of the other phytochemicals, particularly in human. Future investigations should be performed to clarify their clinical efficacy.

Interestingly, previous studies revealed that mulberry leaves extracts exhibited the stronger glucose-lowering effects in glucose uptake assay in Caco-2 cell and carbohydrate loading test than purified DNJ [252, 300]. A possible explanation might be from multiple effects of several compounds in the whole extracts on glycemic profiles. It is noteworthy that a combination therapy regimen provides synergistic benefits, improved desired outcomes, and lessen adverse effects. The data might support superiority of the whole mulberry leaves extract to the purified DNJ on achieving glycemic control with the lower undesirable effects.

5.2 Study I (Phase I clinical trial): To determine effect of a single administration of mulberry leaves on plasma glucose excursion following carbohydrate load among healthy non-diabetic persons and determining the effective dose with minimal adverse effects of mulberry DNJ

This randomized controlled study found that mulberry leaves could be effective for management of postprandial hyperglycemia. Mulberry leaves at the weight equivalent to 6 to 18 mg of DNJ dose-dependently modulated the elevated PPG-30, PPG-60, and $AUC_{0\text{-}180\ min}$ of glucose in response to the 50-g sucrose ingestion in healthy non-diabetic persons ($N = 85$) without serious adverse effect. In

addition, 12 mg of mulberry DNJ was considered the effective dose with minimal adverse effects in our study.

5.2.1 Efficacy of mulberry leaves in lowering postprandial hyperglycemia in response to carbohydrate load

After the 50-g sucrose ingestion, the elevated postprandial glucose, including PPG-30, PPG-60, and AUC $_{0\text{-}180\ min}$ of glucose, were suppressed by mulberry leaves containing 6 to 18 mg of DNJ in the dose-response relationship. The significantly suppressive action of mulberry leaves that observed during the first hour after receiving the tested beverages was in accordance with pharmacokinetic profile of DNJ. Animal studies suggested that DNJ was poorly absorbed and rapidly excreted from the body [243, 244]. The favorable effects of mulberry leaves on PPG following the sucrose loading test were consistently reported in clinical researches [32, 33, 255, 257, 258]. Besides sucrose, mulberry leaves were capable of lowering postprandial hyperglycemia regardless of carbohydrate foods, including maltose [31], maltodextrin [259], boiled white rice [34], and Japanese confections [255]. The findings indicate that antihyperglycemic efficacy of mulberry leaves is not affected by types and sources of carbohydrates.

Effect of mulberry leaves on postprandial hyperglycemia is explained by the inhibitory effect against α-glucosidase activities of mulberry DNJ. Regarding the sugar-mimicking structure of DNJ, it competitively binds to the active sites of the carbohydrate digestive enzymes. Mulberry DNJ retards hydrolysis of polysaccharides and disaccharides into monosaccharide in the upper part of small intestine and leads to the delay of glucose absorption into blood circulation [30]. Kim *et al.* [250] early demonstrated that the inhibitory effect on carbohydrate digestion against rat α-glucosidases of mulberry leaves was comparable to acarbose as measured by the IC_{50} values (41.0 and 19.0 μg/mL for mulberry leaves extract and acarbose, respectively). Furthermore, Yatsunami *et al.* [249] showed the relevance between the presence of DNJ in mulberry leaves extract and its biological activity. The content of DNJ in mulberry leaves extract was positively correlated to the magnitude of α-glucosidases inhibition ($r = 0.90$, $p < 0.001$). This was in agreement with the study of Vichasilp *et*

al. [238], showing that the higher concentration of DNJ in mulberry leaves samples was correlated to the higher inhibitory effect on α-glucosidases ($r = 0.84$, $p < 0.05$).

Postprandial hyperglycemia is the common metabolic defect found in the early stage of metabolic impairment even the FPG level remains in the normal range [122]. Importance of management of postprandial hyperglycemia have been addressed. First, PPG is considered as another component, in addition to FPG, for achieving the optimal goal of A1C because the value of A1C is an integration of blood glucose during fasting and postprandial states in the 3-month duration [135]. Second, postprandial hyperglycemia is an independent risk factor for diabetic complications and cardiovascular events [122]. The 2-hour PPG, postprandial glucose peak, and AUC of postprandial glucose are the three strongest predictors for atherosclerotic risks when compared with FPG and A1C [125]. Asai *et al.* [34] and Nakamura *et al.* [255, 256] showed the significant suppression on glucose peak at 30 min and the AUC of blood glucose after carbohydrate load in the mulberry leaves-treated group in comparison with the control. This was in line with our findings as mentioned. We found that mulberry leaves significantly blunted the peak glucose level (PPG-30) and suppressed the $AUC_{0\text{-}180\ min}$ of glucose during postprandial state, suggesting that mulberry leaves administration may have potential for lowering the risks for diabetic complications and cardiovascular diseases. Third, the modulation of postprandial hyperglycemia reduces the excessive insulin secretion stimulated by glucose, which subsequently prevents insulin resistance and β-cell dysfunction.

5.2.2 Adverse events of a single administration of mulberry leaves containing 6, 12, and 18 mg of DNJ

A single administration of mulberry leaves mostly caused bloating and flatulence. The incidence was dependent upon the administered doses. Loose stools and nausea were also observed, but these side effects appeared not to be related to the doses of mulberry DNJ. Overall, the adverse events were tolerated and self-recovered. No toxicity, allergy, and serious side effect of mulberry leaves was found.

Gastrointestinal side effects are the well-known adverse effects of α-glucosidases inhibitors [134, 301]. Evidence suggested that the ideal administered

dose of α-glucosidases inhibitors is the one that allow all carbohydrates to be digested. Otherwise, the carbohydrates that escape digestion and absorption contribute to the unwanted outcomes [302]. The undigested carbohydrates remaining in the large intestine are substrates of bacterial fermentation, leading to formation of the large amount of gas and osmotic pressure in the gastrointestinal tract that cause bloating, flatulence, and loose stool [293]. In the meantime, one person in the control group complained of feeling nausea. A plausible explanation is that taking a lot of sugary beverage in the short period of time may rapidly raise osmotic pressure in the gastrointestinal system and stimulate Vagus nerve. This phenomenon promotes pyloric sphincter relaxation and accelerates stomach emptying [303].

5.2.3 The effective dose with minimal adverse effects of mulberry DNJ

In this study, mulberry leaves containing 12 mg of DNJ was considered the most optimal dose because it exhibited clinically significant effect on PPG with the minimal rates of gastrointestinal symptoms. In other words, we suggested that 12 mg of DNJ was not only the effective dose for modulating postprandial hyperglycemia, but it was also safe and well tolerated.

The suitable dose of mulberry DNJ was firstly extrapolated from an animal experiment. The results showed that 6-24 mg of mulberry DNJ could be capable of modulating postprandial hyperglycemia in human [34]. Kimura *et al.* [32] further supported that mulberry leaves containing DNJ 12 and 18 mg significantly reduced the elevated PPG during the sucrose loading test among healthy volunteers (N = 24) in comparison with placebo. Chung *et al.* [31] also found that mulberry DNJ at the dose of 9 and 18 mg were effective to attenuate hyperglycemia following maltose load in non-diabetic population (N = 50). Meanwhile, Asai *et al.* [34] supported that mulberry DNJ 6 and 9 mg lowered the level of PPG-30 after ingestion of boiled white rice in individuals with impaired glucose metabolism (N = 12).

One of the major knowledge gaps nowadays is lack of the well-defined recommended dose of mulberry DNJ. Previously, the administered dose of DNJ was reported in the limited numbers of published studies [32, 34, 35]. Moreover, the recommended dose of DNJ was normally considered on the basis of clinical efficacy

on blood glucose reduction, whereas its safety and tolerability remained questionable. Thus, consideration of the optimal dose based on safety-efficacy balance strengthens our findings to be more practical.

5.3 Study II (Phase II clinical trial): To determine efficacy and safety of a long-term supplementation of mulberry leaves on glycemic control in persons who had impaired glucose metabolism including obese persons with prediabetes and patients with early-stage type 2 diabetes

This 12-week prospective randomized controlled study found that mulberry leaves containing 12 mg of DNJ slightly improved glycemic control and lipid profiles in obese persons with prediabetes and patients with newly diagnosed type 2 diabetes (N = 54). Long-term administration of mulberry leaves caused gastrointestinal symptoms; nonetheless, no serious side effect was observed throughout the study.

5.3.1 Efficacy of daily administration of mulberry leaves on glycemic control

The present study found the small but significant improvement in glycemic control after the 12-week daily administration of mulberry leaves in obese persons with prediabetes and patients with newly diagnosed type 2 diabetes. Although no difference in any glycemic outcomes between the treatment group and the control group was observed, the mean concentrations of PPG-30 following the 75-g OGTT, FPG, and A1C of the treatment group significantly lowered than the baseline levels. In addition, none of participant in the two groups developed type 2 diabetes during the study period.

To the best of our knowledge, this is the first study investigating effects of mulberry leaves on postprandial glucose excursion following the standard OGTT. The 75-g OGTT aims at identifying impaired glucose tolerance and determining ability of glucose uptake by insulin target tissues, which are strongly related to pathophysiology of prediabetes and the early-stage of type 2 diabetes [139]. We found a significant decrease in PPG-30 of the treatment group by -10.75 mg/dL ($p = 0.018$) from the

baseline level at the endpoint. However, we failed to observe changes in PPG at the other timepoints of measurement. Absence of the significant improvement in glucose tolerance may result from the insufficient study duration [9]. The optimal interval for evaluating the improvement in glucose tolerance and the reversion of prediabetic state to normal condition has been not clearly defined. Nonetheless, studies were commonly conducted for over one year with annually screening by the standard OGTT, for example, 3.2 years for the Finnish Diabetes Prevention study [11], 2.8 years for the US DPP study [120], and 3.3 years for the STOP-NIDDM study [20]. In order to clarify the effects of mulberry leaves administration on glucose tolerance, further works should be performed in the longer study duration.

The 12-week administration of mulberry leaves significantly reduced A1C by -0.11% ($p = 0.011$) from the initial level although no difference between the groups was detected. The result represents clinical relevance between postprandial glucose excursion and long term glycemic control because PPG is a variable affecting the value of A1C [135]. The reduction in A1C of the treatment group could be at least from the modulation on postprandial hyperglycemia by mulberry DNJ as described early. Also, it might be attributed to the decreased FPG throughout the study period. In comparison with the baseline level, the magnitude of reduction in FPG ranged from -3.86 to -5.61 mg/dL and the maximum reduction was reached at week 8. Although the result showed the small reduction in FPG, it is not unexpected to observe the slight effect of mulberry leaves on FPG concentration. Due to the inhibition on α-glucosidases of DNJ, mulberry leaves administration could be more likely affect the concentration of PPG. The improvement in insulin resistance of peripheral cells in response to insulin action might be a plausible explanation for the reduction in FPG.

Our findings did not differ from the study of Asai *et al.* [34], who showed that daily supplementation of mulberry leaves (6 mg DNJ; t.i.d.) for 12 weeks lowered A1C by -0.2% ($p < 0.05$) from baseline in persons with mild fasting hyperglycemia (FPG 100-140 mg/dL) ($N = 65$). The greater magnitude of reduction could result from excellent compliance to the study intervention of the participants: 99.4 ± 1.4% in the treatment group and 99.0 ± 1.8% in the placebo group. Riche *et al*. [261] consistently showed the trend of A1C reduction when compared with baseline ($p = 0.098$) in

patients with type 2 diabetes (N = 17) who taking mulberry leaves (1,000 mg of standardized mulberry leaves extract; t.i.d.) for 12 weeks. In contrast, Kim *et al.* [35] reported that no alteration in the concentrations of A1C and FPG was observed in prediabetic individuals who taking mulberry leaves (6 mg DNJ; t.i.d.) for four weeks.

In comparison with acarbose, we observed the relatively small reduction in A1C and FPG by mulberry leaves. Previous evidence showed that acarbose decreased the levels of A1C by -0.58% ($p < 0.001$) and FPG by -13.1 mg/dL ($p < 0.001$) [304]. This may be attributed to the low baseline blood glucose of participants in our study. Blood glucose concentration is established as an important factor affecting efficacy of antihyperglycemic agents. In general, persons who had the higher baseline levels better respond to the medications than those with the lower degree of hyperglycemia [305]. In addition, the study duration appeared to be too short to detect the clinically significant reduction in A1C. Chiasson *et al.* [301] revealed that the maximum effect of acarbose on A1C was observed after six months of the treatment. Another plausible reason is that mulberry DNJ 12 mg was probably not sufficient to suppress the elevated PPG in response to the regular diets and the higher dose may be required for achieving the desirable effects. Although it was considered the effective dose regarding the results of study I, it should be noted that it was relied on the 50-g sucrose loading test. Regular diets generally contain complicated compositions other than digested carbohydrate such as fat, protein, and fiber that may interfere carbohydrate digestion and absorption. Furthermore, sources and quantity of the ingested carbohydrate itself are the key factors influencing postprandial glycemic responses in the different ways. For example, carbohydrate-rich meals and high glycemic index foods produce the high magnitude of postprandial hyperglycemia [14, 25].

5.3.2 Effects of daily administration of mulberry leaves on insulin resistance

This is the first study evaluating effect of mulberry leaves on insulin resistance in human using the HOMA-IR method. We observed that the HOMA-IR of the treatment group tended to decrease from baseline with the borderline significant level

($p = 0.057$) at the end of study. Since the reduction in HOMA-IR indicates the improvement of insulin sensitivity [140], our results suggest that daily administration of mulberry leaves may alleviate insulin resistance.

The present study failed to observe the significant effect of daily administration of mulberry leaves on insulin resistance. It could be explained by the small sample size and the short treatment duration. However, we found that effect of mulberry leaves on insulin resistance index was comparable to other herbal extracts. Choi *et al.* [142] demonstrated that the HOMA-IR level in obese persons with impaired glucose metabolism (N = 136) did not alter from baseline (3.5 ± 0.2 *vs.* 3.1 ± 0.2, $p = 0.16$) after supplementation of aloe vera gel for 8 weeks and the mean value of HOMA-IR in the treatment group was not different from the control group at the end of study. On the other hand, a 3-year clinical trial in individuals who met the criteria of prediabetes (N = 240) of Chuengsamarn *et al.* [144] showed that curcumin extract was capable of lowering HOMA-IR (3.60 *vs.* 3.22, p value was not mentioned) in the treatment group. Moreover, the study reported the significant difference in HOMA-IR between the groups ($p < 0.001$ *vs.* the placebo group) at the end of study. A slight but significant improvement in insulin resistance among persons with prediabetes and patients with newly diagnosed type 2 diabetes (N = 41) was also evident by Bang *et al.* [306]. The authors showed that Korean red ginseng supplementation significantly reduced HOMA-IR (1.81 ± 0.12 *vs.* 1.53 ± 0.12, $p < 0.05$) at the end of 12-week study [306].

Benefits of mulberry leaves on insulin resistance could be a result of the decreased postprandial hyperglycemia. A prolonged high response of glucose excursion after meals aggressively provokes insulin secretion, eventually leading to impaired insulin sensitivity and β cell function. Therefore, the blunted PPG excursion may lessen stimulation of insulin release and preserve β cells. Another plausible explanation for alleviation in insulin resistance in the mulberry leaves-treated group was the reduction in BMI at week 12 (-0.26 ± 0.46 kg/m^2, $p = 0.075$). Excess body weight is involved in the development of insulin resistance in several pathways [12, 53]. Thus, the robust relationship between weight loss and insulin sensitivity was well evident [307].

In accordance with our work, preclinical studies suggested that mulberry leaves improved insulin sensitivity in insulin-target tissues via several mechanisms. The extracts modified expressions of genes and proteins involved in hepatic glucose homeostasis. Activities of gluconeogenic enzymes: G6Pase and PEPCK were suppressed [38], whereas activities of glycolysis enzymes: glucokinase, phosphofructokinase, and pyruvate kinase were promoted in a dose-dependent fashion by mulberry leaves treatment [308]. The extracts also activated PI3K/Akt and GSK-3β signaling pathways [36] and elevated GLUT-4 translocation [39] in skeletal muscles and adipose tissues. In addition, mulberry leaves increased circulating adiponectin, which is an endogenous insulin sensitizer, in rat plasma [309]. In the meantime, mulberry leaves also reduced the level of circulating leptin, contributing to modulation of leptin resistance [309]. It was postulated that phenolic acids and flavonoids in the extracts were responsible for benefits of mulberry leaves on insulin resistance [38, 309].

By contrast, effects of mulberry leaves and the relevant mechanisms on alleviating insulin resistance were not widely elucidated in human studies. Kim *et al.* [35] investigated effects of mulberry leaves administration on postprandial insulin response in human in order to clarify whether mulberry leaves decreased the PPG concentration by stimulating insulin secretion or not. The results showed the higher AUC of postprandial plasma insulin during carbohydrate tolerance test in the control group when compared with the mulberry leaves-treated group ($p < 0.05$). The favorable effect was consistent with postprandial response of C-peptide. The findings indicate that mulberry leaves can decrease blood glucose with no effect on insulin secretion. Prolonged stimulation of insulin secretion aggravates β-cell exhaustion and dysfunction as described [52]. As a result, one of the desired antihyperglycemic actions is lowering blood glucose without enhancing pancreatic insulin release. This could suggest that mulberry leaves could be a desired agent for treating hyperglycemia.

5.3.3 Effects of daily administration of mulberry leaves on blood lipids

In the present study, mulberry leaves possessed the significant lowering effect on TC, but it did not affect the values of TG, HDL-C, and LDL-C. At the endpoint, the significant difference between the groups (-17.91 mg/dL; $p = 0.034$) and the significant change from the baseline level (-13.42 mg/dL; $p = 0.002$) were detected in the treatment group. It is important to note that the above results were relied on the mean values of blood lipids from all participants (N = 54) including those who had blood lipids in the normal range and those with the abnormal lipid profiles.

The case selection analysis was additionally performed to determine the effect of mulberry leaves on lipids only in persons with dyslipidemia (N = 41). Similarly, the results showed the significant difference in TC in the treatment group when compared with the control group (-19.52 mg/dL; $p = 0.024$) and the baseline level (-17.10 mg/dL; $p < 0.001$). Moreover, the significant reduction in LDL-C from the initial level (-10.33 mg/dL; $p = 0.038$) was found in the treatment group. Although no improvement in TG and HDL-C by mulberry leaves treatment was observed in this study, the level of TG tended to lower from baseline (-12.36 mg/dL; $p = 0.067$).

A similar pattern of results and degree of reductions in TC, TG, and LDL-C was obtained in the study of Aramwit *et al*. [275] that conducted in patients with early-stage dyslipidemia (N = 23). After 12 weeks of the treatment period, mulberry leaves significantly improved lipid profiles by reducing TC (-12.8 mg/dL; $p < 0.05$), TG (-19.1 mg/dL; $p < 0.05$), and LDL-C (-9.7 mg/dL; $p < 0.05$) from baseline. The study of Andallu *et al*. [277] also showed that the 4-week treatment of mulberry leaves significantly lowered TC (-23.4 mg/dL; $p < 0.01$), TG (-32.4 mg/dL; $p < 0.01$), and LDL-C (-23.4 mg/dL; $p < 0.01$) in comparison with the initial levels among patients with type 2 diabetes (N = 24). Furthermore, our findings were in agreement with Chiasson *et al.* [132] and Malaguarnera *et al*. [310], showing lipid-lowering efficacy of acarbose on TC and LDL-C. The studies proposed that the reduction in cholesterol levels resulted from the decreased lipogenic effect of insulin [310]. The relatively small reduction in blood lipids that found in this study could be explained by the severity of dyslipidemia. Individuals who have the markedly high blood lipids better respond to antihyperlipidemic agents than those with the lower baseline of

dyslipidemia [311]. In our study, the baseline lipid profiles of participants were not extremely high; therefore, the marked changes in blood lipids were not expected.

There are several pathways associated with the lipid-lowering effect of mulberry leaves. First, it was proposed that mulberry leaves inhibited the function of HMG-CoA reductase [264], leading to the inhibition of endogenous cholesterol synthesis. Second, DNJ-rich mulberry leaves extract acted as a PPAR-α agonist [262-264]. This could promote lipolysis in hepatic cells. Third, being the α-glucosidase inhibitor could be a possible explanation. Regarding the suppressive effect on PPG and insulin release, mulberry DNJ reduced blood glucose and hepatic glucose influx, leading to the reductions in TG and cholesterols [251]. Many phytochemicals, including DNJ, DNJ derivatives, and polyphenols were hypothesized to be the functional compounds responsible for lipid-lowering effect of mulberry leaves [262, 263, 265-267]. Mulberry leaves also contain soluble fiber that interfere the absorption of dietary lipids by binding with bile acid and being excreted in feces [272].

5.3.4 Effects of daily administration of mulberry leaves on anthropometric profiles

Mulberry leaves did not exhibit anti-obesity effect in the current study, which was in agreement with previous clinical trials. No evidence has indicated ability of mulberry leaves in reducing body weight in human. Interestingly, we however found the trend of BMI reduction in the treatment group ($p = 0.075$ *vs.* baseline) after the intervention period even the numbers of caloric intake were maintained. We hypothesized that there might be unknown favorable effects of mulberry leaves on fat metabolism and fat accumulation. Other obesity-related markers such as body fat mass, lean body mass index, and adipokine level should be evaluated in the future work in order to obtain insight explanations of anti-obesity effect of mulberry leaves.

In contrast to the clinical findings, mulberry leaves moderately reduced body weight in animals. After induction of obesity by high-fat diet feeding, Chang *et al.* [265] found that mice treated with mulberry leaves extract had the lower body weight and fat/body weight ratio than the control mice. Various mechanisms of anti-obesity action of mulberry leaves were proposed. Mulberry leaves significantly decreased

lipid accumulation in adipocytes as evaluated by histological examination [265, 266]. Chang *et al.* [265] and Peng *et al.* [312] consistently suggested that mulberry leaves inhibited adipogenesis, retarded adipocytes maturation, and promoted apoptosis of functional adipocytes, resulting in the reduction in mature adipocytes. In addition, it has further evident that mulberry leaves regulated activities of the key adipokines involved in energy metabolism and body weight regulation, including adiponectin and leptin [309]. Moreover, mulberry leaves modulated expressions of genes involved in adipogenesis, lipogenesis, and lipolysis [265, 266, 312]. Inconsistency between our finding and the animal researches might be from a major difficulty of conducting human studies. It is difficult to strictly control and completely avoid confounding variables that may influence anthropometric outcomes, for example, diet intake and physical activity in the real-world situation. Unlike, variables can be carefully controlled and monitored throughout the study period in the animal experiments.

5.3.5 Safety of daily administration of mulberry leaves

Daily administration of mulberry leaves was relatively safe without the occurrence of hypoglycemia and serious side effect. Biochemical analysis revealed no adverse effect on hepatic and renal function by mulberry leaves. Meanwhile, self-report adverse events were gastrointestinal symptoms: bloating, flatulence, abdominal pain, loose stools, and constipation. The most prominent events occurring in 50% of participants in the treatment group at the first four weeks of administration were bloating and flatulence. The rates however declined to 39.3% at week 8 and 28.6% at week 12, suggesting recovery of the side effects over time. Meanwhile, the incidences of loose stools and constipation were approximately 2-7% and 4-6%, respectively, during the treatment period. Although the high proportion of participants who complained of gastrointestinal side effects were observed, the actual withdrawal rate was relatively low. Only 3.45% in the treatment group prematurely discontinued participation to the study, indicating that tolerability of mulberry leaves was good.

As previously described, gastrointestinal symptoms were the most frequently reported problem of α-glucosidase inhibitors because the agents leads to the large production of gas and osmotic pressure in the digestive tract [293]. We found that the

gastrointestinal side effects from long-term mulberry leaves administration were in line with the prior studies [34, 35]. Nonetheless, when compared with acarbose, mulberry leaves appeared to cause the lower rates of gastrointestinal symptoms and study withdrawal regarding the relevant side effects. Chiasson *et al.* [301] showed that acarbose was more likely to cause gastrointestinal side effects than placebo: 73.2% *vs.* 39.0% for bloating, 43.6% *vs.* 20.3% for diarrhea, and 25.0% *vs.* 8.8% for abdominal cramp. Similarly, these were the main reasons for study withdrawal in the study of Holman *et al.* [313], which found the over 50% drop-out rate among the patients taking acarbose because of flatulence and diarrhea. A plausible explanation is that acarbose exhibited the potent inhibitory effect on both α-glucosidase and α-amylase enzymes [250]. This could result in the higher amount of undigested carbohydrates remaining in the gastrointestinal tract by acarbose. The findings indicate that mulberry leaves could be more well-tolerated than the synthetic drug.

5.3.6 Diet control

We postulated that eating patterns of study participants could not be the confounder affecting clinical outcomes because we found no change in total caloric intake and portions of macronutrients intake along the study period. However, limitations of the evaluation methods are acknowledged. First, measures of caloric and macronutrients intake by the food diary can cause recording and measurement biases. Participants were asked to estimate serving sizes of foods using the standard household measures. The method could be complicated for some participants, especially in those who are not familiar with weighing food and household tools. Second, respondents may intentionally alter or not report their actual eating patterns. These can be the results of underreport and misinterpretation in types and amount of the consumed diets.

5.4 Study III (proteomic analysis): to determine effect of the long-term supplementation of mulberry leaves on expressions of plasma proteins in persons who had impaired glucose metabolism, including obese persons with prediabetes and patients with early-stage type 2 diabetes

As far as our knowledge, this is the first work incorporating clinical study and proteomic analysis for investigating effects of mulberry leaves administration in human. The longitudinal changes in expression of plasma proteins in response to the 12-week mulberry leaves administration among the selected participants who enrolled in the study II (N = 12) was determined using LC-MS/MS coupled with the label-free proteomic technique. Type 2 diabetes is the multifactorial disease associated with multi-organ dysregulation. Regarding the advantages of proteomics, the results reveal the comprehensive view of multiple proteins that linked to the ongoing cellular modification. Plasma specimen was used. Blood is a connective tissue traveling throughout the body, suggesting that the proteins that secreted or leaking from the different tissues are present in the blood circulation. It has been addressed that the label-free strategy is suitable for quantitative determination of proteins in blood-based specimen, which is the highly dynamic biological system, because of the wide-range detectable capacity of the technique [173]. In addition, blood collection procedure is relatively minimally invasive and convenient. Accordingly, blood-based specimen was considered the optimal option of our study.

Following the 12-week treatment period, we found the differential expression of numerous proteins in the after-treatment samples when compared with the before-treatment ones. In other words, the changes in protein expression reflected biological modification at the cellular level in response to the treatment. The results showed that the candidate proteins are functionally involved in metabolic regulation, ECM constituents and organization, immune system, and inflammatory response.

5.4.1 Proteins involved in metabolic regulation

After the 12-week supplementation of mulberry leaves, expressions of the following proteins that involved in metabolic regulation changed from baseline.

- Phosphatidylinositol 3-kinase regulatory subunit β (↓)

- Insulin receptor (↓)
- Insulin receptor substrate 2 (↑)
- Hexokinase domain-containing protein 1 (↓)
- Oxysterols receptor LXR-α (↑)
- Retinol-binding protein 4 (↓)

Phosphatidylinositol 3-kinase regulatory subunit β (PI3K-β) is a component of PI3Ks. It is one of the downstream proteins of insulin receptor and IRS-1 and 2 and it serves as the signaling protein of PI3K/Akt signaling pathway. In response to elevated blood glucose, the binding of insulin and insulin receptor mediates signal transduction through IRS and subsequently facilitates the interaction between IRS and PI3Ks. This leads to the activation of phosphatidylinositol 3,4,5-trisphosphate (PIP3), which is the key intermediate promoting glucose transport and glycogenesis in the peripheral cells [314]. Also, hexokinase domain-containing protein 1, a protein belonging to the hexokinase family, is closely associated with glucose uptake in liver cells via GLUT-2 translocation [315]. Dysregulation of the mentioned proteins therefore contributes to hyperglycemia and the subsequent insulin resistance because of the defects in peripheral glucose utilization.

The down-expression of PI3K-β, insulin receptor, and hexokinase was observed in the samples. Our findings differ from the studies of Hamdy *et al.* [37] and Liu *et al.* [39] showed that mulberry leaves water extract promoted expression of proteins that regulate hepatic glucose metabolism, including G6Pase and PEPCK. However, the results reflect metabolic dysregulation in the study population. In other words, this suggests that mulberry leaves supplementation seemed not to have effect on peripheral glucose uptake via the modulation of the mentioned proteins.

Published data demonstrated that activity of PI3K, IRS, and hexokinases dramatically decreased in various models of insulin resistance and type 2 diabetes [315, 316]. The crucial roles of the proteins that involved in the PI3K/Akt signaling pathway were clarified in experimental studies. Suppression of PI3K activity inhibited the translocation of GLUT-4 to the cell membrane, leading to the reduction in glucose

uptake into the cells [317-319]. PI3K inhibitor, wortmannin, also blunted insulin action and caused energy imbalance and impaired lipid metabolism [319]. Consistently, transgenic PI3K- and IRS-deficient animals exhibited glucose intolerance, excessive fat accumulation in adipocytes, and loss of body weight control when compared with the wild-type controls [318, 319]. The impaired glucose tolerance following the OGTT was also observed in mice with down-expressing *HKDC1*, which is the encoding gene of hexokinase domain-containing protein 1 [315, 320]. Moreover, depletion of *HKDC1* gene appeared associated with insulin resistance since it resulted in the reduction in TG clearance [320]. On the other hand, pharmacological treatments that increased the expression of the mentioned proteins led to the improvement in insulin-resistant conditions [321, 322].

Meanwhile, oxysterols receptor LXR-α (LXR-α) is the protein regulating cholesterol, lipid, and carbohydrate metabolism in liver and adipose tissue [323]. Studies showed that the up-regulated LXR-α mediates reverse cholesterol transport by HDL particles, lowers intestinal fat absorption, and enhances TG synthesis [324, 325]. LXR-α agonists alleviated hyperglycemia and glucose intolerance in animal models since the interventions decreased gluconeogenesis and increased GLUT-4 expression [326, 327]. The activated LXR-α also enhanced insulin production and secretion from β cells [328]. By contrast, transgenic down-expression of LXR-α in mice exhibited the significantly impaired functions of HMG-CoA reductase, SREBP, and fatty acid synthase, which are the key enzymes responsible for cholesterol and fatty acid metabolism [329].

In this analysis, we found the up-expression of LXR-α, reflecting the modulation of metabolic regulation related to LXR-α activity. This brought us to assume that the reductions in plasma lipid of those in the treatment group might be associated with the modulation of LXR-α expression by mulberry leaves treatment. Also, we assumed that the up-expressed LXR-α might exert favorable effects on glycemic outcomes and insulin resistance profile. Besides metabolic regulation, evidences suggested that LXR-α modulated inflammation through many mechanisms: lowering local lipid accumulation, inhibiting unsaturated FFA formation, and suppressing gene expression of proinflammatory cytokines [323, 325]. These might

be another reflection of the reversed inflammation-induced insulin resistance in the study participants. However, Ann *et al.* [266] reported the controversial finding from ours. Ethanolic mulberry leaves extract promoted adipocyte apoptosis by the suppression of LXR-α expression [266].

Next, the down-expression of retinol-binding protein 4 (RBP-4) by mulberry leaves treatment might be also associated with the favorable outcomes of the phase-II study. RBP-4 is considered a novel adipocytokine. The protein impaired the activation of IRS-1 and PI3K/Akt signaling pathway and increased hepatic gluconeogenesis, leading to hyperglycemia [330, 331]. These findings indicated that the over-expression of RBP-4 possesses deleterious effects on glucose-insulin homeostasis [331]. Furthermore, the significant elevation of serum RBP-4 in various models of insulin resistance, glucose intolerance, and obesity [330-333]. Similarly, the concentration of circulating RBP-4 in type 2 diabetic patients was higher than that of non-diabetic controls [332], which was consistent with the gene expression level in visceral adipose tissue collected from diabetic persons [334]. RBP-4 is also positively associated with various components of metabolic syndromes such as high BMI, waist-to-hip ratio, and TG level [331]. Conversely, studies revealed that suppression of RBP-4 activity by fenretinide and pioglitazone was capable of improving insulin sensitivity and reversing glucose intolerance [330, 335], which was in line with the effect of mulberry leaves in our study. For these reasons, we suggested that mulberry leaves might modify RBP-4 expression and it might be subsequently associated with the improvement in glycemic control and insulin resistance in the study population.

5.4.2 Proteins involved in ECM constituents and organization

After the treatment of mulberry leaves, we found the differential expression of eight proteins as summarized below. All of these are classified as the ECM constituents, except integrin β-6, which is the cell surface receptor of collagens.

- Collagen alpha-1(II) chain (↓)
- Collagen alpha-3(IV) chain (↓)
- Collagen alpha-3(VI) chain (↓)

- Cartilage oligomeric matrix protein (↓)
- Tripartite motif-containing protein 46 (↓)
- Emilin-2 (↓)
- A disintegrin and metalloproteinase with thrombospondin motifs 12 (↓)
- Integrin β-6 (↑)

ECM remodeling is known as another result of uncontrolled hyperglycemia and excessive lipid accumulation [57]. The alterations in ECM constituents and organization, particularly in adipose tissue, liver, and skeletal muscle, are linked to insulin resistance and type 2 diabetes [58-60]. The expression of ECM proteins increases several folds in obesity and type 2 diabetes [57]. Also, several encoding genes of ECM constituents highly expressed in insulin-resistant condition when compared with the controls [60]. In contrast to the prior reports, we observed the down-expression of ECM proteins: collagen alpha-1(II) chain, collagen alpha-3(IV) chain, collagen alpha-3(VI) chain, cartilage oligomeric matrix protein, tripartite motif-containing protein 46, emilin-2, and a disintegrin and metalloproteinase with thrombospondin motifs 12 in the samples. We assumed that this could reflect the modulating effects of mulberry leaves on ECM remodeling towards metabolic disturbance. This assumption is supported by the published evidences. The overexpressed ECM proteins were attenuated by various interventions. The gene expression of collagens significantly decreased in diabetic skeletal muscle after physical training [336]. Also, relaxin treatment was capable of reducing hepatic collagen induced by high-fat diet intake, resulting in the subsequent alleviation of hepatic insulin resistance [337].

In addition, we found the only cell surface protein, integrin β-6, in the samples. Expression of integrin proteins in relation to insulin resistance has been controversially reported among the studies. William *et al*.[338] showed that integrin-deficient mice exhibited the improved glucose uptake and insulin signaling in obese mice fed with high-fat diet. However, Kang *et al.* [339] showed that genetic depletion of integrins exacerbated insulin resistance and impaired hepatic glucose output in

high-fat diet-induced insulin-resistant mice. On the other hand, the overexpression of integrins was observed in hepatocytes isolated from mice fed with high-fat diet [338]. The up-regulated integrin genes: *ITGBL1*, *ITGA4*, and *ITGA5* were also reported in various models of obesity, insulin resistance, and type 2 diabetes [66, 340]. The studies further suggested that it might be a consequence of the excessive ECM deposition in the cells [66]. According to our findings, we suggested that the up-expressed integrin β-6 might reflect the early compensation feedback in response to the decreased expression of ECM proteins.

5.4.3 Proteins involved in immune response

The analysis showed that there were four proteins that involved in immune system expressed differentially when compared with the baseline profile as shown below. The two first proteins are the members of pattern recognition proteins family, whereas the latter ones serve as inflammasome and the component of inflammasome complex, respectively.

- Nucleotide-binding oligomerization domain-containing protein 2 (↓)
- Ficolin-3 (↓)
- Pyrin (↑)
- NACHT, LRR and PYD domains-containing protein 7 (↑)

It has been proposed that the interactions of specific pathogens and pattern recognition proteins together with inflammasome, are the major triggers of immune response that closely linked to metabolic inflammation in the peripheral tissues [63]. Du *et al.* [341] found that the overexpression of nucleotide-binding oligomerization domain-containing protein 2 (NOD-2) resulted in the impaired glucose uptake and insulin signaling. Meanwhile, Schertzer *et al*. [64] demonstrated that *NOD2* knockout mice were protected from lipid accumulation and insulin resistance induced by high-fat diet feeding. The protective effect of *NOD2* depletion against tissue inflammation induced by hyperglycemia was also reported [64]. Meanwhile, ficolin-3 is one of the proteins belonging to the ficolin family and is highly specific to carbohydrate derived from bacteria and metabolic products [342]. Serum ficolin-3 concentration of persons

with prediabetic and type 2 diabetic statuses was higher than that of healthy controls [165, 343]. The expression of ficolin-3 was also in accordance with the increased values of FPG, TC, and TG [343]. Nonetheless, the study of Liu *et al.* [343] found that the up-regulated ficolin-3 was suppressed after receiving exercise intervention and its expression among those in the treatment group was significantly lower when compared with that of the non-treated one at the end of the study [343].

These findings were in agreement with ours, suggesting the favorable effects of the down-expression of NOD-2 and ficolin-3 in response to the given treatment. Moreover, ficolin-3 is the major activator of lectin-complement system [165], which is the hallmark of low-grade inflammation including insulin resistance [344]. We proposed that the decreased expression of ficolin-3 could further reflect another benefit of mulberry leaves on inflammation-induced insulin resistance. By contrast, the controversial results regarding the down-expression/depletion of the proteins were reported. Denou *et al*. [345] showed that transgenic NOD-2-deficient mice exhibited inflamed adipose tissue and liver, leading to the exacerbation of insulin resistance.

To date, little is known about the specific roles of pyrin and NACHT, LRR and PYD domains-containing protein 7 (NLRP7) in metabolic diseases. Furthermore, the certain expression of pyrin and NLRP7 in relation to insulin resistance and type 2 diabetes remains poorly understood. To the best of our knowledge, this is the first study reporting the detection of pyrin and NLRP7 in persons with impaired glucose metabolism. According to the existing data, inflammasomes are up-regulated during hyperglycemic and hyperlipidemic conditions [62, 63] and it is associated with the increased risk of insulin resistance [67]. For these reasons, we suggest that the over-expression of pyrin and NLRP7 could indicate hyperglycemic and hyperlipidemic states in the study participants and mulberry leaves treatment seemed not link to the modification of inflammasome expression.

5.4.4 Proteins involved in inflammatory response

In this work, we observed the down-expression of eight proteins that associated with inflammatory response, including IKK/NF-κB signaling pathway, JNK signaling pathway, and acute-phase reactants as follows.

- *IKK/NF-κB signaling pathway:* TNF receptor superfamily member 1A (↓), inhibitor of NF-κB kinase subunit α (↓), and NF-κB essential modulator (↓)
- *JNK signaling pathway:* dual specificity mitogen-activated protein kinase kinase 6 (↓)
- *Acute-phase reactants:* angiotensinogen (↓), haptoglobin (↓), α-1-acid glycoprotein 2 (↓), and α-2-HS-glycoprotein (↓)

First, TNF receptor superfamily member 1A (TNF-R1) is the major receptor of TNF-α. The interaction of TNF-α and TNF-R1 mediates transcription factor of NF-κB. Second and third, inhibitor of NF-κB kinase subunit α (IKK-α) and NF-κB essential modulator (NEMO/IKK-γ) are the two of the three subunits of the IKK complex, which serves as the distinct activator of NF-κB [78]. The robust association between the overexpression of IKK/NF-κB signaling pathway and peripheral insulin resistance has been well established [61, 79]. Previous cross-sectional studies observed that the up-expression of TNF-R1 in subcutaneous adipose tissue isolated from obese adults when compared with the controls [79]. Also, TNF-R1 serum level in Thai obese persons tended to be higher than that of the lean controls [93].

In addition to IKK/NF-κB cascade, JNK signaling pathway also takes the crucial part in metabolic inflammation. The only differentially expressed protein associated with the JNK pathway we found was dual specificity mitogen-activated protein kinase kinase 6 (MAPK kinase 6). The protein is established as the key element mediating signal transduction via p38 MAPK, which is the upstream activator of the JNK inflammatory pathway [61, 76]. The up-regulated MAPK kinase 6 moderately impaired insulin signal transduction in adipocytes due to the dramatic reductions in IRS-1 and IRS-2 expressions and PI3K/Akt activity in *in vitro* model [346]. The overexpression of *MKK6*, the encoding gene of MAPK kinase 6 expression, was detected in white adipose tissue of obese adults [347]. MAPK kinase 6 is considered the promising target of various inflammatory conditions to date.

Many evidences suggested that the decreased expression of TNF-R1, IKK-α NEMO/IKK-γ, and MAPK kinase 6 ameliorates inflammation-induced insulin

resistance. Diet-induced obesity animals treated with TNF-R1 antagonist exhibited the reversion of insulin resistance as well as the decreased fat accumulation and body weight [348, 349]. The treatment also alleviated the overexpression of TNF-α [349]. Consistently, TNF-R1 knockdown mice exhibited the improved glucose intolerance and the increased insulin secretion following the intraperitoneal glucose tolerance test [350]. Deficiency of *NEMO* gene exerted the protective effect against obesity-induced insulin resistance and glucose intolerance in transgenic mice [351]. Meanwhile, *MKK6* deficient mice were protected from diet-induced obesity and insulin resistance [347]. These findings were consistent with ours, showing the down-expression of TNF-R1, IKK-α NEMO/IKK-γ, and MAPK kinase 6 after receiving the intervention. Therefore, we suggested that mulberry leaves supplementation could have favorable effects against metabolic impairments through the modulation of IKK/NF-κB and JNK signaling responses.

Besides, we found that the expression of angiotensinogen, haptoglobin, α-1-acid glycoprotein 2, and α-2-HS-glycoprotein, were lower than the baseline. All of the four proteins are classified as positive acute-phase reactants, meaning that their expression levels significantly increase during the inflammatory period. Scientific studies supported that the over-expressed angiotensinogen, haptoglobin, α-1-acid glycoprotein 2, and α-2-HS-glycoprotein were linked to metabolic dysregulation. The hyperactivated angiotensinogen suppressed IRS-1 activity and led to insulin resistance in insulin-target tissues and endothelial cells [132]. Likewise, transgenic overexpression of α-2-HS-glycoprotein gene (*AHSG*) resulted in adipocyte dysfunction and impaired insulin signaling [352]. Meanwhile, patients with prediabetes and type 2 diabetes exhibited the elevated expressions and levels of haptoglobin and orosomucoids in serum [85, 165, 353]. The increased orosomucoids and α-2-HS-glycoprotein blunted the capacity of glucose clearance and insulin secretion following the standard glucose tolerance test among prediabetic persons [88, 353-355]. Conversely, the decreased expression of angiotensinogen modulated insulin resistance and lowered diabetic risk [356]. Haptoglobin gene (*HP*) knockout mice exhibited the improved glucose intolerance, insulin secretion, insulin action, and adiponectin expression [357]. According to the consistency between these reports and

our results, this could reflect the alleviation of inflammatory reactions that underlying insulin resistance by mulberry leaves intervention.

As far as our knowledge, this is the first proteomic study investigating the effects of mulberry leaves on metabolic impairment. Hence, our novel observation could suggest the advanced benefits of mulberry leaves beyond the well-known mechanism of action, which is inhibition of α-glucosidase activity. Limitations of the present analysis have been addressed, including the absence of controls (those who assigned to control the diets alone) as well as lack of non-diabetic controls, and small sample size. Moreover, there are a wide variation in proteomic data regarding the nature of the analysis. It is important to validate these preliminary findings by complementary techniques such as western blot analysis and enzyme linked immunosorbent assay (ELISA).

In summary, proteomic analysis reveals the changes in expression of many plasma proteins in response to mulberry leaves supplementation. Interestingly, we observed the novel proteins in individuals who had metabolic impairment including NOD-2 and NLRP7. The findings further reflect the cellular modification in several biological pathways that strongly linked to pathophysiology and pathogenesis of type 2 diabetes. We suggested that mulberry leaves exerted promising effects against insulin resistance and type 2 diabetes by modulating expression of LXR-α, RBP-4, ECM remodeling, pattern recognition protein expression, and inflammatory response via the JNK and IKK/NF-κB pathways, but the leaves seemed not to have effect on glucose utilization in peripheral tissues via PI3K/Akt signaling transduction.

5.5 Limitations

Study intervention

- The whole of mulberry leaves product was prepared by the single batch production for the overall study period and lack of product stability testing.

Study I

- Open-label trial

- Lack of identical matching placebo
- Lack of variety in carbohydrate source used in carbohydrate loading test: consideration of other carbohydrates (disaccharides and complex carbohydrates) might be more informative.

Study II

- Open-label trial
- Lack of identical matching placebo
- Insufficient study period to observe the significant improvements in glucose tolerance and insulin sensitivity
- Lack of the measurement of physical activity intensity
- No measurement of hemoglobin level at the time of the participant recruitment as well as throughout the study period: the misinterpretation of A1C might result from the abnormal hemoglobin homeostasis such as in patients with anemia.

Study III

- Within-group comparison
- Lack of control group
- Small sample size

5.6 Conclusion

In conclusion, we suggest that mulberry leaves have promising metabolic potentials for the management of the early-stage metabolic impairment in type 2 diabetes. First, daily supplementation of mulberry leaves containing 12 mg of DNJ effectively suppressed the elevation of PPG following the single administration of 50-g sucrose solution. Moreover, the certain treatment regimen of mulberry leaves was capable of reducing postprandial glucose peak, A1C, and FPG, and improving blood lipid profile in obese persons with prediabetes and patients with early-stage type 2 diabetes. Furthermore, it seemed to have favorable effects against insulin resistance. Second, mulberry leaves administration was relatively safe and well tolerated. There were no reports of hypoglycemia, adverse effects on renal and hepatic functions, as

well as life-threatening side effects following the mulberry leaves treatment. Last, regarding proteomic analysis, mulberry leaves might play the role in the modulation in expressions of plasma proteins that involved in metabolic regulation, ECM remodeling, immunity, and inflammatory response.

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APPENDICES

Appendix A

Certificate of ethic approval (Study I)

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel/Fax: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 160/2016

Certificate of Approval

Study Title No. 121.1/59 : EFFECT OF MULBERRY LEAF ON BLOOD GLUCOSE CONTROL IN HEALTHY VOLUNTEERS

Principal Investigator : PROF. PORNANONG ARAMWIT, Pharm.D., Ph.D.

Place of Proposed Study/Institution : Faculty of Pharmaceutical Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP).

Signature: ..... Signature: .....

(Associate Professor Prida Tasanapradit, M.D.) (Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.)

Chairman Secretary

Date of Approval : 15 September 2016 Approval Expire date : 14 September 2017

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire

Protocol No. 121.1/59
Date of Approval 15 SEP 2016
Approval Expire Date 14 SEP 2017

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

Appendix B

Clinical record forms (Study I)

แบบบันทึกข้อมูลทั่วไป

คำชี้แจง

แบบบันทึกนี้เป็นแบบบันทึกข้อมูลทั่วไปของผู้เข้าร่วมวิจัย เพื่อประเมินคุณสมบัติของท่านในการคัดเข้าในโครงการวิจัย รวมถึงเพื่อใช้ประกอบการประเมินประสิทธิภาพและความปลอดภัยของผงใบหม่อนที่ใช้ในการศึกษาครั้งนี้ **จึงขอความร่วมมือทุกท่าน**กรอกแบบบันทึกโดยเลือกหรือเติมข้อความในช่องว่างที่เว้นไว้ ให้ตรงกับความเป็นจริงของตัวท่าน

1. วันที่...../...../..... รหัสผู้เข้าร่วมวิจัย.....
2. เพศ ( ) ชาย ( ) หญิง
3. อายุ.....ปี วัน/เดือน/พ.ศ.เกิด...../...../.....
4. โรคประจำตัว *(หมายถึง โรคที่เป็นอยู่ในอดีตจนถึงปัจจุบัน เช่น เบาหวาน ความดันโลหิตสูง โรคหัวใจ โรคไต ภูมิแพ้ หอบหืด มะเร็ง ฯลฯ)*

   ( ) ไม่มี

   ( ) มี โปรดระบุโรค……

5. ยา สมุนไพร อาหารเสริมที่ใช้ประจำในปัจจุบัน *(ที่ได้รับทั้งจากโรงพยาบาล คลินิกแพทย์ ร้านยา หรือหาซื้อเองตามแหล่งอื่น)*

   ( ) ไม่มี

   ( ) มี โปรดระบุชื่อ……

6. ยา สมุนไพร อาหารเสริม ที่นอกเหนือจากยาประจำ รับประทานเป็นครั้งคราวเพื่อบรรเทาอาการใด ๆ **ในช่วง 2 สัปดาห์ที่ผ่านมา**

   ( ) ไม่มี

( ) มี โปรดระบุชื่อ……..…

7. ประวัติการแพ้ยา/สมุนไพร/อาหารเสริม/สารเคมี

( ) ไม่มี

( ) มี โปรดระบุชื่อ……..…

อาการ.....

8. การสูบบุหรี่

( ) ไม่เคยสูบ ( ) เคยสูบ แต่เลิกแล้ว……ปี ( ) ปัจจุบันสูบ……มวน/วัน

9. การดื่มแอลกอฮอล์

( ) ไม่เคยดื่ม ( ) เคยดื่ม แต่เลิกแล้ว……ปี ( ) ปัจจุบันดื่ม……แก้ว/วัน

10. การออกกำลังกาย

( ) ไม่เคยออกกำลังกาย

( ) ออกกำลังกายบ้างเป็นครั้งคราว ชนิดกีฬา.....

จำนวน.....ครั้ง/เดือน ระยะเวลา……ชั่วโมง/วัน

( ) ออกกำลังกายเป็นประจำ ชนิดกีฬา.....

จำนวน.....ครั้ง/เดือน ระยะเวลา……ชั่วโมง/วัน

ขอบคุณที่ให้ความร่วมมือ

Appendix B

Clinical record forms (Study I)

ID…….. Date……..../……/……	
Anthropometric profiles and vital signs	
Weight (kg)	
Height (cm)	
BMI (kg/m^2)	
SBP (mmHg)	
DBP (mmHg)	
HR (bpm)	
Blood tests	
FPG (mg/dL)	
HbA1C (%)	
BUN (mmol/L)	
SCr (mg/dL)	
AST (U/L)	
ALT (U/L)	
ALK (U/L)	
Capillary blood glucose	
PPG-0 (mg/dL)	
Time for taking test beverage ….....	
PPG-30 (mg/dL)	(Time……)
PPG-60 (mg/dL)	(Time……)
PPG-90 (mg/dL)	(Time……)
PPG-120 (mg/dL)	(Time……)
PPG-180 (mg/dL)	(Time……)

Appendix B

Clinical record forms (Study I)

แบบบันทึกการเกิดอาการไม่พึงประสงค์จากการรับประทานผงใบหม่อน

<u>ส่วนที่ 1</u> วันที่ทำการศึกษา (สำหรับผู้วิจัย)

เวลา	อาการไม่พึงประสงค์ที่พบ	การดำเนินการ / การรักษา

ผลการประเมินการเกิดอาการไม่พึงประสงค์

( ) เกิดจากผงใบหม่อน ( ) ไม่ได้เกิดจากผงใบหม่อน ( ) ไม่แน่ใจ

หมายเหตุ

……

……

(…….)

แพทย์ผู้ประเมิน / ผู้วิจัย

<u>ส่วนที่ 2</u> การติดตามอาการไม่พึงประสงค์ 7 วันหลังวันที่ทำการศึกษา (สำหรับผู้เข้าร่วมวิจัย)

คำชี้แจง

แบบบันทึกนี้ใช้สำหรับรายงานการเกิดอาการไม่พึงประสงค์จากการรับประทานผงใบหม่อน ในช่วง 7 วันหลังวันที่ทำการศึกษา เพื่อประเมินความปลอดภัยของผงใบหม่อนที่ใช้ในการศึกษาครั้งนี้ หากท่านพบอาการผิดปกติซึ่งสงสัยว่าอาจเกิดจากการรับประทานผงใบหม่อน โปรดกรอกแบบบันทึกโดยเลือกหรือเติมข้อความในช่องว่างที่เว้นไว้ให้

อาการไม่พึงประสงค์ที่พบ	วัน/เดือน/ ปี	เวลา
( ) น้ำมูกไหล คัดจมูก จาม รายละเอียดเพิ่มเติม (ถ้ามี)…… ……		
( ) ผื่นที่ผิวหนัง รายละเอียดเพิ่มเติม (ถ้ามี)…… ……		
( ) ปวดเกร็งท้อง รายละเอียดเพิ่มเติม (ถ้ามี)…… ……		
( ) ท้องอืด รายละเอียดเพิ่มเติม (ถ้ามี)…… ……		
( ) อื่น ๆ โปรดระบุ…… รายละเอียดเพิ่มเติม (ถ้ามี)…… ……		

โปรดส่งคืนผู้วิจัยเมื่อครบ 7 วันหลังที่ทำการศึกษา ขอบคุณที่ให้ความร่วมมือ

Appendix B

Naranjo's algorithm

อาการไม่พึงประสงค์…….....

คำถาม	ใช่	ไม่ใช่	ไม่ทราบ	คะแนน
1. เคยมีสรุปหรือรายงานการปฏิกิริยานี้มาแล้วหรือไม่	+1	0	0	
2. อาการไม่พึงประสงค์นี้เกิดขึ้นภายหลังจากได้รับยาที่คิดว่าเป็นสาเหตุหรือไม่	+2	-1	0	
3. อาการไม่พึงประสงค์นี้ดีขึ้นเมื่อหยุดยาดังกล่าว หรือเมื่อให้ยาต้านที่จำเพาะเจาะจง (specific antagonist) หรือไม่	+1	0	0	
4. อาการไม่พึงประสงค์ดังกล่าวเกิดขึ้นเมื่อเริ่มให้ยาใหม่หรือไม่	+2	-1	0	
5. ปฏิกิริยาที่เกิดขึ้นสามารถเกิดจากสาเหตุอื่น (นอกเหนือจากยา) ของผู้ป่วยได้หรือไม่	-1	+2	0	
6. ปฏิกิริยาดังกล่าวเกิดขึ้นอีก เมื่อให้ยาหลอกหรือไม่	-1	+1	0	
7. สามารถตรวจวัดปริมาณยาได้ในเลือด (หรือของเหลวอื่น) ในปริมาณความเข้มข้นที่เป็นพิษหรือไม่	+1	0	0	
8. ปฏิกิริยารุนแรงเกิดขึ้น เมื่อเพิ่มขนาดยาหรือลดความรุนแรงลงเมื่อลดขนาดยาหรือไม่	1	0	0	
9. ผู้ป่วยเคยมีปฏิกิริยาเหมือนหรือคล้ายคลึงกันนี้มาก่อนในการได้รับยาครั้งก่อน ๆ หรือไม่	+1	0	0	
10. อาการไม่พึงประสงค์นี้ ได้รับการยืนยันโดยหลักฐานที่เป็นรูปธรรม (objective evidence) หรือไม่	+1	0	0	
รวม				

ระดับคะแนน	คะแนนมากกว่าหรือเท่ากับ 9	Definite ใช่แน่
	คะแนนเท่ากับ 5-8	Probable ใช่
	คะแนนเท่ากับ 1-4	Possible อาจจะใช่
	คะแนนน้อยกว่าหรือเท่ากับ 0	Doubtful น่าสงสัย

Appendix C

Chemical analysis of phytochemicals in mulberry leaves powder

A. Quantitative determination of DNJ

(1) Liquid chromatographic condition	
Analytical column:	Inertsil NH2, 75 x 2.1-mm, 3-μm
Guard column:	Inertsil NH2, 10 x 2.1-mm, 3-μm
Mobile phase:	Mixture of 5 mM ammonium formate and 0.1% formic acid in acetonitrile, gradient
Autosampler temperature:	15°C
Column oven temperature:	40°C
Flow rate:	0.4 mL/min
Injection volume:	1 μL
Run time:	8 min

(2) Mass spectrometric condition	
MS mode:	Positive electrospray ionization (ESI+) mode Multiple reaction monitoring (MRM), m/z 164.05 → 110.15

Result of analysis:

Phytochemicals (Chemical formula)	**Categories**	**Concentration (mg/g in dried leaves)**
DNJ ($C_6H_{13}NO_4$)	Alkaloids	2.60 ± 0.78

B. Quantitative determination of phytochemicals

(1) Liquid chromatographic condition

Analytical column:	Zorbax RRHD Eclipse Plus C18 column from Agilent (100 × 2.1 mm, particle size: 1.8 μm)
Mobile phase:	Acetonitrile (B) and 0.01% formic acid in water (A)
Column oven temperature:	45°C
Flow rate:	0.4 mL/min
Injection volume:	2 μL
Gradient profile:	0/5, 3/5, 7/40, 11/80, 11.5/100, 13.5/100, 14/5, 16/5 min/B%
(2)Time-of-flight mass spectrometer condition	
MS mode:	Positive ion electrospray ionization (full scan mode at 50-800 m/z; spectra rate at 4 Hz)
MS parameters:	Nitrogen nebulizer gas pressure, 3 bar; drying gas flow rate, 12 L/min; End plate offset, 500 V; capillary voltage, +4500 V; dry temperature, 200 °C; funnel 1 RF and funnel 2 RF, 300 Vpp; CID energy, O eV; hexaploe RF, 50 Vpp; quadrople ion energy, 5 eV and low mass filtering at 50 m/z
Collision cell parameters:	Collision energy, 3 eV; collision RF, 500 Vpp; transfer time 50 μS and pre pulse storage at 6 μs
Data analysis:	Bruker software (Daltonics, Bremen, Germany)

Result of analysis:

Phytochemicals (Chemical formula)	**Categories**	**Concentration (mg/g in dried leaves)**
Chlorogenic acid ($C_{16}H_{18}O_9$)	Phenolic acid	1.59 ± 0.06
Quercetin ($C_{15}H_{10}O_7$)	Flavonoids	NQ
Kaempferol ($C_{15}H_{10}O_6$)	Flavonoids	NQ
Rutin ($C_{27}H_{30}O_{16}$)	Flavonol glycosides	0.82 ± 0.05

Isoquercitrin ($C_{21}H_{20}O_{12}$)	Flavonol glycosides	0.58 ± 0.05
Kaempferol 7-glucoside ($C_{21}H_{20}O_{11}$)	Flavonol glycosides	NQ
Kaempferol 3-*O*-β-D-(6"-*O*-malonyl)-glucoside ($C_{24}H_{22}O_{14}$)	Flavonol glycosides	NQ
Quercetin 3-(6”-malonyl-glucoside) ($C_{24}H_{22}O_{15}$)	Flavonol glycosides	NQ

Data are expressed as mean ± S.D.

NQ: cannot be quantified

Appendix D

Clinical outcomes (Study I)

Participants ID	Assigned group	Weight (kg)	Height (cm)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	HR (bpm)	FPG (mg/dL)	A1C (%)	AST (U/L)	ALT (U/L)	Cr (mg/dL)	BUN (mg/dL)	PPG 0 (mg/dL)	PPG 30 (mg/dL)	PPG 60 (mg/dL)	PPG 90 (mg/dL)	PPG 120 (mg/dL)	PPG 180 (mg/dL)	AUC $_{0\text{-}180\ min}$ (mg/dL.min)
6	**1**	56	164	20.82	107	76	75	83	5.3	16	9	0.8	8	83	110	89	97	94	100	240.75
4	**1**	51.8	163	19.50	107	78	65	83	5	18	11	0.69	10	83	126	111	105	139	88	283.25
12	**1**	48	156	19.72	131	76	97	83	4.3	21	10	0.74	14	83	141	131	139	108	91	303.00
13	**1**	57	172	19.27	106	66	60	75	4.8	20	12	0.96	10	75	143	178	114	70	78	290.75
19	**1**	60	166	21.77	103	61	72	83	5.1	30	18	0.88	10	83	135	101	82	86	83	243.50
22	**1**	55	160	21.48	102	68	62	74	4.9	36	13	0.77	11	74	153	118	89	68	70	250.00
25	**1**	60	175	19.59	122	67	74	99	5.3	18	8	0.97	11	99	157	130	87	88	85	277.00
28	**1**	57	167	20.44	89	68	79	85	5.3	24	14	0.6	7	85	139	92	89	82	88	244.25
32	**1**	66	175	21.55	115	69	80	85	5.2	36	21	1.01	15	85	145	97	78	88	99	250.00
35	**1**	55	165	20.20	104	65	72	86	5.2	20	12	0.78	12	86	153	143	106	79	88	284.00
41	**1**	52	160	20.31	102	67	72	84	5.1	16	5	0.7	13	84	120	129	79	74	79	241.75
46	**1**	53	150	23.56	100	65	68	77	5	16	7	0.8	12	77	99	110	89	90	79	233.00
48	**1**	59	167	21.16	120	77	81	84	4.9	17	19	0.6	9	84	143	104	77	95	77	249.75
55	**1**	47	158	18.83	102	73	60	84	5.1	25	12	0.76	10	84	154	113	86	77	72	254.00
57	**1**	52	162	19.81	103	69	62	83	5	15	9	0.73	9	83	145	114	94	93	82	264.25
63	**1**	47	150	20.89	104	69	88	77	5.3	22	10	0.63	8	77	135	109	80	66	72	232.25
65	**1**	51	163	19.20	97	66	85	76	5.1	23	11	0.62	14	76	139	129	112	75	91	269.25
70	**1**	47	158	18.83	97	65	92	82	5	16	10	0.56	11	82	157	134	99	100	89	287.75
73	**1**	58	170	20.07	96	67	70	88	4.6	37	18	0.84	13	88	155	132	89	66	70	260.50

Participants ID	Assigned group	Weight (kg)	Height (cm)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	HR (bpm)	FBS (mg/dL)	A1C (%)	AST (U/L)	ALT (U/L)	Cr (mg/dL)	BUN (mg/dL)	PPG 0 (mg/dL)	PPG 30 (mg/dL)	PPG 60 (mg/dL)	PPG 90 (mg/dL)	PPG 120 (mg/dL)	PPG 180 (mg/dL)	$AUC_{0\text{-}180\ min}$ (mg/dL.min)
76	**1**	62	165	22.77	95	64	80	81	4.8	27	14	0.65	9	81	142	138	108	83	73	274.00
84	**1**	48	166	17.42	97	64	75	85	5.4	20	8	0.85	10	85	144	138	105	99	73	282.50
3	**2**	52	162	19.81	112	70	93	89	5	15	8	0.71	7	89	115	92	94	102	91	246.50
5	**2**	54.4	155	22.64	120	77	71	79	5.5	25	26	0.72	9	79	125	108	115	83	80	255.25
9	**2**	48	158	19.23	96	60	80	87	5	28	15	0.66	11	87	106	99	89	96	80	236.75
16	**2**	57	160	22.27	110	67	70	96	5.2	29	9	0.59	10	96	130	128	101	91	77	268.25
18	**2**	55	168	19.49	98	50	88	100	5.3	19	10	0.82	9	100	117	106	104	93	76	254.00
21	**2**	51	158	20.43	106	69	66	83	4.6	16	9	0.71	10	83	108	101	89	95	89	239.50
27	**2**	65	177	20.75	128	68	79	89	5.1	28	19	0.95	12	89	113	117	89	85	93	247.50
29	**2**	59	163	22.21	92	48	58	74	4.8	23	8	0.65	9	74	118	106	113	100	90	259.50
34	**2**	65	170	22.49	128	86	101	83	5.4	30	22	0.83	11	83	165	174	131	111	72	329.25
36	**2**	64	173	21.38	108	79	68	80	5.5	36	19	0.94	10	80	133	96	98	93	86	251.50
39	**2**	50	160	19.53	117	83	84	84	5.3	16	7	0.75	12	84	105	101	108	102	96	253.00
42	**2**	60	168	21.26	102	53	60	82	5.2	21	11	0.7	8	82	114	109	99	91	85	248.25
47	**2**	53	163	19.95	107	68	61	76	4.8	12	9	0.64	10	76	122	97	92	107	84	249.00
49	**2**	45.2	156	18.57	109	70	62	78	5.2	18	12	0.67	10	78	116	109	106	107	73	256.75
54	**2**	55	158	22.03	103	72	77	72	4.6	23	12	0.62	9	72	103	90	91	102	79	230.75
58	**2**	52	160	20.31	111	77	81	78	5.1	15	5	0.7	12	78	106	99	97	82	76	230.50
62	**2**	52	156	21.37	101	60	68	83	4.3	19	18	0.72	13	83	106	117	107	90	85	252.00
64	**2**	50	158	20.03	98	61	60	81	4.3	21	8	0.76	13	81	109	125	109	87	92	258.25
68	**2**	58	176	18.72	112	73	92	88	5.7	22	20	1.01	18	88	138	120	83	97	80	261.00
75	**2**	55	164	20.45	97	62	63	86	5	18	11	0.7	14	86	118	98	93	86	82	239.50
81	**2**	45	154	18.97	96	68	92	75	4.8	18	10	0.89	10	75	99	107	97	93	85	238.00

Participants ID	Assigned group	Weight (kg)	Height (cm)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	HR (bpm)	FBS (mg/dL)	A1C (%)	AST (U/L)	ALT (U/L)	Cr (mg/dL)	BUN (mg/dL)	PPG 0 (mg/dL)	PPG 30 (mg/dL)	PPG 60 (mg/dL)	PPG 90 (mg/dL)	PPG 120 (mg/dL)	PPG 180 (mg/dL)	AUC $_{0\text{-}180\ min}$ (mg/dL.min)
2	**3**	48	160	18.75	90	60	91	79	4.6	29	30	0.8	9	79	91	85	89	81	78	212.25
7	**3**	59	168	20.90	101	61	80	63	4.7	20	13	1.05	15	63	82	94	104	95	80	223.25
11	**3**	54	163	20.32	104	63	82	84	5	13	26	0.68	8	84	105	100	92	103	88	243.00
15	**3**	55	158	22.03	108	78	97	83	5.2	18	9	0.69	13	83	99	101	97	88	82	233.75
23	**3**	47	158	18.83	97	63	70	83	5.4	18	8	0.81	9	83	102	98	99	89	90	237.25
26	**3**	56	165	20.57	91	64	78	86	4.7	26	9	0.7	10	86	116	103	98	96	94	251.50
30	**3**	54	167	19.36	119	82	72	84	5.3	32	26	1.11	12	84	110	120	96	78	61	238.25
33	**3**	70	177	22.34	123	74	72	80	5.6	36	39	1.13	13	80	100	88	95	98	90	233.00
38	**3**	43	153	18.37	105	65	70	82	5.4	22	7	0.52	12	82	111	84	89	92	79	228.25
45	**3**	60	167	21.51	127	84	88	87	5.4	17	13	0.61	11	87	123	119	103	99	85	265.00
51	**3**	48	160	18.75	95	71	88	81	4.6	17	14	0.57	11	81	114	96	89	89	99	239.00
53	**3**	47	157	19.07	111	79	93	88	5.4	14	11	0.75	19	88	85	96	100	98	98	236.00
56	**3**	78	187	22.31	110	61	53	85	5.3	14	10	0.8	15	85	114	92	103	104	75	246.50
61	**3**	50	164	18.59	98	69	100	84	5.3	17	13	0.74	9	84	83	103	87	88	85	222.75
66	**3**	50	164	18.59	86	57	67	79	5.2	17	11	0.59	11	79	99	107	94	89	80	234.25
69	**3**	48	157	19.47	98	55	75	87	4.7	15	10	0.71	7	87	103	115	107	107	106	264.25
72	**3**	44	154	18.55	89	61	61	74	4.9	32	30	0.69	15	74	105	91	83	92	80	224.00
77	**3**	60	165	22.04	96	68	74	76	5.2	17	10	0.64	11	76	130	111	87	91	69	245.75
78	**3**	43	152	18.61	117	73	87	79	5.6	18	6	0.65	12	79	107	108	107	100	97	255.00
80	**3**	56	170	19.38	115	71	70	84	5.1	20	13	0.58	8	84	103	113	112	93	86	253.00
82	**3**	59	164	21.94	124	75	84	82	5.1	18	10	0.62	9	82	124	146	134	121	88	305.00
1	**4**	61	167	21.87	93	62	75	90	5	22	10	1.01	10	90	97	89	96	100	95	237.25
8	**4**	69	185	20.16	111	60	95	83	4.6	27	11	1	10	83	95	91	95	90	86	227.75

Participants ID	Assigned group	Weight (kg)	Height (cm)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	HR (bpm)	FBS (mg/dL)	A1C (%)	AST (U/L)	ALT (U/L)	Cr (mg/dL)	BUN (mg/dL)	PPG 0 (mg/dL)	PPG 30 (mg/dL)	PPG 60 (mg/dL)	PPG 90 (mg/dL)	PPG 120 (mg/dL)	PPG 180 (mg/dL)	AUC $_{0\text{-}180\ min}$ (mg/dL.min)
10	**4**	50	150	22.22	89	56	72	82	5.2	32	20	0.82	12	82	112	114	117	102	96	267.00
14	**4**	53	165	19.47	100	55	69	84	5.4	13	10	0.62	15	84	89	79	88	87	90	215.00
17	**4**	57	169	19.96	108	72	68	91	5.2	26	10	0.94	9	91	89	95	92	99	97	234.50
20	**4**	58	166	21.05	104	51	69	75	5.1	35	19	0.72	7	75	88	85	96	87	94	220.25
24	**4**	48	154	20.24	92	55	79	83	5.1	25	15	0.65	9	83	90	99	102	89	99	235.50
31	**4**	60	160	23.44	127	67	65	88	5.7	18	9	0.85	17	88	102	99	97	101	106	248.00
37	**4**	48	152	20.78	101	84	87	82	5	17	11	0.72	11	82	108	114	95	87	93	245.75
40	**4**	45	153	19.22	83	41	64	80	5.3	20	14	0.77	11	80	105	125	93	75	78	238.50
43	**4**	41	150	18.22	108	64	86	84	5	19	11	0.62	10	84	99	95	98	96	104	241.00
44	**4**	45	153	19.22	102	67	70	101	5.6	18	18	0.59	8	101	108	125	106	119	106	280.75
50	**4**	52	150	23.11	133	77	79	87	5.2	21	8	0.64	11	87	116	95	103	106	86	253.25
52	**4**	54	163	20.32	110	75	88	84	4.9	30	7	0.75	16	84	90	95	108	86	79	230.25
59	**4**	49	162	18.67	97	66	62	90	5.2	21	10	0.62	14	90	83	90	99	81	80	219.00
60	**4**	62	168	21.97	120	77	74	82	5	18	20	0.75	12	82	95	103	98	86	85	232.75
67	**4**	58	168	20.55	97	68	73	78	5.2	18	10	0.48	12	78	89	98	83	89	83	219.75
71	**4**	67	189	18.76	118	64	61	87	5.2	18	13	0.81	15	87	107	110	110	94	81	252.50
74	**4**	63	175	20.57	108	81	73	78	5.4	24	16	0.7	10	78	103	100	89	102	85	237.75
79	**4**	59	167	21.16	114	78	86	88	5.2	22	10	0.59	6	88	100	94	84	98	85	231.25
83	**4**	49	150	21.78	106	74	80	88	4.5	16	6	0.67	9	88	96	96	96	100	100	241.00
85	**4**	50	164	18.59	106	77	85	85	5	28	30	0.77	9	85	106	87	89	83	89	226.00

Appendix E

Certificate of ethic approval (Study II)

RL 01_2560

คณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก

317/5 ถนนราชวิถี เขตราชเทวี กรุงเทพฯ 10400

ที่ IRBRTA.....๑๙๗...../2561 **รหัสโครงการ: Q038h/60**

ชื่อโครงการวิจัย : ประสิทธิศักย์และความปลอดภัยของใบหม่อนในการควบคุมระดับน้ำตาลในเลือดในผู้ที่มีภาวะอ้วนและผู้ป่วยเบาหวานชนิดที่ 2
[EFFICACY AND SAFETY OF MULBERRY LEAVES ON GLYCEMIC CONTROL IN PATIENTS WITH OBESITY AND PATIENTS WITH TYPE 2 DIABETES]

เลขที่โครงการวิจัย : -

ชื่อผู้วิจัยหลัก: เภสัชกรหญิง ธัญชนิต ไทยพิทักษ์วงศ์

สังกัดหน่วยงาน : คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สถานที่ทำการวิจัย: แผนกผู้ป่วยนอก โรงพยาบาลพระมงกุฎเกล้า

เอกสารรับรอง :

(1) แบบรายงานการส่งโครงร่างการวิจัยเพื่อพิจารณาครั้งแรก ฉบับที่ 1 ลงวันที่ 29 ธันวาคม 2560
(2) โครงร่างการวิจัย ฉบับที่ 2 ลงวันที่ 6 กุมภาพันธ์ 2561
(3) เอกสารชี้แจงข้อมูลแก่ผู้เข้าร่วมโครงการวิจัย และหนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย ฉบับที่ 2 ลงวันที่ 6 กุมภาพันธ์ 2561
(4) แบบบันทึกข้อมูล ฉบับที่ 2 ลงวันที่ 6 กุมภาพันธ์ 2561
(5) ประวัติผู้วิจัย เภสัชกรหญิง ธัญชนิต ไทยพิทักษ์วงศ์ ฉบับที่ 2 ลงวันที่ 6 กุมภาพันธ์ 2561
(6) ประวัติที่ปรึกษา เภสัชกรหญิง พรอนงค์ อร่ามวิทย์ ฉบับที่ 2 ลงวันที่ 6 กุมภาพันธ์ 2561
(7) ประวัติที่ปรึกษา พ.อ.อุปถัมภ์ ศุภสินธุ์ ฉบับที่ 2 ลงวันที่ 6 กุมภาพันธ์ 2561

ขอรับรองว่าโครงการดังกล่าวข้างต้นได้ผ่านการพิจารณารับรองจากคณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบกว่าสอดคล้องกับแนวทางจริยธรรมสากล ได้แก่ ปฏิญญาเฮลซิงกิ รายงานเบลมองต์แนวทางจริยธรรมสากลสำหรับการวิจัยในมนุษย์ขององค์การสากลด้านวิทยาศาสตร์การแพทย์ (CIOMS) และแนวทางการปฏิบัติการวิจัยที่ดี (ICH GCP)

วันที่รับรองด้านจริยธรรมของโครงร่างการวิจัย: 12 กุมภาพันธ์ 2561
วันสิ้นสุดการรับรอง: 11 กุมภาพันธ์ 2562
ความถี่ของการส่งรายงานความก้าวหน้าของการวิจัย: 1 ปี

พันเอกหญิง
(แสงแข ชำนาญวนกิจ)
ประธานคณะอนุกรรมการพิจารณาโครงการวิจัย
กรมแพทย์ทหารบก

สำนักงานคณะอนุกรรมการพิจารณาโครงการวิจัย พบ.

Appendix F

Clinical record forms (Study II)

แบบบันทึกข้อมูลทั่วไป

คำชี้แจง

แบบบันทึกนี้เป็นแบบบันทึกข้อมูลทั่วไปของผู้เข้าร่วมวิจัย เพื่อประเมินคุณสมบัติของท่านในการคัดเข้าในโครงการวิจัยเรื่อง **ประสิทธิศักย์และความปลอดภัยของใบหม่อนในการควบคุมระดับน้ำตาลในเลือดในผู้ที่มีภาวะอ้วนและผู้ป่วยเบาหวานชนิดที่ 2** รวมถึงเพื่อใช้ประกอบการประเมินประสิทธิภาพและความปลอดภัยของใบหม่อนที่ใช้ในการวิจัยครั้งนี้เบื้องต้น จึงขอความร่วมมือทุกท่านกรอกแบบบันทึกโดยเลือกหรือเติมข้อความในช่องว่างที่เว้นไว้ให้ตรงกับความเป็นจริงของตัวท่านเฉพาะในส่วนที่ 1 ของแบบบันทึกนี้

ส่วนที่ 1

11. เพศ ( ) ชาย ( ) หญิง

12. อายุ.....ปี วัน/เดือน/พ.ศ.เกิด...../...../.....

13. โรคประจำตัว ที่นอกเหนือจากโรคเบาหวาน *(หมายถึง โรคที่เป็นอยู่ในอดีตจนถึงปัจจุบัน เช่น ความดันโลหิตสูง ไขมันสูง โรคหัวใจ โรคกระเพาะอาหาร โรคไต ภูมิแพ้ หอบหืด มะเร็ง ฯลฯ)*

    ( ) ไม่มี

    ( ) มี โปรดระบุโรค……..

14. ประวัติการผ่าตัดทางเดินอาหาร

    ( ) ไม่เคย

    ( ) เคย โปรดระบุรายละเอียด……..……...…

15. ยา สมุนไพร อาหารเสริมที่ใช้ประจำในปัจจุบัน *(ที่ได้รับทั้งจากโรงพยาบาล คลินิกแพทย์ ร้านยา หรือหาซื้อเองตามแหล่งอื่น)*

    ( ) ไม่มี

    ( ) มี โปรดระบุชื่อ……..……..……

16. ยา สมุนไพร อาหารเสริม ที่นอกเหนือจากยาประจำ รับประทานเป็นครั้งคราวเพื่อบรรเทาอาการใด ๆ **ในช่วง 1 เดือนที่ผ่านมา**

( ) ไม่มี

( ) มี โปรดระบุชื่อ……

17. ประวัติการแพ้ยา/สมุนไพร/อาหารเสริม/สารเคมี

( ) ไม่มี

( ) มี โปรดระบุชื่อ……

อาการ.....

18. การสูบบุหรี่

( ) ไม่เคยสูบ

( ) เคยสูบ แต่เลิกแล้วเป็นระยะเวลา…….....(โปรดระบุจำนวนเดือน หรือ ปี)

( ) ปัจจุบันสูบ……มวนต่อวัน

19. การดื่มแอลกอฮอล์ เช่น เหล้า เบียร์ ไวน์ เหล้าขาว ยาดอง

( ) ไม่เคยดื่ม

( ) เคยดื่ม แต่เลิกแล้วเป็นระยะเวลา…….....(โปรดระบุจำนวนเดือน หรือ ปี)

( ) ปัจจุบันดื่ม……แก้ว (โปรดระบุความถี่ ได้แก่ ต่อวัน สัปดาห์ เดือน หรือ ปี)

20. การออกกำลังกาย

( ) ไม่เคยออกกำลังกาย

( ) ออกกำลังกายบ้างเป็นครั้งคราว ชนิดกีฬา.....

จำนวน.....ครั้ง/เดือน ระยะเวลา……ชั่วโมง/วัน

( ) ออกกำลังกายเป็นประจำ ชนิดกีฬา.....

จำนวน.....วัน/สัปดาห์ ระยะเวลา……ชั่วโมง/วัน

ขอบคุณที่ให้ความร่วมมือ

<u>ส่วนที่ 2</u> (สำหรับผู้วิจัย เพื่อการคัดกรองผู้เข้าร่วมงานวิจัย)

( ) ไม่เคยได้รับการวินิจฉัยว่าเป็นเบาหวานมาก่อน Height……cm Weight……kg BMI…..…kg/m2 (เมื่อวันที่.....)
( ) ได้รับการวินิจฉัยว่าเป็นเบาหวานชนิดที่ 2 1. ได้รับการวินิจฉัยเมื่อวันที่……..…… 2. ระยะเวลาที่ได้รับการวินิจฉัยว่าเป็นเบาหวานชนิดที่ 2 นาน……ปี…….เดือน 3. FPG.....mg/dL (เมื่อวันที่.....) 4. 2-hour PPG after 75-g OGTT……..…mg/dL (เมื่อวันที่.....) 5. HbA1C……...% (เมื่อวันที่.....) 6. การรักษาเบาหวานชนิดที่ 2 ที่เคยที่ได้รับ ( ) ไม่เคย ( ) อื่น ๆ ระบุ..... 7. Height……cm Weight……kg BMI……kg/m^2 (เมื่อวันที่.....) 8. AST…..U/L ALT…….U/L (เมื่อวันที่.....) 9. Cr.....mg/dL BUN……mg/dL (เมื่อวันที่.....)
( ) Signs and symptoms of hyperglycemia
( ) Diabetic complications
( ) Pregnancy / lactation

Appendix F

Clinical record forms (Study II)

ผลการตรวจร่างกายและผลตรวจทางห้องปฏิบัติการ

Outcomes	Experimental period			
	Week 0	Week 4	Week 8	Week 12
Date				
Height (cm)				
Weight (kg)				
BMI (kg/m^2)				
Waist circumference (cm)				
SBP (mmHg)				
DBP (mmHg)				
HR (bpm)				
FPG (mg/dL)				
PPG-30 (mg/dL)				
PPG-60 (mg/dL)				
PPG-90 (mg/dL)				
PPG-120 (or 2-hour PPG) (mg/dL)				
A1C (%)				
FPI (µIU/mL)				
HOMA-IR				
TC (mg/dL)				
TG (mg/dL)				
HDL-C (mg/dL)				
LDL-C (mg/dL)				
AST (U/L)				
ALT (U/L)				
Cr (mg/dL)				

Appendix F

Clinical record forms (Study II)

แบบบันทึกการบริโภคอาหาร

คำชี้แจง

วิธีการบันทึก

1) จดบันทึกอาหารทุกชนิด ซึ่งรวมถึงขนมและเครื่องดื่มที่รับประทานทั้งในมื้อหลักและมื้อของว่างในวันใดวันหนึ่งในช่วง 1 สัปดาห์ก่อนวันนัดครั้งต่อไป ทั้งนี้ไม่แนะนำให้ทำในวันพิเศษ เช่น วันที่ไปงานแต่งงาน จัดงานเลี้ยงวันเกิด หรือวันที่ไม่สบาย เป็นต้น

2) ควรบันทึกทันทีที่บริโภคในแต่ละครั้งเพื่อป้องกันการลืม

3) บันทึกส่วนประกอบอาหารต่าง ๆ อย่างละเอียด ดังนี้

ก. ชนิด เช่น ไข่เป็ด ไข่ไก่ ก๋วยเตี๋ยวเส้นใหญ่ บะหมี่

ข. วิธีการปรุง เช่น ดิบ ต้ม นึ่ง ทอด

ค. ยี่ห้อ เช่น มันฝรั่งทอดกรอบเลย์ บะหมี่กึ่งสำเร็จรูปตราไวไว

ง. รสชาติ เช่น นมสดพลาสเจอไรซ์รสจืด โยเกิร์ตรสผลไม้รวม

จ. ลักษณะพิเศษ เช่น โค้กซีโร่

ฉ. ขนาด เช่น ตับหมูขนาด กว้าง ยาว หนา

ช. เครื่องปรุงรส

4) ระบุปริมาณส่วนประกอบอาหารต่าง ๆ อย่างละเอียด เช่น ถ้วย ช้อนชา ช้อนโต๊ะ ทัพพี ชิ้น ห่อ ขวด

5) โปรดส่งคืนแบบบันทึกนี้ให้กับนักกำหนดอาหารในนัดครั้งต่อไป (วันที่.....)

ตัวอย่างการบันทึกการบริโภคอาหาร

วันที่บันทึก.....1.....เดือน....ตุลาคม.....พ.ศ....2560.... ( ) วันจันทร์-ศุกร์ (√) วันเสาร์-อาทิตย์

มื้ออาหาร/เวลา	**ชนิดอาหารที่รับประทานและส่วนประกอบ**	**ปริมาณ (เช่น ถ้วย, ชิ้น, ช้อนชา, ฯลฯ)**
เช้า เวลา...8.00...น.	ข้าวต้มหมูสับ - ข้าวขาว - หมูสับ - ตับหมูหั่นบาง - น้ำตาลทราย - น้ำปลา - น้ำส้มสายชู น้ำเต้าหู้ หวานน้อย ใส่เม็ดแมงลัก - เม็ดแมงลัก - น้ำตาลทรายแดง	 - 2 ทัพพี ไม่รวมน้ำ - 5 ชิ้น - 3 ชิ้น - 2 ช้อนชา - 1 ช้อนชา - 2 ช้อนชา 1 ถุง - 1 ช้อนโต๊ะ - 1 ช้อนชา
ของว่างช่วงเช้า เวลา...10.30..น.	ส้มเขียวหวาน	2 ผล
เที่ยง เวลา...12.30..น.	ข้าวกล้อง ปลาดุกผัดเผ็ด - ปลาดุก - มะเขือกรอบ (แบ่ง 4 ส่วน/ลูก) - ถั่วฝักยาว (ยาวประมาณครึ่งนิ้ว) ไข่เจียว โอเลี้ยง (ชาต้นพะยอม)	2 ทัพพี - 4 ชิ้น - 2 ชิ้น - 4-5 ชิ้น 1 ฟอง 1 แก้ว
เย็น เวลา...18.30..น.	เกาเหลาลูกชิ้นหมูน้ำใส - ลูกชิ้นหมู - หมูสับ - ถั่วงอก - ผักบุ้ง	 - 4 ลูก - 2 ช้อนโต๊ะ - 1 ทัพพี - 1 ทัพพี

วันที่บันทึก.....เดือน.....พ.ศ..... ( ) วันจันทร์-ศุกร์ ( ) วันเสาร์-อาทิตย์

มื้ออาหาร/ เวลา	ชนิดอาหารที่รับประทานและส่วนประกอบ	ปริมาณ (เช่น ถ้วย, ชิ้น, ช้อนชา, ฯลฯ)

Appendix F

Clinical record forms (Study II)

แบบบันทึกความร่วมมือในการปฏิบัติตามแผนการวิจัย

<u>ส่วนที่ 1</u> ความร่วมมือในการควบคุมอาหาร

	Experimental period			
	Week 0	**Week 4**	**Week 8**	**Week 12**
Date				
Total energy intake (kcal)				
Total fat intake (%)				
Total carbohydrate intake (%)				
Total protein intake (%)				

<u>ส่วนที่ 2</u> ความร่วมมือในการรับประทานผงใบหม่อน (เฉพาะกลุ่มทดลอง)

	Experimental period			
	Week 0	**Week 4**	**Week 8**	**Week 12**
Date				
จำนวนซองที่ได้รับจากนัดครั้งก่อน				
จำนวนซองที่เหลือในนัดครั้งนี้				
% Compliance				

Appendix F

Clinical record forms (Study II)

แบบสอบถามการเกิดอาการไม่พึงประสงค์จากการรับประทานใบหม่อน

คำชี้แจง แบบบันทึกนี้ใช้สำหรับรายงานการเกิดอาการไม่พึงประสงค์จากการรับประทานผงใบหม่อน เพื่อประเมินความปลอดภัยของผงใบหม่อนที่ใช้ในการศึกษาครั้งนี้ ขอให้ท่านระบุอาการไม่พึงประสงค์ที่เกิดขึ้น พร้อมทั้งประเมินระดับความรุนแรงจากน้อยไปมาก โดยการให้คะแนนตั้งแต่ 0 ถึง 5

อาการไม่พึงประสงค์ที่พบ	**ระดับความรุนแรง**					
	ไม่มี	น้อยมาก	น้อย	ปานกลาง	มาก	มากที่สุด
อาการจากภาวะน้ำตาลในเลือดต่ำ เช่น หัวใจเต้นเร็ว เวียนศีรษะ สายตาพร่า มือสั่น เหงื่อออกมาก หิวบ่อย อ่อนเพลีย คล้ายจะหน้ามืดเป็นลม รายละเอียดเพิ่มเติม (ถ้ามี).....	(0)	(1)	(2)	(3)	(4)	(5)
ปวดเกร็งท้อง รายละเอียดเพิ่มเติม (ถ้ามี).....	(0)	(1)	(2)	(3)	(4)	(5)
ท้องอืด มีแก๊สในท้องมาก รายละเอียดเพิ่มเติม (ถ้ามี).....	(0)	(1)	(2)	(3)	(4)	(5)
ถ่ายเหลว รายละเอียดเพิ่มเติม (ถ้ามี).....	(0)	(1)	(2)	(3)	(4)	(5)
ท้องผูก รายละเอียดเพิ่มเติม (ถ้ามี).....	(0)	(1)	(2)	(3)	(4)	(5)
อื่น ๆ รายละเอียดเพิ่มเติม	(0)	(1)	(2)	(3)	(4)	(5)

ขอบคุณที่ให้ความร่วมมือ

Appendix G

Clinical outcomes (Study II)

(i) Anthropometric profile and vital signs

Participants ID	Assigned group	Weight (kg)-0	Weight (kg)-12	BMI (kg/m^2)-0	BMI (kg/m^2)-12	WC (cm)-0	WC (cm)-12	SBP (mmHg)-0	SBP (mmHg)-12	DBP (mmHg)-0	DBP (mmHg)-12	HR (bpm)-0	HR (bpm)-12
1	**1**	96.1	95.1	37.54	37.15	103	103	138	131	80	77	76	72
4	**1**	87.95	88	36.14	36.16	110	110	139	131	83	85	85	82
6	**1**	64.5	65.2	26.85	27.14	84	84	141	128	91	88	89	82
7	**1**	63	60.1	28.00	26.71	89	86.5	128	132	87	85	94	85
8	**1**	62.4	60.65	27.37	26.60	85	84.5	129	129	87	95	68	57
11	**1**	68.1	67.35	29.09	28.77	92	92	117	126	66	71	65	65
14	**1**	79.85	79.9	34.56	34.58	103	103.5	122	139	92	91	73	72
16	**1**	93.5	90.75	38.18	37.05	122	121	130	139	88	91	65	72
17	**1**	61	58.7	25.39	24.43	91	89	109	109	64	67	82	90
18	**1**	63.2	62.3	25.32	24.96	89	89	101	103	55	56	73	73
22	**1**	68.8	68.35	27.56	27.38	94	93	124	129	74	79	93	81
23	**1**	90	89.36	34.29	34.05	118	118.5	143	155	95	106	90	97
28	**1**	63.7	63.75	26.51	26.53	94	94	131	128	68	73	83	76
29	**1**	77	77	26.03	26.03	100	100	113	119	78	71	84	96
31	**1**	79	78	30.86	30.47	103.5	104	127	138	80	70	66	63
33	**1**	85	84.25	28.08	27.83	104	104	129	133	80	84	86	82
34	**1**	84.2	84	29.48	29.41	98	98	127	121	83	84	95	77
35	**1**	70.9	68	31.51	30.22	99	99	148	147	83	85	89	87
37	**1**	76.9	77.75	27.25	27.55	101.5	101.5	143	146	91	95	91	79
39	**1**	65	64.8	27.41	27.32	104	105	105	115	54	50	86	78
41	**1**	93.45	92.35	31.59	31.22	111	111	123	108	74	63	79	77
42	**1**	115	115.35	37.55	37.67	130.5	130.5	132	110	71	71	76	86
46	**1**	67.1	66.5	25.25	25.03	92	92	136	126	70	67	82	91
47	**1**	76.45	75	27.74	27.22	99	99	123	130	77	75	82	80
49	**1**	77.75	77.25	31.95	31.74	102	102	138	139	72	70	72	75
53	**1**	81.5	82.2	28.88	29.12	102	102.5	128	127	76	72	73	69
56	**1**	68.5	69.2	26.76	27.03	90	90.5	142	144	85	83	68	68
57	**1**	105.9	107	34.58	34.94	115	115	115	120	71	71	70	69
2	**2**	73.4	72.2	30.16	29.67	103	101	94	113	54	67	75	72
3	**2**	65.8	65.4	28.67	28.49	86	86	130	141	80	75	80	72
5	**2**	70.5	69.5	27.54	27.15	96	95.5	142	148	83	77	71	77
10	**2**	82.2	82.5	30.94	31.05	102.5	102	128	124	74	69	78	78
13	**2**	89.35	91.9	30.56	31.43	110	109	110	118	78	81	79	67
15	**2**	85	82	33.62	32.44	102	102	132	145	65	67	63	82

Participants ID	Assigned group	Weight (kg)-0	Weight (kg)-12	BMI (kg/m^2)-0	BMI (kg/m^2)-12	WC (cm)-0	WC (cm)-12	SBP (mmHg)-0	SBP (mmHg)-12	DBP (mmHg)-0	DBP (mmHg)-12	HR (bpm)-0	HR (bpm)-12
19	**2**	71.2	71.9	32.07	32.39	94	94.5	123	127	71	77	76	81
20	**2**	90.35	90.25	36.65	36.61	114	114.5	100	110	60	63	70	70
21	**2**	75.9	76.5	28.92	29.15	95	96.5	145	118	90	84	63	102
24	**2**	83	83.7	30.49	30.74	96	94.5	140	146	79	97	79	57
25	**2**	85	86	34.05	34.45	106	107	113	115	62	63	73	73
26	**2**	85.5	86.4	32.98	33.33	105	107	158	149	87	87	63	69
27	**2**	72.55	73.3	28.70	28.99	101	99.5	130	116	81	74	65	78
30	**2**	72.15	71.8	26.83	26.70	93	93	120	122	70	71	82	79
32	**2**	92.3	92	34.74	34.63	109	109	139	131	82	72	81	84
36	**2**	57.8	58	25.35	25.44	95	95	137	145	74	80	69	77
38	**2**	69.3	69	25.45	25.34	92.5	93	141	139	91	90	84	88
40	**2**	67.05	67.5	26.86	27.04	91	91	128	122	66	65	97	92
43	**2**	107.3	105.3	38.02	37.31	111	111	122	124	66	68	78	81
44	**2**	80	79.55	33.30	33.11	101	100.5	134	103	86	60	84	89
45	**2**	89.1	89.5	27.50	27.62	112	112.5	161	139	88	88	89	85
48	**2**	110	111.5	38.06	38.58	122.5	123	159	149	109	101	95	95
50	**2**	72.55	73	26.65	26.81	99.5	99	143	140	94	95	74	76
54	**2**	76.6	77	31.48	31.64	96.5	96.5	102	110	57	67	59	61
55	**2**	165.45	114.65	54.02	37.44	165	134	140	151	90	93	91	93
58	**2**	66.9	67.35	28.21	28.40	85	85	129	125	65	69	84	81

Appendix G

Clinical outcomes (Study II)

(ii) Glycemic profile and insulin resistance

Participants ID	Assigned group	FPG (mg/dL)-0	FPG (mg/dL)-4	FPG (mg/dL)-8	FPG (mg/dL)-12	PPG 30 (mg/dL)-0	PPG 30 (mg/dL)-12	PPG 60 (mg/dL)-0	PPG 60 (mg/dL)-12	PPG 90 (mg/dL)-0	PPG 90 (mg/dL)-12	PPG 120 (mg/dL)-0	PPG 120 (mg/dL)-12	AUC (mg/dL.min)-0	AUC (mg/dL.min)-12	A1C (%)-0	A1C (%)-12	FPI (µIU/mL)-0	FPI (µIU/mL)-12	HOMA-IR-0	HOMA-IR-12
1	**1**	106	103	96	102	174	192	206	199	181	192	148	166	344.00	358.50	6	5.9	21.5	18.4	5.63	4.63
4	**1**	81	80	82	88	133	132	174	164	176	178	155	185	300.50	301.75	5.7	5.7	19.6	13.9	3.92	3.02
6	**1**	111	115	101	107	176	177	194	201	191	165	162	141	348.75	331.00	5.7	5.7	7.7	2.7	2.11	0.71
7	**1**	108	118	90	100	179	170	192	208	208	226	186	195	363.00	386.00	5.6	5.4	6.6	8.1	1.76	2.00
8	**1**	115	109	111	103	166	169	198	166	171	174	121	130	326.50	312.75	5.4	5.6	4.5	6.3	1.28	1.60
11	**1**	89	84	84	81	183	189	196	171	157	174	165	134	331.50	320.75	5.6	5.4	N/A	N/A	N/A	N/A
14	**1**	107	109	96	103	187	154	156	178	166	132	122	131	311.75	290.50	5.5	5.6	10.7	6.4	2.83	1.63
16	**1**	95	96	93	92	175	165	189	200	186	188	176	161	345.25	255.25	5.7	5.6	8.8	4.9	2.06	1.11
17	**1**	93	94	94	94	187	184	178	187	162	174	145	146	323.00	332.50	5.8	5.6	11.0	8.7	2.53	2.02
18	**1**	108	98	102	112	185	168	201	189	177	185	118	134	338.00	328.75	5.4	5.4	9.1	6.5	2.43	1.80
22	**1**	108	99	110	105	208	193	235	184	167	143	89	131	354.25	319.00	5.5	5.3	16.5	17.9	4.40	4.64
23	**1**	112	110	106	113	180	189	220	212	207	187	129	119	363.75	351.00	5.7	5.7	23.1	21.9	6.39	6.11
28	**1**	100	97	99	96	171	155	180	193	179	131	156	104	329.00	308.50	5.7	5.6	6.6	12.0	1.63	2.84
29	**1**	109	101	101	102	158	155	189	211	145	150	118	109	302.75	328.25	5.6	5.6	19.3	21.3	5.19	5.36
31	**1**	116	106	118	125	176	211	226	256	232	215	197	129	395.25	404.50	5.7	5.7	3.9	2.5	1.12	0.77
33	**1**	112	99	118	94	169	131	147	141	107	115	95	59	263.30	231.75	6	5.8	12.2	18.1	3.38	4.20

Participants ID	Assigned group	FPG (mg/dL)-0	FPG (mg/dL)-4	FPG (mg/dL)-8	FPG (mg/dL)-12	PPG 30 (mg/dL)-0	PPG 30 (mg/dL)-12	PPG 60 (mg/dL)-0	PPG 60 (mg/dL)-12	PPG 90 (mg/dL)-0	PPG 90 (mg/dL)-12	PPG 120 (mg/dL)-0	PPG 120 (mg/dL)-12	AUC (mg/dL.min)-0	AUC (mg/dL.min)-12	A1C (%)-0	A1C (%)-12	FPI (μIU/mL)-0	FPI (μIU/mL)-12	HOMA-IR-0	HOMA-IR-12
34	**1**	126	123	112	116	156	196	204	248	188	188	152	134	344.75	378.50	6.7	6.6	5.2	9.9	1.62	2.84
35	**1**	125	123	113	115	187	172	234	197	252	184	220	195	422.75	362.50	6.9	6.5	16.7	16.7	5.15	4.74
37	**1**	110	103	92	102	161	174	112	188	99	173	128	110	246.50	320.50	6	5.1	13.6	15.5	3.69	3.90
39	**1**	99	96	91	99	189	182	156	168	166	128	153	94	318.50	287.25	5.9	5.6	7.6	5.6	1.86	1.37
41	**1**	105	108	114	100	189	155	156	177	138	140	71	125	389.50	292.25	5.9	5.9	36.8	19.8	9.54	4.89
42	**1**	127	113	98	115	188	179	196	193	173	224	149	191	347.50	373.00	5.7	5.7	28.8	27.6	9.03	7.84
46	**1**	106	99	100	100	158	143	160	164	133	145	133	116	285.25	277.75	6.2	5.8	13.8	10.8	3.61	2.67
47	**1**	111	99	96	114	133	142	134	153	124	125	97	114	247.50	265.50	5.7	5.7	6.4	5.0	1.75	1.41
49	**1**	102	101	107	98	186	113	219	148	160	205	151	187	345.75	304.25	6.2	5.9	17.5	16.2	4.41	3.92
53	**1**	101	98	99	100	154	164	186	161	184	151	154	134	325.75	301.50	6	5.9	17.9	18.8	4.46	4.64
56	**1**	107	96	106	108	198	186	214	202	177	168	114	133	349.75	338.25	5.5	5.8	8.0	9.2	2.11	2.45
57	**1**	108	100	111	105	163	180	173	175	188	180	148	150	327.25	335.75	5.9	5.9	12.5	10.5	3.33	2.72
2	**2**	104	106	94	97	162	122	178	123	140	102	129	99	298.25	222.50	5.5	5.4	11.9	8.8	3.06	2.11
3	**2**	78	83	82	76	153	180	174	183	187	193	149	154	313.75	335.50	5.6	5.8	11.5	9.2	2.21	1.73
5	**2**	105	107	88	102	154	184	202	214	159	201	126	177	315.25	369.25	5.6	5.6	14.9	17.9	3.86	4.51
10	**2**	96	89	93	93	168	160	179	180	165	164	155	151	318.75	313.00	5.6	5.6	8.8	11.3	2.09	2.59
13	**2**	117	109	97	109	188	174	204	199	200	187	159	153	365.00	345.50	5.9	5.8	15.8	16.2	4.56	4.36
15	**2**	105	99	101	109	136	133	176	180	165	176	166	154	306.25	310.25	5.4	5.4	16.0	15.3	4.15	4.12
19	**2**	109	116	116	112	182	157	182	198	175	171	149	184	334.00	337.00	6.2	6.2	14.2	12.3	3.82	3.40
20	**2**	110	105	103	111	204	176	242	240	282	263	264	228	457.50	424.25	6	6.3	9.4	7.3	2.55	2.00
21	**2**	114	105	98	120	202	176	229	245	224	242	169	227	398.25	418.25	6.1	6.4	14.8	14.7	4.17	4.36

Participants ID	Assigned group	FPG (mg/dL)-0	FPG (mg/dL)-4	FPG (mg/dL)-8	FPG (mg/dL)-12	PPG 30 (mg/dL)-0	PPG 30 (mg/dL)-12	PPG 60 (mg/dL)-0	PPG 60 (mg/dL)-12	PPG 90 (mg/dL)-0	PPG 90 (mg/dL)-12	PPG 120 (mg/dL)-0	PPG 120 (mg/dL)-12	AUC (mg/dL.min)-0	AUC (mg/dL.min)-12	A1C (%)-0	A1C (%)-12	FPI (µIU/mL)-0	FPI (µIU/mL)-12	HOMA-IR-0	HOMA-IR-12
24	2	101	105	102	104	176	184	207	223	207	150	183	162	366.00	345.00	5.4	5.5	11.1	13.5	2.77	3.47
25	2	113	111	107	104	187	180	171	181	178	166	159	151	336.00	327.25	5.9	5.8	16.5	17.5	4.60	4.49
26	2	114	99	109	92	158	160	186	228	198	248	190	237	347.00	400.25	5.5	6	8.8	6.7	2.48	1.52
27	2	100	100	114	104	162	162	207	180	180	185	147	141	336.25	324.75	5.5	5.7	17.3	20.1	4.27	5.16
30	2	119	107	116	111	174	131	196	200	158	131	136	79	327.75	278.50	5.5	5.1	15.0	15.6	4.41	4.28
32	2	113	116	111	113	167	175	177	168	138	125	99	122	294.00	292.75	5.6	5.6	24.4	35.7	6.81	9.96
36	2	119	114	107	101	194	183	246	182	207	167	196	159	402.25	331.00	5.8	5.7	6.3	4.4	1.85	1.10
38	2	96	100	93	106	171	155	173	190	165	170	149	128	315.75	316.00	5.4	5.5	9.5	8.0	2.25	2.09
40	2	105	106	107	102	121	143	146	122	131	111	100	78	250.25	233.00	5.5	5.5	10.3	10.3	2.67	2.59
43	2	106	106	111	120	168	146	176	178	154	142	141	120	310.75	293.00	5.5	5.5	27.3	29.4	7.15	8.71
44	2	109	106	103	104	156	173	131	130	120	107	134	101	264.25	256.25	5.4	5.3	16.8	9.0	4.52	2.31
45	2	115	115	124	120	216	199	271	242	262	250	213	222	456.50	431.00	6.5	6.6	28.5	20.2	8.09	5.99
48	2	105	117	109	119	168	197	203	238	224	232	192	201	371.75	413.50	6.2	6.3	20.7	18.7	5.37	5.49
50	2	118	114	112	109	175	167	173	189	134	142	113	110	298.75	303.75	5.8	5.8	6.2	6.7	1.81	1.80
54	2	101	104	108	106	188	176	197	199	160	195	145	157	334.00	350.75	5.8	6.1	6.7	5.6	1.67	1.47
55	2	116	109	110	115	194	187	212	195	178	167	174	149	364.50	340.50	5.8	5.8	19.4	20.1	5.56	5.71
58	2	101	106	111	109	149	161	140	150	123	138	96	116	255.25	280.75	5.4	5.7	11.9	10.8	2.97	2.91

Appendix G

Clinical outcomes (Study II)

(iii) Lipid profile and renal and hepatic function

Participants ID	Assigned group	TC (mg/dL)-0	TC (mg/dL)-12	TG (mg/dL)-0	TG (mg/dL)-12	HDL-C (mg/dL)-0	HDL-C (mg/dL)-12	LDL-C (mg/dL)-0	LDL-C (mg/dL)-12	Cr (mg/dL)-0	Cr (mg/dL)-12	AST (U/L)-0	AST (U/L)-12	ALT (U/L)-0	ALT (U/L)-12
1	**1**	125.6	150	102.4	111.2	40.5	46	74.4	110.9	0.63	0.74	29.5	26.4	32.1	30.1
4	**1**	125.4	125.3	103.7	144	47.3	40.7	72.1	72	0.69	0.71	17.3	19.3	19.1	23.3
6	**1**	187.4	168.9	173.4	128.1	53.8	51.2	126.4	113.6	0.91	0.86	24.9	22.8	26.5	23.2
7	**1**	202	182.4	147.6	150.7	44.1	53.9	145.9	124.6	0.72	0.8	26.3	17.4	15.5	15
8	**1**	270.9	246.3	156.6	126.4	59.9	58	196.2	207.4	0.88	0.84	19.5	16.4	16.1	15.7
11	**1**	165.1	161.7	114.1	89.1	80.1	77.3	84.7	99.3	0.8	0	20	21.9	19.9	22.1
14	**1**	244.8	208	52.5	52.2	86.6	78.3	162.4	132.8	0.85	0.72	22.2	20.6	14	18.1
16	**1**	192	179.7	168.5	149	50.9	46.4	143.5	129.9	0.61	0.67	23.7	14.6	27.5	15.2
17	**1**	233.4	206.6	142.4	215.3	47.2	52.9	176.3	150.4	0.67	0.54	19.8	17.5	19.8	21.6
18	**1**	166.5	166.5	74.2	74.2	65.2	65.2	108.7	108.7	0.63	0.67	21	22.4	23.5	22.5
22	**1**	178.2	174	147.8	134.6	68.4	60.2	110.5	118.3	0.7	0.73	20.6	18.1	24.8	18.8
23	**1**	226	190.4	149.7	149.8	45.7	47.8	187.5	145.6	0.88	0.89	18.4	18.8	21.7	24.9
28	**1**	170.6	175.4	86.7	100.3	71.8	55.7	135	133.8	0.74	0.75	15.7	18.3	11	12.5
29	**1**	187.3	174.2	121.4	95	55.2	47.3	123.4	118	1.03	1	19.3	20.7	24.4	26.4
31	**1**	223.9	218.8	248.5	176.9	45	42	170.2	163.9	0.96	0.93	16.5	27.6	15.5	48.5
33	**1**	156.9	150.2	61	60.8	52.4	52.8	108.8	103	0.91	0.92	30.8	22.7	36.1	22.4

Participants ID	Assigned group	TC (mg/dL)-0	TC (mg/dL)-12	TG (mg/dL)-0	TG (mg/dL)-12	HDL-C (mg/dL)-0	HDL-C (mg/dL)-12	LDL-C (mg/dL)-0	LDL-C (mg/dL)-12	Cr (mg/dL)-0	Cr (mg/dL)-12	AST (U/L)-0	AST (U/L)-12	ALT (U/L)-0	ALT (U/L)-12
34	**1**	221.4	193.1	105.3	76.1	45.9	49.4	187.1	159.8	1.12	1.06	21.1	21	16	17
35	**1**	218.5	180.2	82.8	114.6	84	65.4	143.2	113	0.53	0.53	23.4	16.2	28.4	29
37	**1**	201.5	160.4	169.7	145.6	35.6	38	155.8	108.7	0.87	0.73	23.5	21.9	30.2	24.8
39	**1**	245.2	191.8	116.3	81.7	74.1	61	180.1	132.9	0.72	0.65	20.5	24.3	13.4	11
41	**1**	165.6	169.3	95.4	126.9	54.9	44.3	127.5	107.6	0.94	0.88	23.5	26.7	39.3	32.2
42	**1**	182.2	222.2	142.2	99.1	43.3	58.4	133.8	168.8	0.89	0.92	23.8	20.6	27.1	25
46	**1**	158.3	154.5	98.6	103.4	52.1	53.9	110.2	128.7	1.24	1.22	24.5	34.5	32.4	46.8
47	**1**	218.3	188.1	154.7	113	57.1	53	160.2	171.3	1.14	1.11	18.1	16	24.3	22.1
49	**1**	187.4	161.8	116.8	113.7	54.1	46.5	131.6	114.1	0.61	0.52	12.7	13.6	13.8	12.6
53	**1**	170	168.6	172	152.1	48.4	48.9	117	108.4	0.67	0.69	15.5	18	14	17
56	**1**	206.4	186.7	162.2	147.4	45.4	46.8	147	167	0.68	0.72	28.1	40.6	33.6	73.4
57	**1**	229	229	219.2	219.2	28	28	174.1	174.1	0.75	0.71	33.8	32.5	30.1	35.6
2	**2**	146.7	178	89.1	73.9	42	48.8	102.8	122.3	0.7	0.72	13.8	12.8	15.7	14.7
3	**2**	199.8	199.2	186.5	235.8	48.6	45.5	141.5	135.8	0.75	0.75	18.4	22.2	18.7	23.3
5	**2**	140.3	139.4	113.7	112.9	50	48	88.4	91.7	0.95	0.96	23.8	24.4	22	21.1
10	**2**	221.4	222.3	151.3	109.7	68	69.8	143.6	142.2	0.65	0.64	21.3	17.3	15	14.5
13	**2**	175.3	190.8	79.6	168.5	58.6	63.5	115.1	114.1	1.42	1.2	24.3	19.4	20.5	22.9
15	**2**	244	211.7	210.5	134.6	63.5	65.2	155.6	135.9	0.71	0.81	27	24.6	27.8	27.8
19	**2**	162.1	170.5	101	134.4	49	47.3	109.5	120.7	0.6	0.62	18.1	16.1	25.9	23
20	**2**	200.4	176.5	170.4	136.9	55.8	54.4	132.2	112.5	0.74	0.66	27.5	21.7	30.2	28.1
21	**2**	260	263.9	76.8	109.4	68.2	65.4	193.1	199.4	0.75	0.73	19.1	18.9	17.9	26.9
24	**2**	225.8	225.8	190.4	190.4	46.1	46.1	161.7	161.7	0.79	0.76	14.8	16	14.5	15.9

Participants ID	Assigned group	TC (mg/dL)-0	TC (mg/dL)-12	TG (mg/dL)-0	TG (mg/dL)-12	HDL-C (mg/dL)-0	HDL-C (mg/dL)-12	LDL-C (mg/dL)-0	LDL-C (mg/dL)-12	Cr (mg/dL)-0	Cr (mg/dL)-12	AST (U/L)-0	AST (U/L)-12	ALT (U/L)-0	ALT (U/L)-12
25	**2**	175.7	181.4	114.5	149.2	65.2	66.2	113.6	108.3	0.48	0.45	33.2	33.5	28.7	30.1
26	**2**	226.9	241.3	115.7	241.8	63.7	65.1	156.4	147	0.79	0.54	16.1	14.6	18.8	11.6
27	**2**	179.5	185.8	314.5	206	49	59.3	73	104.5	0.65	0.59	15.2	16.4	14	14.1
30	**2**	199.5	174.6	125.2	116.1	45.7	42.6	153.21	123.6	0.84	0.95	19.4	16.6	25.4	13.6
32	**2**	181.5	170.7	184.2	108.8	38	50	125	119.6	0.63	0.6	18.6	18.2	16.5	18.7
36	**2**	204.8	150.8	84.7	68.4	69.1	56.6	139.9	93.5	0.74	0.7	17.9	20.1	16.6	16.4
38	**2**	224.5	216	231.5	158.5	68.4	64.5	139.4	154.8	0.74	0.65	12.7	28	7.2	10
40	**2**	232.2	198.8	124.7	80	56.1	52.5	171.2	148.1	0.62	0.62	15.4	33.2	11.5	12.6
43	**2**	147.1	146.2	114.2	153.1	36.9	36.4	104.3	102.3	0.51	0.47	19.7	15.9	15.5	10.9
44	**2**	188.4	199.5	104.7	66	45.8	53.7	139.1	149.6	0.6	0.63	13.2	11.6	8.5	9.8
45	**2**	138.3	193.3	114.5	132.6	46.5	55.7	79.9	129.8	1	1.02	20.2	42	25.6	32.7
48	**2**	268.4	260.9	245.1	167.5	45.5	47.4	193.3	195.9	0.71	0.82	29.6	35.3	26	65.4
50	**2**	239.4	227.4	87.8	121.3	61.6	61.5	188.2	171.7	0.69	0.67	15.7	16.7	9.1	11.6
54	**2**	207.8	216	114.7	107.3	52	54.3	153.6	157.8	0.66	0.68	23.2	39	34.5	87.5
55	**2**	256.9	256.9	207.1	207.1	45.8	45.8	189	189	0.54	0.46	20.5	24	18.8	34.9
58	**2**	170.5	189.1	118.4	127	53.4	54.9	111.3	136.8	0.57	0.57	19.7	20.6	15.4	14.9

Appendix G

Clinical outcomes (Study II)

(iv) Adverse events

Participants ID	Assigned group	Hypoglycemia			Abdominal pain			Bloating, frequent belching and fart			Watery stool			Constipation			Allergic reaction		
		Week 4	Week 8	Week 12	Week 4	Week 8	Week 12	Week 4	Week 8	Week 12	Week 4	Week 8	Week 12	Week 4	Week 8	Week 12	Week 4	Week 8	Week 12
1	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	**1**	0	0	0	0	0	0	3	2	1	2	2	1	3	1	1	0	0	0
6	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**1**	0	0	0	0	0	1	2	1	1	2	0	0	0	0	0	0	0	0
8	**1**	0	0	0	0	0	0	3	2	1	0	1	0	1	0	0	0	0	0
11	**1**	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0
14	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	**1**	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
17	**1**	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
18	**1**	0	0	0	0	0	0	4	2	3	1	0	0	0	1	1	0	0	0
22	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	**1**	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
28	**1**	0	0	0	0	0	0	2	2	1	0	0	0	0	0	4	0	0	0
29	**1**	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
31	**1**	0	0	0	0	0	0	3	0	0	3	0	0	1	0	0	0	0	0
33	**1**	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	**1**	0	0	0	0	0	0	2	1	0	2	1	1	2	1	0	0	0	0
35	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

37	**1**	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0
39	**1**	0	0	0	0	1	0	2	3	0	0	2	0	0	0	0	0	0	0
41	**1**	0	0	0	0	0	0	0	0	0	4	0	0	3	2	2	0	0	0
42	**1**	0	0	0	0	0	0	3	1	1	0	0	0	2	0	0	0	0	0
46	**1**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	**1**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
49	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	**1**	0	0	0	0	0	0	3	0	1	0	0	0	0	0	1	0	0	0
56	**1**	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0
57	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix H

Differentially expressed plasma proteins (Study III)

(i) Up-expressed proteins (n = 210)

	Accession number		Protein name	Encoding gene	Mass (Da)	Protein family	Protein function	Fold change	
	Swiss-Prot	Uniprot						mean	SD
1	K1C14_HUMAN	P02533	Keratin, type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4	*KRT14*	51,561	Intermediate filament family	Structural constituents (intermediate filament structure)	2.72	3.04
2	K2C5_HUMAN	P13647	Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3	*KRT5*	62,378	Intermediate filament family	Structural constituents (intermediate filament structure)	3.15	2.32
3	K2C6C_HUMAN	P48668	Keratin, type II cytoskeletal 6C OS=Homo sapiens GN=KRT6C PE=1 SV=3	*KRT6C*	60,025	Intermediate filament family	Structural constituents (intermediate filament structure)	2.12	2.46
4	HV304_HUMAN	Q7Z3Y8	Ig heavy chain V-III region TIL OS=Homo sapiens PE=1 SV=1	*IGHV3-23*	12,582	Unknown	Immune response	1.50	1.30
5	K1C27_HUMAN	P04259	Keratin, type I cytoskeletal 27 OS=Homo sapiens GN=KRT27 PE=1 SV=1	*KRT27*	49,822	Intermediate filament family	Structural constituents (intermediate filament structure)	1.67	0.58
6	K2C6B_HUMAN	P01860	Keratin, type II cytoskeletal 6B OS=Homo sapiens GN=KRT6B PE=1 SV=5	*KRT6B*	60,067	Intermediate filament family	Structural constituents (intermediate filament structure)	2.51	2.00
7	IGHG3_HUMAN	P68032	Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2	*IGHG3*	41,287	Unknown	Immune response	1.61	0.78
8	ACTC_HUMAN	P27169	Actin, alpha cardiac muscle 1 OS=Homo sapiens GN=ACTC1 PE=1 SV=1	*ACTC1*	42,019	Actin family	Structural constituents (actin cytoskeletal structure)	N/A	N/A
9	PON1_HUMAN	Q92608	Serum paraoxonase/arylesterase 1 OS=Homo sapiens GN=PON1 PE=1 SV=2	*PON1*	39,731	Paraoxonase family	Lipid metabolism	N/A	N/A
10	DOCK2_HUMAN	Q6F5E8	Dedicator of cytokinesis protein 2 OS=Homo sapiens GN=DOCK2 PE=1 SV=2	*DOCK2*	211,948	DOCK family	Immune response	N/A	N/A
11	LR16C_HUMAN	Q96HN2	Leucine-rich repeat-containing protein 16C OS=Homo sapiens GN=RLTPR PE=1 SV=2	*CARMIL2*	154,689	CARMIL family	Structural constituents (actin cytoskeletal structure)	N/A	N/A
12	SAHH3_HUMAN	Q71F56	Putative adenosylhomocysteinase 3 OS=Homo sapiens GN=AHCYL2 PE=1 SV=1	*AHCYL2*	66,721	Adenosylhomocysteinase family	Others (one-carbon metabolic process)	N/A	N/A

13	MD13L_HUMAN	Q2M385	Mediator of RNA polymerase II transcription subunit 13-like OS=Homo sapiens GN=MED13L PE=1 SV=1	*MED13L*	242,602	Mediator complex subunit 13 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
14	MPEG1_HUMAN	Q15468	Macrophage-expressed gene 1 protein OS=Homo sapiens GN=MPEG1 PE=2 SV=1	*MPEG1*	78,587	MPEG1 family	Immune response (response to bacteria)	N/A	N/A
15	STIL_HUMAN	P01594	SCL-interrupting locus protein OS=Homo sapiens GN=STIL PE=1 SV=2	*STIL*	142,955	Unknown	Cellular process (organ development)	N/A	N/A
16	KV102_HUMAN	P49790	Ig kappa chain V-I region AU OS=Homo sapiens PE=1 SV=1	*IGKV1-33*	12,848	Unknown	Immune response	N/A	N/A
17	NU153_HUMAN	Q9NQ75	Nuclear pore complex protein Nup153 OS=Homo sapiens GN=NUP153 PE=1 SV=2	*NUP153*	153,938	NUP153 family	Cell interaction and communication	N/A	N/A
18	CASS4_HUMAN	Q9H165	Cas scaffolding protein family member 4 OS=Homo sapiens GN=CASS4 PE=1 SV=2	*CASS4*	87,144	CAS family	Cell interaction and communication (cell adhesion)	N/A	N/A
19	BC11A_HUMAN	O94875	B-cell lymphoma/leukemia 11A OS=Homo sapiens GN=BCL11A PE=1 SV=2	*BCL11A*	91,197	Unknown	Cellular process (organ development)	N/A	N/A
20	SRBS2_HUMAN	Q9NT68	Sorbin and SH3 domain-containing protein 2 OS=Homo sapiens GN=SORBS2 PE=1 SV=3	*SORBS2*	124,108	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
21	TEN2_HUMAN	Q8NET4	Teneurin-2 OS=Homo sapiens GN=ODZ2 PE=1 SV=3	*TENM2*	307,787	Tenascin family, Teneurin subfamily	Cell interaction and communication (cell adhesion)	N/A	N/A
22	RGAG1_HUMAN	Q9HAU5	Retrotransposon gag domain-containing protein 1 OS=Homo sapiens GN=RGAG1 PE=1 SV=1	*RTL9*	144,280	Unknown	Unknown	N/A	N/A
23	RENT2_HUMAN	Q70CQ4	Regulator of nonsense transcripts 2 OS=Homo sapiens GN=UPF2 PE=1 SV=1	*UPF2*	147,810	Unknown	Cellular process (organ development)	N/A	N/A
24	UBP31_HUMAN	Q5HYW2	Ubiquitin carboxyl-terminal hydrolase 31 OS=Homo sapiens GN=USP31 PE=1 SV=2	*USP31*	146,651	Peptidase C19 family	Protein metabolism (ubiquitin-dependent protein catabolic process)	N/A	N/A
25	NHSL2_HUMAN	Q9BX84	NHS-like protein 2 OS=Homo sapiens GN=NHSL2 PE=2 SV=1	*NHSL2*	133,286	NHS family	Cellular process (cell proliferation and differentiation)	N/A	N/A
26	TRPM6_HUMAN	P29597	Transient receptor potential cation channel subfamily M member 6 OS=Homo sapiens GN=TRPM6 PE=1 SV=2	*TRPM6*	231,708	Protein kinase superfamily, TRPM6 sub-subfamily	Cell interaction and communication	N/A	N/A
27	TYK2_HUMAN	Q96NH3	Non-receptor tyrosine-protein kinase TYK2 OS=Homo sapiens GN=TYK2 PE=1 SV=3	*TYK2*	133,650	Protein kinase superfamily, JAK subfamily	Signaling pathway (cytokines)	N/A	N/A

28	CF170_HUMAN	Q149M9	Uncharacterized protein C6orf170 OS=Homo sapiens GN=C6orf170 PE=2 SV=3	*TBC1D32*	144,756	Unknown	Cellular process (organ development)	N/A	N/A
29	NWD1_HUMAN	Q99707	NACHT and WD repeat domain-containing protein 1 OS=Homo sapiens GN=NWD1 PE=2 SV=2	*NWD1*	174,552	Unknown	Inflammatory response	N/A	N/A
30	METH_HUMAN	Q6YHU6	Methionine synthase OS=Homo sapiens GN=MTR PE=1 SV=2	*MTR*	140,527	Vitamin-B12 dependent methionine synthase family	Cellular process (organ development)	N/A	N/A
31	THADA_HUMAN	O15553	Thyroid adenoma-associated protein OS=Homo sapiens GN=THADA PE=1 SV=1	*THADA*	219,607	THADA family	Lipid metabolism	N/A	N/A
32	MEFV_HUMAN	Q13489	Pyrin OS=Homo sapiens GN=MEFV PE=1 SV=1	*MEFV*	86,444	Unknown	Inflammatory response	N/A	N/A
33	BIRC3_HUMAN	Q9P2E2	Baculoviral IAP repeat-containing protein 3 OS=Homo sapiens GN=BIRC3 PE=1 SV=2	*BIRC3*	68,372	IAP family	Signaling pathway (NF-kB)	N/A	N/A
34	KIF17_HUMAN	Q9P2N2	Kinesin-like protein KIF17 OS=Homo sapiens GN=KIF17 PE=1 SV=2	*KIF17*	115,068	TRAFAC class myosin-kinesin ATPase superfamily, Kinesin family	Cell interaction and communication	N/A	N/A
35	RHG28_HUMAN	Q5JPB2	Rho GTPase-activating protein 28 OS=Homo sapiens GN=ARHGAP28 PE=2 SV=2	*ARHGAP28*	82,060	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
36	ZN831_HUMAN	A0A0C4DH32	Zinc finger protein 831 OS=Homo sapiens GN=ZNF831 PE=2 SV=4	*ZNF831*	177,949	Unknown	Ion binding and transport (metal)	N/A	N/A
37	HV320_HUMAN	O15439	Ig heavy chain V-III region GAL OS=Homo sapiens PE=1 SV=1	*IGHV3-20*	12,673	Unknown	Immune response	N/A	N/A
38	MRP4_HUMAN	Q5T4T6	Multidrug resistance-associated protein 4 OS=Homo sapiens GN=ABCC4 PE=1 SV=3	*ABCC4*	149,527	ABC transporter superfamily, Conjugate transporter subfamily	Cell interaction and communication	N/A	N/A
39	SYC2L_HUMAN	Q96RY7	Synaptonemal complex protein 2-like OS=Homo sapiens GN=SYCP2L PE=1 SV=2	*SYCP2L*	93,599	SYCP2 family	Cellular process (cell division)	N/A	N/A
40	IF140_HUMAN	Q5VYP0	Intraflagellar transport protein 140 homolog OS=Homo sapiens GN=IFT140 PE=1 SV=1	*IFT140*	165,193	Unknown	Cellular process (organ development)	N/A	N/A
41	F75A3_HUMAN	Q2TBE0	Protein FAM75A3 OS=Homo sapiens GN=FAM75A3 PE=2 SV=1	*SPATA31A3*	148,706	SPATA31 family	Cellular process (cell proliferation and differentiation)	N/A	N/A
42	C19L2_HUMAN	P01042	CWF19-like protein 2 OS=Homo sapiens GN=CWF19L2 PE=1 SV=3	*CWF19L2*	103,787	CWF19 family	Cellular process (mRNA splicing)	N/A	N/A

43	KNG1_HUMAN	Q9NZB2	Kininogen-1 OS=Homo sapiens GN=KNG1 PE=1 SV=2	*KNG1*	71,957	Unknown	Immune response	N/A	N/A
44	F120A_HUMAN	P52179	Constitutive coactivator of PPAR-gamma-like protein 1 OS=Homo sapiens GN=FAM120A PE=1 SV=2	*FAM120A*	121,888	Constitutive coactivator of PPAR-gamma family	Unknown	N/A	N/A
45	MYOM1_HUMAN	Q8IW75	Myomesin-1 OS=Homo sapiens GN=MYOM1 PE=1 SV=2	*MYOM1*	187,627	Unknown	Cellular process (organ development)	N/A	N/A
46	SPA12_HUMAN	Q502W6	Serpin A12 OS=Homo sapiens GN=SERPINA12 PE=2 SV=1	*SERPINA12*	47,175	Serpin family	Lipid metabolism	N/A	N/A
47	VWA3B_HUMAN	Q9UBW7	von Willebrand factor A domain-containing protein 3B OS=Homo sapiens GN=VWA3B PE=2 SV=2	*VWA3B*	145,748	Unknown	Unknown	N/A	N/A
48	ZMYM2_HUMAN	Q9UMD9	Zinc finger MYM-type protein 2 OS=Homo sapiens GN=ZMYM2 PE=1 SV=1	*ZMYM2*	154,911	Unknown	Ion binding and transport (zinc)	N/A	N/A
49	COHA1_HUMAN	Q8TE56	Collagen alpha-1(XVII) chain OS=Homo sapiens GN=COL17A1 PE=1 SV=3	*COL17A1*	150,419	Unknown	Structural constituents (extracellular matrix)	N/A	N/A
50	ATS17_HUMAN	Q8IWE5	A disintegrin and metalloproteinase with thrombospondin motifs 17 OS=Homo sapiens GN=ADAMTS17 PE=2 SV=1	*ADAMTS17*	121,127	Unknown	Structural constituents (extracellular matrix)	N/A	N/A
51	PKHM2_HUMAN	P32314	Pleckstrin homology domain-containing family M member 2 OS=Homo sapiens GN=PLEKHM2 PE=1 SV=2	*PLEKHM2*	112,780	Unknown	Protein metabolism (protein localization)	N/A	N/A
52	FOXN2_HUMAN	Q9P243	Forkhead box protein N2 OS=Homo sapiens GN=FOXN2 PE=2 SV=3	*FOXN2*	47,161	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
53	ZFAT_HUMAN	P29279	Zinc finger protein ZFAT OS=Homo sapiens GN=ZFAT PE=1 SV=2	*ZFAT*	139,034	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
54	CTGF_HUMAN	Q8IYE0	Connective tissue growth factor OS=Homo sapiens GN=CTGF PE=1 SV=2	*CCN2*	38,091	CCN family	Cellular process (organ development)	N/A	N/A
55	CC146_HUMAN	Q5T7N2	Coiled-coil domain-containing protein 146 OS=Homo sapiens GN=CCDC146 PE=2 SV=2	*CCDC146*	112,806	Unknown	Unknown	N/A	N/A
56	LITD1_HUMAN	Q8NI27	LINE-1 type transposase domain-containing protein 1 OS=Homo sapiens GN=L1TD1 PE=2 SV=1	*L1TD1*	98,850	Transposase 22 family	Unknown	N/A	N/A
57	THOC2_HUMAN	Q9H6A9	THO complex subunit 2 OS=Homo sapiens GN=THOC2 PE=1 SV=2	*THOC2*	182,775	THOC2 family	Cellular process (organ development)	N/A	N/A

58	PCX3_HUMAN	Q86VY9	Pecanex-like protein 3 OS=Homo sapiens GN=PCNXL3 PE=1 SV=2	*PCNX3*	222,039	Pecanex family	Structural constituents (integral component of membrane)	N/A	N/A
59	T200A_HUMAN	P17844	Transmembrane protein 200A OS=Homo sapiens GN=TMEM200A PE=2 SV=1	*TMEM200A*	54,356	TMEM200 family	Structural constituents (integral component of membrane)	N/A	N/A
60	DDX5_HUMAN	Q86YC3	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1	*DDX5*	69,148	DEAD box helicase family, DDX5/DBP2 subfamily	Signaling pathway	N/A	N/A
61	LRC33_HUMAN	O43432	Leucine-rich repeat-containing protein 33 OS=Homo sapiens GN=LRRC33 PE=2 SV=1	*NRROS*	76,366	LRRC32/LRRC33 family	Immune response	N/A	N/A
62	IF4G3_HUMAN	Q7Z7F7	Eukaryotic translation initiation factor 4 gamma 3 OS=Homo sapiens GN=EIF4G3 PE=1 SV=2	*EIF4G3*	176,652	Eukaryotic initiation factor 4G family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
63	RM55_HUMAN	Q9NSJ1	39S ribosomal protein L55, mitochondrial OS=Homo sapiens GN=MRPL55 PE=1 SV=1	*MRPL55*	15,128	Mitochondrion-specific ribosomal protein mL55 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
64	ZN834_HUMAN	Q9H694	Putative zinc finger protein 834 OS=Homo sapiens GN=ZNF834 PE=5 SV=2	*ZNF355P*	49,689	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
65	BICC1_HUMAN	Q494R0	Protein bicaudal C homolog 1 OS=Homo sapiens GN=BICC1 PE=1 SV=2	*BICC1*	104,844	BicC family	Cellular process (organ development)	N/A	N/A
66	YP028_HUMAN	Q9BVV6	Putative uncharacterized protein encoded by NCRNA00095 OS=Homo sapiens GN=NCRNA00095 PE=5 SV=1	*FBXL19-AS1*	13,218	Unknown	Unknown	N/A	N/A
67	K0586_HUMAN	Q7Z4L5	Uncharacterized protein KIAA0586 OS=Homo sapiens GN=KIAA0586 PE=1 SV=2	*KIAA0586*	169,307	TALPID3 family	Structural constituents (cilia structure)	N/A	N/A
68	TT21B_HUMAN	Q9UIF8	Tetratricopeptide repeat protein 21B OS=Homo sapiens GN=TTC21B PE=2 SV=1	*TTC21B*	150,937	TTC21 family	Structural constituents (cilia structure)	N/A	N/A
69	BAZ2B_HUMAN	Q9HBT7	Bromodomain adjacent to zinc finger domain protein 2B OS=Homo sapiens GN=BAZ2B PE=1 SV=3	*BAZ2B*	240,459	WAL family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
70	ZN287_HUMAN	Q9Y4H2	Zinc finger protein 287 OS=Homo sapiens GN=ZNF287 PE=2 SV=1	*ZNF287*	88,339	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
71	IRS2_HUMAN	P78316	Insulin receptor substrate 2 OS=Homo sapiens GN=IRS2 PE=1 SV=2	*IRS2*	137,334	Unknown	Glucose metabolism	N/A	N/A
72	NOP14_HUMAN	O00192	Nucleolar protein 14 OS=Homo sapiens GN=NOP14 PE=1 SV=3	*NOP14*	97,668	NOP14 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A

73	ARVC_HUMAN	O15085	Armadillo repeat protein deleted in velo-cardio-facial syndrome OS=Homo sapiens GN=ARVCF PE=1 SV=1	*ARVCF*	104,642	Beta-catenin family	Cell interaction and communication	N/A	N/A
74	ARHGB_HUMAN	O94832	Rho guanine nucleotide exchange factor 11 OS=Homo sapiens GN=ARHGEF11 PE=1 SV=1	*ARHGEF11*	167,704	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
75	MYO1D_HUMAN	Q9UIC8	Myosin-Id OS=Homo sapiens GN=MYO1D PE=1 SV=2	*MYO1D*	116,202	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family	Structural constituents (actin cytoskeletal structure)	N/A	N/A
76	LCMT1_HUMAN	Q6P4R8	Leucine carboxyl methyltransferase 1 OS=Homo sapiens GN=LCMT1 PE=1 SV=2	*LCMT1*	38,379	Methyltransferase superfamily, LCMT family	Cellular process (cell apoptosis)	N/A	N/A
77	NFRKB_HUMAN	Q9UMN6	Nuclear factor related to kappa-B-binding protein OS=Homo sapiens GN=NFRKB PE=1 SV=2	*NFRKB*	139,001	NFRKB family	Inflammatory response	N/A	N/A
78	MLL4_HUMAN	A6NMS7	Histone-lysine N-methyltransferase MLL4 OS=Homo sapiens GN=WBP7 PE=1 SV=1	*KMT2B*	293,515	Class V-like SAM-binding methyltransferase superfamily	Cellular process (cell proliferation and differentiation)	N/A	N/A
79	L37A1_HUMAN	Q14004	Leucine-rich repeat-containing protein 37A OS=Homo sapiens GN=LRRC37A PE=2 SV=2	*LRRC37A*	188,258	LRRC37A family	Structural constituents (integral component of membrane)	N/A	N/A
80	CDK13_HUMAN	Q9Y644	Cell division protein kinase 13 OS=Homo sapiens GN=CDK13 PE=1 SV=2	*CDK13*	164,923	Protein kinase superfamily, CMGC Ser/Thr protein kinase family	Cellular process (cell proliferation and differentiation)	N/A	N/A
81	RFNG_HUMAN	Q7Z5M5	Beta-1,3-N-acetylglucosaminyltransferase radical fringe OS=Homo sapiens GN=RFNG PE=2 SV=2	*RFNG*	36,424	Glycosyltransferase 31 family	Cellular process (cell proliferation and differentiation)	N/A	N/A
82	TMC3_HUMAN	Q9Y2G9	Transmembrane channel-like protein 3 OS=Homo sapiens GN=TMC3 PE=1 SV=2	*TMC3*	125,685	TMC family	Structural constituents (integral component of membrane)	N/A	N/A
83	SBNO2_HUMAN	Q9HBR0	Protein strawberry notch homolog 2 OS=Homo sapiens GN=SBNO2 PE=2 SV=3	*SBNO2*	150,275	SBNO family	Cellular process (organ development)	N/A	N/A

84	S38AA_HUMAN	Q58F21	Putative sodium-coupled neutral amino acid transporter 10 OS=Homo sapiens GN=SLC38A10 PE=1 SV=2	*SLC38A10*	119,762	Amino acid/polyamine transporter 2 family	Cell interaction and communication	N/A	N/A
85	BRDT_HUMAN	Q8IYI8	Bromodomain testis-specific protein OS=Homo sapiens GN=BRDT PE=1 SV=4	*BRDT*	107,954	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
86	ZN440_HUMAN	Q5TYW2	Zinc finger protein 440 OS=Homo sapiens GN=ZNF440 PE=2 SV=1	*ZNF440*	69,106	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
87	A20A1_HUMAN	O75110	Ankyrin repeat domain-containing protein 20A1 OS=Homo sapiens GN=ANKRD20A1 PE=1 SV=1	*ANKRD20A1*	94,048	Unknown	Unknown	N/A	N/A
88	ATP9A_HUMAN	Q07890	Probable phospholipid-transporting ATPase IIA OS=Homo sapiens GN=ATP9A PE=1 SV=3	*ATP9A*	118,583	Cation transport ATPase (P-type) (TC 3.A.3) family, Type IV subfamily	Cell interaction and communication (endocytosis)	N/A	N/A
89	SOS2_HUMAN	Q9Y2G4	Son of sevenless homolog 2 OS=Homo sapiens GN=SOS2 PE=1 SV=2	*SOS2*	152,979	Unknown	Signaling pathway (G protein-coupled receptor)	N/A	N/A
90	ANKR6_HUMAN	Q5CZ79	Ankyrin repeat domain-containing protein 6 OS=Homo sapiens GN=ANKRD6 PE=1 SV=2	*ANKRD6*	79,971	Unknown	Signaling pathway	N/A	N/A
91	AN20B_HUMAN	Q6IQ55	Ankyrin repeat domain-containing protein 20B OS=Homo sapiens GN=ANKRD20B PE=2 SV=2	*ANKRD20A8P*	93,909	Unknown	Unknown	N/A	N/A
92	TTBK2_HUMAN	Q4AC94	Tau-tubulin kinase 2 OS=Homo sapiens GN=TTBK2 PE=1 SV=2	*TTBK2*	137,412	Protein kinase superfamily, CK1 Ser/Thr protein kinase family	Cellular process (organ development)	N/A	N/A
93	C2CD3_HUMAN	Q5XXA6	C2 domain-containing protein 3 OS=Homo sapiens GN=C2CD3 PE=1 SV=3	*C2CD3*	260,389	Unknown	Cellular process (organ development)	N/A	N/A
94	ANO1_HUMAN	Q9H4L5	Anoctamin-1 OS=Homo sapiens GN=ANO1 PE=1 SV=1	*ANO1*	114,078	Anoctamin family	Cell interaction and communication	N/A	N/A
95	OSBL3_HUMAN	Q08AN1	Oxysterol-binding protein-related protein 3 OS=Homo sapiens GN=OSBPL3 PE=1 SV=1	*OSBPL3*	101,224	OSBP family	Lipid metabolism	N/A	N/A
96	ZN616_HUMAN	Q9C075	Zinc finger protein 616 OS=Homo sapiens GN=ZNF616 PE=2 SV=2	*ZNF616*	90,273	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
97	K1C23_HUMAN	Q9H6R7	Keratin, type I cytoskeletal 23 OS=Homo sapiens GN=KRT23 PE=1 SV=2	*KRT23*	48,131	Intermediate filament family	Structural constituents (intermediate filament structure)	N/A	N/A

98	CB044_HUMAN	Q13873	WD repeat-containing protein C2orf44 OS=Homo sapiens GN=C2orf44 PE=1 SV=1	*WDCP*	79,136	Unknown	Protein metabolism (protein complex oligomerization)	N/A	N/A
99	BMPR2_HUMAN	Q6ZUX3	Bone morphogenetic protein receptor type-2 OS=Homo sapiens GN=BMPR2 PE=1 SV=2	*BMPR2*	115,201	Protein kinase superfamily, TKL Ser/Thr protein kinase family, TGFB receptor subfamily	Cellular process (organ development)	N/A	N/A
100	F179A_HUMAN	Q13459	Protein FAM179A OS=Homo sapiens GN=FAM179A PE=2 SV=2	*TOGARAM2*	111,153	Crescerin family	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
101	MYO9B_HUMAN	P55157	Myosin-IXb OS=Homo sapiens GN=MYO9B PE=1 SV=2	*MYO9B*	243,401	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family	Structural constituents (actin cytoskeletal structure)	N/A	N/A
102	MTP_HUMAN	Q9C026	Microsomal triglyceride transfer protein large subunit OS=Homo sapiens GN=MTTP PE=1 SV=1	*MTTP*	99,351	Unknown	Lipid metabolism	N/A	N/A
103	TRIM9_HUMAN	Q16222	Tripartite motif-containing protein 9 OS=Homo sapiens GN=TRIM9 PE=1 SV=1	*TRIM9*	79,177	TRIM/RBCC family	Cell interaction and communication	N/A	N/A
104	UAP1_HUMAN	Q9UK32	UDP-N-acetylhexosamine pyrophosphorylase OS=Homo sapiens GN=UAP1 PE=1 SV=3	*UAP1*	58,769	UDPGP type 1 family	Others (UDP-N-acetylglucosamine biosynthetic process)	N/A	N/A
105	KS6A6_HUMAN	Q4V348	Ribosomal protein S6 kinase alpha-6 OS=Homo sapiens GN=RPS6KA6 PE=1 SV=1	*RPS6KA6*	83,872	Protein kinase superfamily, AGC Ser/Thr protein kinase family, S6 kinase subfamily	Cellular process (organ development)	N/A	N/A
106	Z658B_HUMAN	Q92793	Zinc finger protein 658B OS=Homo sapiens GN=ZNF658B PE=2 SV=1	*ZNF658B*	94,331	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
107	CBP_HUMAN	Q8WX94	CREB-binding protein OS=Homo sapiens GN=CREBBP PE=1 SV=3	*CREBBP*	265,351	Unknown	Signaling pathway (Notch)	N/A	N/A
108	NALP7_HUMAN	Q3SY89	NACHT, LRR and PYD domains-containing protein 7 OS=Homo sapiens GN=NLRP7 PE=1 SV=1	*NLRP7*	111,807	NLRP family	Inflammatory response	N/A	N/A
109	EA3L1_HUMAN	Q07889	RNA polymerase II transcription factor SIII subunit A3-like-1 OS=Homo sapiens GN=TCEB3CL PE=2 SV=1	*ELOA3BP*	59,760	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
110	SOS1_HUMAN	O43795	Son of sevenless homolog 1 OS=Homo sapiens GN=SOS1 PE=1 SV=1	*SOS1*	152,464	Unknown	Signaling pathway (G protein-coupled receptor)	N/A	N/A
111	MYO1B_HUMAN	Q9H8G1	Myosin-Ib OS=Homo sapiens GN=MYO1B PE=1 SV=3	*MYO1B*	131,985	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family	Structural constituents (actin cytoskeletal structure)	N/A	N/A

112	ZN430_HUMAN	Q9BRS2	Zinc finger protein 430 OS=Homo sapiens GN=ZNF430 PE=2 SV=2	*ZNF430*	66,319	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
113	RIOK1_HUMAN	Q08999	Serine/threonine-protein kinase RIO1 OS=Homo sapiens GN=RIOK1 PE=1 SV=2	*RIOK1*	65,583	Protein kinase superfamily, RIO-type Ser/Thr kinase family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
114	RBL2_HUMAN	P10242	Retinoblastoma-like protein 2 OS=Homo sapiens GN=RBL2 PE=1 SV=3	*RBL2*	128,367	Retinoblastoma protein (RB) family	Cellular process (cell division)	N/A	N/A
115	MYB_HUMAN	Q6UXX5	Transcriptional activator Myb OS=Homo sapiens GN=MYB PE=1 SV=2	*MYB*	72,341	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
116	ITH5L_HUMAN	P49747	Inter-alpha-trypsin inhibitor heavy chain H5-like protein OS=Homo sapiens GN=ITIH5L PE=2 SV=1	*ITIH6*	143,187	ITIH family	Structural constituents (extracellular matrix)	N/A	N/A
117	DY12L_HUMAN	Q6ZR08	Axonemal dynein heavy chain 12-like protein OS=Homo sapiens GN=DNAH12L PE=1 SV=2	*DNAH12*	356,942	Dynein heavy chain family	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
118	ERR1_HUMAN	Q9HCE3	Steroid hormone receptor ERR1 OS=Homo sapiens GN=ESRRA PE=1 SV=3	*ESRRA*	45,510	Nuclear hormone receptor family, NR3 subfamily	Cellular process (regulation of gene transcription and translation)	N/A	N/A
119	ZN532_HUMAN	Q9BZ68	Zinc finger protein 532 OS=Homo sapiens GN=ZNF532 PE=1 SV=2	*ZNF532*	141,696	Krueppel C2H2-type zinc-finger protein family	Ion binding and transport (metal)	N/A	N/A
120	FKS43_HUMAN	Q9UQB8	Putative FERM domain-containing protein FKSG43 OS=Homo sapiens GN=FKSG43 PE=5 SV=2	*FRMD8P1*	41,136	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
121	BAIP2_HUMAN	Q9P267	Brain-specific angiogenesis inhibitor 1-associated protein 2 OS=Homo sapiens GN=BAIAP2 PE=1 SV=1	*BAIAP2*	60,868	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
122	MBD5_HUMAN	Q8NDV3	Methyl-CpG-binding domain protein 5 OS=Homo sapiens GN=MBD5 PE=2 SV=2	*MBD5*	159,895	Unknown	Glucose metabolism	N/A	N/A
123	SMC1B_HUMAN	Q99460	Structural maintenance of chromosomes protein 1B OS=Homo sapiens GN=SMC1B PE=1 SV=2	*SMC1B*	143,908	SMC family, SMC1 subfamily	Cellular process (cell division)	N/A	N/A
124	PSMD1_HUMAN	Q13133	26S proteasome non-ATPase regulatory subunit 1 OS=Homo sapiens GN=PSMD1 PE=1 SV=2	*PSMD1*	105,836	Proteasome subunit S1 family	Signaling pathway	N/A	N/A
125	NR1H3_HUMAN	Q6WKZ4	Oxysterols receptor LXR-alpha OS=Homo sapiens GN=NR1H3 PE=1 SV=2	*NR1H3*	50,396	Nuclear hormone receptor family, NR1 subfamily	Lipid metabolism	N/A	N/A

126	RFIP1_HUMAN	Q9UPT9	Rab11 family-interacting protein 1 OS=Homo sapiens GN=RAB11FIP1 PE=1 SV=2	*RAB11FIP1*	137,167	Unknown	Lipid metabolism	N/A	N/A
127	UBP22_HUMAN	P16615	Ubiquitin carboxyl-terminal hydrolase 22 OS=Homo sapiens GN=USP22 PE=1 SV=2	*USP22*	59,961	Peptidase C19 family, UBP8 subfamily	Cellular process (cell division)	N/A	N/A
128	AT2A2_HUMAN	Q86TI0	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Homo sapiens GN=ATP2A2 PE=1 SV=1	*ATP2A2*	114,757	Cation transport ATPase (P-type) (TC 3.A.3) family, Type IIA subfamily	Cell interaction and communication	N/A	N/A
129	TBCD1_HUMAN	Q15424	TBC1 domain family member 1 OS=Homo sapiens GN=TBC1D1 PE=1 SV=2	*TBC1D1*	133,084	Unknown	Cell interaction and communication	N/A	N/A
130	SAFB1_HUMAN	P25098	Scaffold attachment factor B1 OS=Homo sapiens GN=SAFB PE=1 SV=4	*SAFB*	102,642	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
131	ARBK1_HUMAN	Q9UKZ1	Beta-adrenergic receptor kinase 1 OS=Homo sapiens GN=ADRBK1 PE=1 SV=2	*GRK2*	79,574	Protein kinase superfamily, AGC Ser/Thr protein kinase family, GPRK subfamily	Signaling pathway (G protein-coupled receptor)	N/A	N/A
132	CB029_HUMAN	O15457	UPF0760 protein C2orf29 OS=Homo sapiens GN=C2orf29 PE=1 SV=1	*CNOT11*	55,215	CNOT11 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
133	MSH4_HUMAN	Q4KWH8	MutS protein homolog 4 OS=Homo sapiens GN=MSH4 PE=2 SV=2	*MSH4*	104,756	DNA mismatch repair MutS family	Cellular process (cell division)	N/A	N/A
134	PLCH1_HUMAN	Q8TD19	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase eta-1 OS=Homo sapiens GN=PLCH1 PE=1 SV=1	*PLCH1*	189,223	Unknown	Signaling pathway (phosphatidylinositol)	N/A	N/A
135	NEK9_HUMAN	P54764	Serine/threonine-protein kinase Nek9 OS=Homo sapiens GN=NEK9 PE=1 SV=2	*NEK9*	107,168	Protein kinase superfamily, NEK Ser/Thr protein kinase family, NIMA subfamily	Cellular process (cell division)	N/A	N/A
136	EPHA4_HUMAN	P12035	Ephrin type-A receptor 4 OS=Homo sapiens GN=EPHA4 PE=1 SV=1	*EPHA4*	109,860	Protein kinase superfamily, Tyr protein kinase family, Ephrin receptor subfamily	Cellular process (organ development)	N/A	N/A
137	K2C3_HUMAN	Q6P2S7	Keratin, type II cytoskeletal 3 OS=Homo sapiens GN=KRT3 PE=1 SV=2	*KRT3*	64,417	Intermediate filament family	Structural constituents (intermediate filament structure)	N/A	N/A
138	GNN_HUMAN	Q7Z7J5	Tetratricopeptide repeat protein GNN OS=Homo sapiens GN=GNN PE=2 SV=3	*TTC41P*	151,684	Unknown	Unknown	N/A	N/A
139	DPPA2_HUMAN	Q6PJP8	Developmental pluripotency-associated protein 2 OS=Homo sapiens GN=DPPA2 PE=2 SV=2	*DPPA2*	33,784	Unknown	Unknown	N/A	N/A
140	DCR1A_HUMAN	Q70EK8	DNA cross-link repair 1A protein OS=Homo sapiens GN=DCLRE1A PE=1 SV=2	*DCLRE1A*	116,400	DNA repair metallo-beta-lactamase (DRMBL) family	Cellular process (cell division)	N/A	N/A

141	UBP53_HUMAN	Q8WVV4	Inactive ubiquitin carboxyl-terminal hydrolase 53 OS=Homo sapiens GN=USP53 PE=2 SV=2	*USP53*	120,806	Peptidase C19 family	Cellular process (cell apoptosis)	N/A	N/A
142	POF1B_HUMAN	O43439	Protein POF1B OS=Homo sapiens GN=POF1B PE=1 SV=2	*POF1B*	68,065	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
143	MTG8R_HUMAN	Q96TA2	Protein CBFA2T2 OS=Homo sapiens GN=CBFA2T2 PE=1 SV=1	*CBFA2T2*	67,133	CBFA2T family	Cellular process (cell proliferation and differentiation)	N/A	N/A
144	YMEL1_HUMAN	P29122	ATP-dependent metalloprotease YME1L1 OS=Homo sapiens GN=YME1L1 PE=1 SV=2	*YME1L1*	86,455	AAA ATPase family; Peptidase M41 family	Cellular process (cell apoptosis)	N/A	N/A
145	PCSK6_HUMAN	Q6DT37	Proprotein convertase subtilisin/kexin type 6 OS=Homo sapiens GN=PCSK6 PE=1 SV=1	*PCSK6*	106,420	Peptidase S8 family	Lipid metabolism	N/A	N/A
146	MRCKG_HUMAN	Q02779	Serine/threonine-protein kinase MRCK gamma OS=Homo sapiens GN=CDC42BPG PE=1 SV=2	*CDC42BPG*	172,459	Protein kinase superfamily, AGC Ser/Thr protein kinase family, DMPK subfamily	Structural constituents (actin cytoskeletal structure)	N/A	N/A
147	M3K10_HUMAN	Q8WYR1	Mitogen-activated protein kinase kinase kinase 10 OS=Homo sapiens GN=MAP3K10 PE=1 SV=3	*MAP3K10*	103,694	Protein kinase superfamily, STE Ser/Thr protein kinase family, MAP kinase kinase kinase subfamily	Cellular process (cell apoptosis)	N/A	N/A
148	PI3R5_HUMAN	Q00610	Phosphoinositide 3-kinase regulatory subunit 5 OS=Homo sapiens GN=PIK3R5 PE=1 SV=1	*PIK3R5*	97,348	Unknown	Signaling pathway (G protein-coupled receptor)	N/A	N/A
149	CLH1_HUMAN	Q8WXS8	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5	*CLTC*	191,615	Clathrin heavy chain family	Cellular process (cell division)	N/A	N/A
150	ATS14_HUMAN	O75581	A disintegrin and metalloproteinase with thrombospondin motifs 14 OS=Homo sapiens GN=ADAMTS14 PE=2 SV=1	*ADAMTS14*	133,888	Unknown	Structural constituents (extracellular matrix)	N/A	N/A
151	LRP6_HUMAN	Q8NDF8	Low-density lipoprotein receptor-related protein 6 OS=Homo sapiens GN=LRP6 PE=1 SV=1	*LRP6*	180,429	LDLR family	Lipid metabolism	N/A	N/A
152	PAPD5_HUMAN	Q8TEM1	PAP-associated domain-containing protein 5 OS=Homo sapiens GN=PAPD5 PE=1 SV=2	*TENT4B*	63,267	DNA polymerase type-B-like family	Glucose metabolism	N/A	N/A
153	PO210_HUMAN	Q8TCJ2	Nuclear pore membrane glycoprotein 210 OS=Homo sapiens GN=NUP210 PE=1 SV=3	*NUP210*	205,111	NUP210 family	Cell interaction and communication	N/A	N/A
154	STT3B_HUMAN	Q9UPZ3	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B OS=Homo sapiens GN=STT3B PE=1 SV=1	*STT3B*	93,674	STT3 family	Inflammatory response (unfolded protein response)	N/A	N/A

155	HPS5_HUMAN	Q8WXF1	Hermansky-Pudlak syndrome 5 protein OS=Homo sapiens GN=HPS5 PE=1 SV=2	*HPS5*	127,449	HPS5 family	Blood coagulation	N/A	N/A
156	PSPC1_HUMAN	P52732	Paraspeckle component 1 OS=Homo sapiens GN=PSPC1 PE=1 SV=1	*PSPC1*	58,744	PSPC family	Immune response	N/A	N/A
157	KIF11_HUMAN	P22413	Kinesin-like protein KIF11 OS=Homo sapiens GN=KIF11 PE=1 SV=2	*KIF11*	119,159	TRAFAC class myosin-kinesin ATPase superfamily, Kinesin family, BimC subfamily	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
158	ENPP1_HUMAN	Q32MH5	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	*ENPP1*	104,924	Nucleotide pyrophosphatase/phosphodiesterase family	Structural constituents (integral component of membrane)	N/A	N/A
159	K1370_HUMAN	P69891	Uncharacterized protein KIAA1370 OS=Homo sapiens GN=KIAA1370 PE=2 SV=2	*FAM214A*	121,670	FAM214 family	Unknown	N/A	N/A
160	HBG1_HUMAN	Q9NXG0	Hemoglobin subunit gamma-1 OS=Homo sapiens GN=HBG1 PE=1 SV=2	*HBG1*	16,140	Globin family	Blood coagulation	N/A	N/A
161	CNTLN_HUMAN	O95785	Centlein OS=Homo sapiens GN=CNTLN PE=2 SV=4	*CNTLN*	161,571	Unknown	Protein metabolism (protein localization)	N/A	N/A
162	WIZ_HUMAN	Q14012	Protein Wiz OS=Homo sapiens GN=WIZ PE=1 SV=2	*WIZ*	178,674	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
163	KCC1A_HUMAN	P48634	Calcium/calmodulin-dependent protein kinase type 1 OS=Homo sapiens GN=CAMK1 PE=1 SV=1	*CAMK1*	41,337	Protein kinase superfamily, CAMK Ser/Thr protein kinase family, CaMK subfamily	Cellular process (cell proliferation and differentiation)	N/A	N/A
164	BAT2_HUMAN	O00429	Large proline-rich protein BAT2 OS=Homo sapiens GN=BAT2 PE=1 SV=2	*PRRC2A*	228,863	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
165	DNM1L_HUMAN	Q9Y2K3	Dynamin-1-like protein OS=Homo sapiens GN=DNM1L PE=1 SV=2	*DNM1L*	81,877	TRAFAC class dynamin-like GTPase superfamily, Dynamin/Fzo/YdjA family	Cellular process (cell apoptosis)	N/A	N/A
166	MYH15_HUMAN	Q9BPX6	Myosin-15 OS=Homo sapiens GN=MYH15 PE=1 SV=4	*MYH15*	224,619	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family	Cellular process (organ development)	N/A	N/A
167	CBAA1_HUMAN	O75808	Calcium-binding atopy-related autoantigen 1 OS=Homo sapiens GN=CBARA1 PE=1 SV=1	*MICU1*	54,351	MICU1 family, MICU1 subfamily	Cell interaction and communication	N/A	N/A
168	CAN15_HUMAN	Q6UW02	Calpain-15 OS=Homo sapiens GN=SOLH PE=1 SV=1	*CAPN15*	117,314	Peptidase C2 family	Protein metabolism (proteolysis)	N/A	N/A

169	CP20A_HUMAN	A6NMK8	Cytochrome P450 20A1 OS=Homo sapiens GN=CYP20A1 PE=1 SV=1	*CYP20A1*	52,432	Cytochrome P450 family	Blood coagulation	N/A	N/A
170	YE035_HUMAN	P02458	Uncharacterized protein LOC100131897 OS=Homo sapiens PE=2 SV=1	*INSYN2B*	59,204	INSYN2 family	Unknown	N/A	N/A
171	LIMA1_HUMAN	Q9UHB6	LIM domain and actin-binding protein 1 OS=Homo sapiens GN=LIMA1 PE=1 SV=1	*LIMA1*	85,226	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
172	E2AK3_HUMAN	Q9NXC2	Eukaryotic translation initiation factor 2-alpha kinase 3	*EIF2AK3*	125,216	Protein kinase superfamily, Ser/Thr protein kinase family, GCN2 subfamily	Inflammatory response (unfolded protein response)	N/A	N/A
173	GFOD1_HUMAN	Q8TDJ6	Glucose-fructose oxidoreductase domain-containing protein 1 OS=Homo sapiens GN=GFOD1 PE=2 SV=1	*GFOD1*	43,158	Gfo/Idh/MocA family	Others (oxidoreductase activity)	N/A	N/A
174	DMXL2_HUMAN	Q5W111	DmX-like protein 2 OS=Homo sapiens GN=DMXL2 PE=1 SV=1	*DMXL2*	339,641	Unknown	Others (vacuolar acidification)	N/A	N/A
175	CLLD6_HUMAN	P01764	Chronic lymphocytic leukemia deletion region gene 6 protein OS=Homo sapiens GN=CLLD6 PE=1 SV=2	*SPRYD7*	21,666	Unknown	Unknown	N/A	N/A
176	HV318_HUMAN	Q14525	Ig heavy chain V-III region TUR OS=Homo sapiens PE=1 SV=1	*IGHV3-23*	12,582	Unknown	Immune response	N/A	N/A
177	KT33B_HUMAN	O94898	Keratin, type I cuticular Ha3-II OS=Homo sapiens GN=KRT33B PE=1 SV=3	*KRT33B*	46,214	Intermediate filament family	Structural constituents (intermediate filament structure)	N/A	N/A
178	LRIG2_HUMAN	O14525	Leucine-rich repeats and immunoglobulin-like domains protein 2 OS=Homo sapiens GN=LRIG2 PE=1 SV=3	*LRIG2*	118,965	Unknown	Protein metabolism (proteolysis)	N/A	N/A
179	ASTN1_HUMAN	Q8WWQ0	Astrotactin-1 OS=Homo sapiens GN=ASTN1 PE=1 SV=3	*ASTN1*	144,913	Astrotactin family	Cell interaction and communication (cell adhesion)	N/A	N/A
180	PHIP_HUMAN	Q9BXT6	PH-interacting protein OS=Homo sapiens GN=PHIP PE=1 SV=1	*PHIP*	206,689	Unknown	Signaling pathway (insulin)	N/A	N/A
181	M10L1_HUMAN	O60284	Putative helicase Mov10l1 OS=Homo sapiens GN=MOV10L1 PE=1 SV=1	*MOV10L1*	135,293	DNA2/NAM7 helicase family, SDE3 subfamily	Cellular process (regulation of gene transcription and translation)	N/A	N/A
182	ST18_HUMAN	Q9BXX2	Suppression of tumorigenicity 18 protein OS=Homo sapiens GN=ST18 PE=1 SV=1	*ST18*	115,155	MYT1 family	Signaling pathway	N/A	N/A
183	AN30B_HUMAN	Q9ULD9	Ankyrin repeat domain-containing protein 30B OS=Homo sapiens GN=ANKRD30B PE=2 SV=2	*ANKRD30B*	158,049	Unknown	Unknown	N/A	N/A
184	ZN608_HUMAN	O60294	Zinc finger protein 608 OS=Homo sapiens GN=ZNF608 PE=1 SV=3	*ZNF608*	162,208	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A

185	LCMT2_HUMAN	Q9Y4C1	Leucine carboxyl methyltransferase 2 OS=Homo sapiens GN=LCMT2 PE=1 SV=3	*LCMT2*	75,602	Methyltransferase superfamily, LCMT family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
186	KDM3A_HUMAN	Q9H799	Lysine-specific demethylase 3A OS=Homo sapiens GN=KDM3A PE=1 SV=3	*KDM3A*	147,341	JHDM2 histone demethylase family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
187	YE031_HUMAN	P18564	Transmembrane protein ENSP00000382582 OS=Homo sapiens PE=4 SV=2	*CPLANE1*	361,746	Unknown	Structural constituents (celia structure)	N/A	N/A
188	ITB6_HUMAN	Q8NHX4	Integrin beta-6 OS=Homo sapiens GN=ITGB6 PE=1 SV=2	*ITGB6*	85,936	Integrin beta chain family	Cell interaction and communication (cell adhesion)	N/A	N/A
189	SPTA3_HUMAN	Q6DN14	Spermatogenesis-associated protein 3 OS=Homo sapiens GN=SPATA3 PE=2 SV=1	*SPATA3*	20,901	Unknown	Unknown	N/A	N/A
190	MCTP1_HUMAN	Q5T5C0	Multiple C2 and transmembrane domain-containing protein 1 OS=Homo sapiens GN=MCTP1 PE=2 SV=1	*MCTP1*	111,624	MCTP family	Cell interaction and communication	N/A	N/A
191	STXB5_HUMAN	Q8NBJ9	Syntaxin-binding protein 5 OS=Homo sapiens GN=STXBP5 PE=1 SV=1	*STXBP5*	127,573	WD repeat L(2)GL family	Cell interaction and communication (exocytosis)	N/A	N/A
192	SIDT2_HUMAN	Q8IYK2	SID1 transmembrane family member 2 OS=Homo sapiens GN=SIDT2 PE=1 SV=2	*SIDT2*	94,454	SID1 family	Glucose metabolism	N/A	N/A
193	CC105_HUMAN	Q9Y2G0	Coiled-coil domain-containing protein 105 OS=Homo sapiens GN=CCDC105 PE=2 SV=2	*CCDC105*	56,909	Unknown	Unknown	N/A	N/A
194	EFR3B_HUMAN	Q9Y4F5	Protein EFR3 homolog B OS=Homo sapiens GN=EFR3B PE=2 SV=2	*EFR3B*	92,487	EFR3 family	Signaling pathway (phosphatidylinositol)	N/A	N/A
195	K0284_HUMAN	P41439	Protein KIAA0284 OS=Homo sapiens GN=KIAA0284 PE=1 SV=4	*CEP170B*	171,688	CEP170 family	Unknown	N/A	N/A
196	FOLR3_HUMAN	Q9NXD2	Folate receptor gamma OS=Homo sapiens GN=FOLR3 PE=1 SV=1	*FOLR3*	27,885	Folate receptor family	Cell interaction and communication (cell adhesion)	N/A	N/A
197	MTMRA_HUMAN	O60391	Myotubularin-related protein 10 OS=Homo sapiens GN=MTMR10 PE=1 SV=3	*MTMR10*	88,273	Protein-tyrosine phosphatase family, Non-receptor class myotubularin subfamily	Signaling pathway (phosphatidylinositol)	N/A	N/A

198	NMD3B_HUMAN	Q14134	Glutamate [NMDA] receptor subunit 3B OS=Homo sapiens GN=GRIN3B PE=2 SV=2	*GRIN3B*	112,992	Glutamate-gated ion channel (TC 1.A.10.1) family, NR3B/GRIN3B subfamily	Cell interaction and communication	N/A	N/A
199	TRI29_HUMAN	Q9NWL6	Tripartite motif-containing protein 29 OS=Homo sapiens GN=TRIM29 PE=1 SV=2	*TRIM29*	65,835	Unknown	Immune response	N/A	N/A
200	ASND1_HUMAN	Q86WI1	Asparagine synthetase domain-containing protein 1 OS=Homo sapiens GN=ASNSD1 PE=2 SV=1	*ASNSD1*	72,080	Unknown	Protein metabolism (amino acid metabolism)	N/A	N/A
201	PKHL1_HUMAN	Q99424	Fibrocystin-L OS=Homo sapiens GN=PKHD1L1 PE=2 SV=2	*PKHD1L1*	465,734	Unknown	Immune response	N/A	N/A
202	ACOX2_HUMAN	Q9NZ56	Peroxisomal acyl-coenzyme A oxidase 2 OS=Homo sapiens GN=ACOX2 PE=1 SV=1	*ACOX2*	76,827	Acyl-CoA oxidase family	Lipid metabolism	N/A	N/A
203	FMN2_HUMAN	O75132	Formin-2 OS=Homo sapiens GN=FMN2 PE=1 SV=4	*FMN2*	180,106	Formin homology family, Cappuccino subfamily	Cellular process (cell division)	N/A	N/A
204	ZBED4_HUMAN	P23471	Zinc finger BED domain-containing protein 4 OS=Homo sapiens GN=ZBED4 PE=1 SV=1	*ZBED4*	130,322	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
205	PTPRZ_HUMAN	P07359	Receptor-type tyrosine-protein phosphatase zeta OS=Homo sapiens GN=PTPRZ1 PE=1 SV=4	*PTPRZ1*	254,587	Protein-tyrosine phosphatase family, Receptor class 5 subfamily	Cellular process (organ development)	N/A	N/A
206	GP1BA_HUMAN	O60271	Platelet glycoprotein Ib alpha chain OS=Homo sapiens GN=GP1BA PE=1 SV=1	*GP1BA*	71,540	Unknown	Blood coagulation	N/A	N/A
207	JIP4_HUMAN	P23352	C-Jun-amino-terminal kinase-interacting protein 4 OS=Homo sapiens GN=SPAG9 PE=1 SV=4	*SPAG9*	146,205	JIP scaffold family	Cellullar process (cell proliferation and differentiation)	N/A	N/A
208	KALM_HUMAN	Q9ULK4	Anosmin-1 OS=Homo sapiens GN=KAL1 PE=1 SV=3	*ANOS1*	76,112	Unknown	Cellular process (organ development)	N/A	N/A
209	MED23_HUMAN	Q96PE3	Mediator of RNA polymerase II transcription subunit 23 OS=Homo sapiens GN=MED23 PE=1 SV=2	*MED23*	156,474	Mediator complex subunit 23 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
210	INP4A_HUMAN		Type I inositol-3,4-bisphosphate 4-phosphatase OS=Homo sapiens GN=INPP4A PE=1 SV=1	*INPP4A*	109,956	Inositol 3,4-bisphosphate 4-phosphatase family	Others (inositol phosphate metabolic process)	N/A	N/A

Appendix H
Differentially expressed plasma proteins (Study III)

(ii) Down-expressed proteins (n = 313)

	Accession number		Protein name	Encoding gene	Mass (Da)	Protein family	Protein function	Fold change	
	Swiss-Prot	Uniprot						mean	S.D.
211	RET4_HUMAN	P02753	Retinol-binding protein 4 OS=Homo sapiens GN=RBP4 PE=1 SV=3	*RBP4*	23,010	Calycin superfamily, Lipocalin family	Cellular process (organ development)	0.35	0.18
212	K2C1_HUMAN	P04264	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	*KRT1*	66,039	Intermediate filament family	Structural constituents (extracellular matrix)	0.45	0.40
213	HPT_HUMAN	P00738	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1	*HP*	45,205	Peptidase S1 family	Inflammatory response	0.64	0.36
214	APOA4_HUMAN	P06727	Apolipoprotein A-IV OS=Homo sapiens GN=APOA4 PE=1 SV=3	*APOA4*	45,372	Apolipoprotein A1/A4/E family	Lipid metabolism	0.66	0.43
215	ANGT_HUMAN	P01019	Angiotensinogen OS=Homo sapiens GN=AGT PE=1 SV=1	*AGT*	53,154	Serpin family	Signaling pathway (MAPK)	N/A	N/A
216	DCTN1_HUMAN	Q14203	Dynactin subunit 1 OS=Homo sapiens GN=DCTN1 PE=1 SV=3	*DCTN1*	141,695	Dynactin 150 kDa subunit family	Inflammatory response (unfolded protein response)	N/A	N/A
217	MYH8_HUMAN	P13535	Myosin-8 OS=Homo sapiens GN=MYH8 PE=1 SV=3	*MYH8*	222,763	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family	Cellular process (organ development)	N/A	N/A
218	IGJ_HUMAN	P01591	Immunoglobulin J chain OS=Homo sapiens GN=IGJ PE=1 SV=4	*JCHAIN*	18,099	Unknown	Immune response	N/A	N/A
219	SMBP2_HUMAN	P38935	DNA-binding protein SMUBP-2 OS=Homo sapiens GN=IGHMBP2 PE=1 SV=2	*IGHMBP2*	109,149	DNA2/NAM7 helicase family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
220	CLH2_HUMAN	P53675	Clathrin heavy chain 2 OS=Homo sapiens GN=CLTCL1 PE=1 SV=2	*CLTCL1*	187,030	Clathrin heavy chain family	Cell interaction and communication (endocytosis)	N/A	N/A
221	GOG6C_HUMAN	A6NDK9	Golgin subfamily A member 6C OS=Homo sapiens GN=GOLGA6C PE=2 SV=1	*GOLGA6C*	79,884	GOLGA6 family	Unknown	N/A	N/A
222	ZF106_HUMAN	Q9H2Y7	Zinc finger protein 106 homolog OS=Homo sapiens GN=ZFP106 PE=1 SV=1	*ZNF106*	208,883	Unknown	Glucose metabolism	N/A	N/A
223	KI26B_HUMAN	Q2KJY2	Kinesin-like protein KIF26B OS=Homo sapiens GN=KIF26B PE=1 SV=1	*KIF26B*	223,883	TRAFAC class myosin-kinesin ATPase superfamily, Kinesin family, KIF26 subfamily	Cell interaction and communication (cell adhesion)	N/A	N/A

224	CC108_HUMAN	Q6ZU64	Cilia- and flagella-associated protein 65 (Coiled-coil domain-containing protein 108)	*CFAP65*	217,250	CFAP65 family	Others (flagellated sperm motility)	N/A	N/A
225	AKAP3_HUMAN	O75969	A-kinase anchor protein 3 OS=Homo sapiens GN=AKAP3 PE=1 SV=2	*AKAP3*	94,751	AKAP110 family	Protein metabolism (protein localization)	N/A	N/A
226	VPS11_HUMAN	Q9H270	Vacuolar protein sorting-associated protein 11 homolog OS=Homo sapiens GN=VPS11 PE=1 SV=1	*VPS11*	107,837	VPS11 family	Cell interaction and communication	N/A	N/A
227	PARD3_HUMAN	Q8TEW0	Partitioning defective 3 homolog OS=Homo sapiens GN=PARD3 PE=1 SV=2	*PARD3*	151,423	PAR3 family	Cellular process (organ development)	N/A	N/A
228	TEC_HUMAN	P42680	Tyrosine-protein kinase Tec OS=Homo sapiens GN=TEC PE=1 SV=2	*TEC*	73,581	Protein kinase superfamily, Tyr protein kinase family, TEC subfamily	Immune response	N/A	N/A
229	DYH9_HUMAN	Q9NYC9	Dynein heavy chain 9, axonemal OS=Homo sapiens GN=DNAH9 PE=1 SV=2	*DNAH9*	511,877	Dynein heavy chain family	Structural constituents (cilia structure)	N/A	N/A
230	MAST4_HUMAN	O15021	Microtubule-associated serine/threonine-protein kinase 4 (EC 2.7.11.1)	*MAST4*	284,097	Protein kinase superfamily, AGC Ser/Thr protein kinase family	Structural constituents	N/A	N/A
231	YE013_HUMAN	Q7Z745	Maestro heat-like repeat-containing protein family member 2B (HEAT repeat-containing protein 7B2) (Sperm PKA-interacting factor) (SPIF)	*MROH2B*	180,781	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
232	SRCAP_HUMAN	Q6ZRS2	Helicase SRCAP (EC 3.6.4.-) (Domino homolog 2) (Snf2-related CBP activator)	*SRCAP*	343,555	SNF2/RAD54 helicase family, SWR1 subfamily	Cellular process (regulation of gene transcription and translation)	N/A	N/A
233	SCN3A_HUMAN	Q9NY46	Sodium channel protein type 3 subunit alpha (Sodium channel protein brain III subunit alpha) (Sodium channel protein type III subunit alpha) (Voltage-gated sodium channel subtype III) (Voltage-gated sodium channel subunit alpha Nav1.3)	*SCN3A*	226,294	Sodium channel (TC 1.A.1.10) family, Nav1.3/SCN3A subfamily	Cell interaction and communication	N/A	N/A
234	VWA3A_HUMAN	A6NCI4	von Willebrand factor A domain-containing protein 3A	*VWA3A*	134,020	Unknown	Unknown	N/A	N/A

235	MYO5A_HUMAN	Q9Y4I1	Unconventional myosin-Va (Dilute myosin heavy chain, non-muscle) (Myosin heavy chain 12) (Myosin-12) (Myoxin)	*MYO5A*	215,405	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family	Structural constituents (actomyosin structure)	N/A	N/A
236	IFT74_HUMAN	Q96LB3	Intraflagellar transport protein 74 homolog (Capillary morphogenesis gene 1 protein) (CMG-1) (Coiled-coil domain-containing protein 2)	*IFT74*	69,239	IFT74 family	Structural constituents (cilia structure)	N/A	N/A
237	UN13C_HUMAN	Q8NB66	Protein unc-13 homolog C (Munc13-3)	*UNC13C*	250,911	Unc-13 family	Cell interaction and communication (signal transduction)	N/A	N/A
238	HTR7A_HUMAN	Q8NDA8	Maestro heat-like repeat-containing protein family member 1 (HEAT repeat-containing protein 7A)	*MROH1*	181,249	Unknown	Unknown	N/A	N/A
239	SYLC_HUMAN	Q9P2J5	Leucine--tRNA ligase, cytoplasmic (EC 6.1.1.4) (Leucyl-tRNA synthetase) (LeuRS)	*LARS1*	134,466	Class-I aminoacyl-tRNA synthetase family	Others (response to stimuli: amino acids)	N/A	N/A
240	EZRI_HUMAN	P15311	Ezrin (Cytovillin) (Villin-2) (p81)	*EZR*	69,413	Unknown	Structural constituents (actin cytoskeletal structure)	N/A	N/A
241	NUP93_HUMAN	Q8N1F7	Nuclear pore complex protein Nup93 (93 kDa nucleoporin) (Nucleoporin Nup93)	*NUP93*	93,488	Nucleoporin interacting component (NIC) family	Cell interaction and communication	N/A	N/A
242	KRT86_HUMAN	O43790	Keratin, type II cuticular Hb6 (Hair keratin K2.11) (Keratin-86) (K86) (Type II hair keratin Hb6) (Type-II keratin Kb26)	*KRT86*	53,501	Intermediate filament family	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
243	PHLB1_HUMAN	Q86UU1	Pleckstrin homology-like domain family B member 1 (Protein LL5-alpha)	*PHLDB1*	151,162	Unknown	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
244	NCKP5_HUMAN	O14513	Nck-associated protein 5 (NAP-5) (Peripheral clock protein)	*NCKAP5*	208,537	Unknown	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
245	CO4A3_HUMAN	Q01955	Collagen alpha-3(IV) chain OS=Homo sapiens GN=COL4A3 PE=1 SV=3	*COL4A3*	161,813	Type IV collagen family	Structural constituents (extracellular matrix)	N/A	N/A
246	RAI14_HUMAN	Q9P0K7	Ankycorbin OS=Homo sapiens GN=RAI14 PE=1 SV=2	*RAI14*	110,041	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A

247	CDC5L_HUMAN	Q99459	Cell division cycle 5-like protein OS=Homo sapiens GN=CDC5L PE=1 SV=2	*CDC5L*	92,251	CEF1 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
248	CAC1A_HUMAN	O00555	Voltage-dependent P/Q-type calcium channel subunit alpha-1A OS=Homo sapiens GN=CACNA1A PE=1 SV=2	*CACNA1A*	282,564	Calcium channel alpha-1 subunit (TC 1.A.1.11) family, CACNA1A subfamily	Cell interaction and communication	N/A	N/A
249	CUX1_HUMAN	P39880	Homeobox protein cut-like 1 OS=Homo sapiens GN=CUX1 PE=1 SV=2	*CUX1*	164,187	CUT homeobox family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
250	U634A_HUMAN	A6QL64	Ankyrin repeat domain-containing protein 36A	*ANKRD36*	214,500	ANKRD36 family	Unknown	N/A	N/A
251	ANR31_HUMAN	Q8N7Z5	Ankyrin repeat domain-containing protein 31	*ANKRD31*	210,816	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
252	PI3R4_HUMAN	Q99570	Phosphoinositide 3-kinase regulatory subunit 4 (PI3-kinase regulatory subunit 4) (EC 2.7.11.1) (PI3-kinase p150 subunit) (Phosphoinositide 3-kinase adaptor protein)	*PIK3R4*	153,103	Protein kinase superfamily, Ser/Thr protein kinase family	Cellular process (autophagy)	N/A	N/A
253	MTG1_HUMAN	Q9BT17	Mitochondrial ribosome-associated GTPase 1 (GTP-binding protein 7) (Mitochondrial GTPase 1)	*MTG1*	37,237	TRAFAC class YlqF/YawG GTPase family, MTG1 subfamily	Cellular process (regulation of gene transcription and translation)	N/A	N/A
254	RAIN_HUMAN	Q5U651	Ras-interacting protein 1 (Rain)	*RASIP1*	103,457	Unknown	Cellular process (autophagy)	N/A	N/A
255	K1549_HUMAN	Q9HCM3	UPF0606 protein KIAA1549	*KIAA1549*	210,755	UPF0606 family	Structural constituents (integral component of membrane)	N/A	N/A
256	TERT_HUMAN	O14746	Telomerase reverse transcriptase (EC 2.7.7.49) (HEST2) (Telomerase catalytic subunit) (Telomerase-associated protein 2) (TP2)	*TERT*	126,997	Reverse transcriptase family, Telomerase subfamily	Cellular process (cell apoptosis)	N/A	N/A
257	AKAP4_HUMAN	Q5JQC9	A-kinase anchor protein 4 (AKAP-4) (A-kinase anchor protein 82 kDa) (AKAP 82) (hAKAP82) (Major sperm fibrous sheath protein) (HI) (Protein kinase A-anchoring protein 4) (PRKA4)	*AKAP4*	94,477	AKAP110 family	Others (flagellated sperm motility)	N/A	N/A
258	NEIL3_HUMAN	Q8TAT5	Endonuclease 8-like 3 (EC 3.2.2.-) (EC 4.2.99.18) (DNA glycosylase FPG2) (DNA glycosylase/AP lyase Neil3) (Endonuclease VIII-like 3) (Nei-like protein 3)	*NEIL3*	67,769	FPG family	Cellular process (nucleotide-excision repair)	N/A	N/A

259	PAR14_HUMAN	Q460N5	Protein mono-ADP-ribosyltransferase PARP14 (EC 2.4.2.-) (ADP-ribosyltransferase diphtheria toxin-like 8) (ARTD8) (B aggressive lymphoma protein 2) (Poly [ADP-ribose] polymerase 14) (PARP-14)	*PARP14*	202,800	Unknown	Immune response	N/A	N/A
260	CN045_HUMAN	Q8ND07	Basal body-orientation factor 1 (Coiled-coil domain-containing protein 176)	*BBOF1*	61,987	BBOF1 family	Structural constituents (cilia structure)	N/A	N/A
261	SIN3A_HUMAN	Q96ST3	Paired amphipathic helix protein Sin3a (Histone deacetylase complex subunit Sin3a) (Transcriptional corepressor Sin3a)	*SIN3A*	145,175	Unknown	Immune response	N/A	N/A
262	TBD2B_HUMAN	Q9UPU7	TBC1 domain family member 2B	*TBC1D2B*	109,880	Unknown	Cell interaction and communication	N/A	N/A
263	GLPK_HUMAN	P32189	Glycerol kinase (GK) (Glycerokinase) (EC 2.7.1.30) (ATP:glycerol 3-phosphotransferase)	*GK*	61,245	FGGY kinase family	Lipid metabolism	N/A	N/A
264	MYH6_HUMAN	P13533	Myosin-6 (Myosin heavy chain 6) (Myosin heavy chain, cardiac muscle alpha isoform) (MyHC-alpha)	*MYH6*	223,735	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family	Cellular process (organ development)	N/A	N/A
265	TAGAP_HUMAN	Q8N103	T-cell activation Rho GTPase-activating protein (T-cell activation GTPase-activating protein)	*TAGAP*	80,703	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A
266	CO6A3_HUMAN	P12111	Collagen alpha-3(VI) chain	*COL6A3*	343,669	Type VI collagen family	Structural constituents (extracellular matrix)	N/A	N/A
267	TET1_HUMAN	Q8NFU7	Methylcytosine dioxygenase TET1 (EC 1.14.11.n2) (CXXC-type zinc finger protein 6) (Leukemia-associated protein with a CXXC domain) (Ten-eleven translocation 1 gene protein)	*TET1*	235,309	TET family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
268	NEUL4_HUMAN	Q96JN8	Neuralized-like protein 4	*NEURL4*	166,907	Unknown	Protein metabolism (ubiquitin protein ligase activity)	N/A	N/A
269	HAIR_HUMAN	O43593	Lysine-specific demethylase hairless (EC 1.14.11.65) ([histone H3]-dimethyl-L-lysine(9) demethylase hairless)	*HR*	127,495	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
270	CCAR1_HUMAN	Q8IX12	Cell division cycle and apoptosis regulator protein 1 (Cell cycle and apoptosis regulatory protein 1) (CARP-1) (Death inducer with SAP domain)	*CCAR1*	132,821	Unknown	Cellular process (cell apoptosis)	N/A	N/A
271	ZN786_HUMAN	Q8N393	Zinc finger protein 786	*ZNF786*	89,815	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A

272	ZER1_HUMAN	Q7Z7L7	Protein zer-1 homolog (Hzyg) (Zyg-11 homolog B-like protein) (Zyg11b-like protein)	*ZER1*	88,169	Zyg-11 family	Protein metabolism (ubiquitin-dependent protein catabolic process)	N/A	N/A
273	A1AG2_HUMAN	P19652	Alpha-1-acid glycoprotein 2 (AGP 2) (Orosomucoid-2) (OMD 2)	*ORM2*	23,603	Calycin superfamily, Lipocalin family	Immune response	N/A	N/A
274	AT2B2_HUMAN	Q01814	Plasma membrane calcium-transporting ATPase 2 (PMCA2) (EC 7.2.2.10) (Plasma membrane calcium ATPase isoform 2) (Plasma membrane calcium pump isoform 2)	*ATP2B2*	136,876	Cation transport ATPase (P-type) (TC 3.A.3) family, Type IIB subfamily	Cell interaction and communication	N/A	N/A
275	LIPA1_HUMAN	Q13136	Liprin-alpha-1 (LAR-interacting protein 1) (LIP-1) (Protein tyrosine phosphatase receptor type f polypeptide-interacting protein alpha-1) (PTPRF-interacting protein alpha-1)	*PPFIA1*	135,779	Liprin family, Liprin-alpha subfamily	Cell interaction and communication (cell adhesion)	N/A	N/A
276	PHF3_HUMAN	Q92576	PHD finger protein 3	*PHF3*	229,481	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
277	MAP7_HUMAN	Q14244	Ensconsin (Epithelial microtubule-associated protein of 115 kDa) (E-MAP-115) (Microtubule-associated protein 7) (MAP-7)	*MAP7*	84,052	MAP7 family	Structural constituents (actin cytoskeletal structure)	N/A	N/A
278	CING_HUMAN	Q9P2M7	Cingulin	*CGN*	136,386	Cingulin family	Signaling pathway (growth factor beta receptor)	N/A	N/A
279	COOA1_HUMAN	Q17RW2	Collagen alpha-1(XXIV) chain	*COL24A1*	175,496	Fibrillar collagen family	Structural constituents (extracellular matrix)	N/A	N/A
280	PRD10_HUMAN	Q9NQV6	PR domain zinc finger protein 10 (EC 2.1.1.-) (PR domain-containing protein 10) (Tristanin)	*PRDM10*	130,136	Class V-like SAM-binding methyltransferase superfamily	Cellular process (regulation of gene transcription and translation)	N/A	N/A
281	MAEA_HUMAN	Q7L5Y9	E3 ubiquitin-protein transferase MAEA (EC 2.3.2.27) (Cell proliferation-inducing gene 5 protein) (Erythroblast macrophage protein) (Human lung cancer oncogene 10 protein) (HLC-10) (Macrophage erythroblast attacher) (P44EMLP)	*MAEA*	45,287	Unknown	Cell interaction and communication (cell adhesion)	N/A	N/A

282	AKA11_HUMAN	Q9UKA4	A-kinase anchor protein 11 (AKAP-11) (A-kinase anchor protein 220 kDa) (AKAP 220) (hAKAP220) (Protein kinase A-anchoring protein 11) (PRKA11)	*AKAP11*	210,512	AKAP110 family	Protein metabolism (protein localization)	N/A	N/A
283	KRT81_HUMAN	Q14533	Keratin, type II cuticular Hb1 (Hair keratin K2.9) (Keratin, hair, basic, 1) (Keratin-81) (K81) (Metastatic lymph node 137 gene protein) (MLN 137) (Type II hair keratin Hb1) (Type-II keratin Kb21) (ghHKb1) (ghHb1)	*KRT81*	54,928	Intermediate filament family	Structural constituents (extracellular matrix)	N/A	N/A
284	CUL7_HUMAN	Q14999	Cullin-7 (CUL-7)	*CUL7*	191,161	Cullin family	Inflammatory response (unfolded protein response)	N/A	N/A
285	MYBA_HUMAN	P10243	Myb-related protein A (A-Myb) (Myb-like protein 1)	*MYBL1*	85,887	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
286	CK035_HUMAN	Q8IXW0	Lamin tail domain-containing protein 2	*LMNTD2*	70,379	Unknown	Unknown	N/A	N/A
287	FSTL5_HUMAN	Q8N475	Follistatin-related protein 5 (Follistatin-like protein 5)	*FSTL5*	95,751	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
288	KIF5C_HUMAN	O60282	Kinesin heavy chain isoform 5C (Kinesin heavy chain neuron-specific 2)	*KIF5C*	109,495	TRAFAC class myosin-kinesin ATPase superfamily, Kinesin family, Kinesin subfamily	Cell interaction and communication (signal transduction)	N/A	N/A
289	ASB2_HUMAN	Q96Q27	Ankyrin repeat and SOCS box protein 2 (ASB-2)	*ASB2*	65,084	Ankyrin SOCS box (ASB) family	Cell interaction and communication (signal transduction)	N/A	N/A
290	CO9_HUMAN	P02748	Complement component C9 [Cleaved into: Complement component C9a; Complement component C9b]	*C9*	63,173	Complement C6/C7/C8/C9 family	Cellular process (cell apoptosis)	N/A	N/A
291	ELYS_HUMAN	Q8WYP5	Protein ELYS (Embryonic large molecule derived from yolk sac) (Protein MEL-28) (Putative AT-hook-containing transcription factor 1)	*AHCTF1*	252,498	ELYS family	Cellular process (cell division)	N/A	N/A
292	ADNP2_HUMAN	Q6IQ32	Activity-dependent neuroprotector homeobox protein 2 (ADNP homeobox protein 2) (Zinc finger protein 508)	*ADNP2*	122,833	Krueppel C2H2-type zinc-finger protein family	Cellullar process (cell proliferation and differentiation)	N/A	N/A
293	TMC5_HUMAN	Q6UXY8	Transmembrane channel-like protein 5	*TMC5*	114,797	TMC family	Structural constituents (integral component of membrane)	N/A	N/A
294	ACSF3_HUMAN	Q4G176	Malonate--CoA ligase ACSF3, mitochondrial (EC 6.2.1.n3) (Acyl-CoA synthetase family member 3)	*ACSF3*	64,130	ATP-dependent AMP-binding enzyme family	Lipid metabolism	N/A	N/A

295	PCCB_HUMAN	P05166	Propionyl-CoA carboxylase beta chain, mitochondrial (PCCase subunit beta) (EC 6.4.1.3) (Propanoyl-CoA:carbon dioxide ligase subunit beta)	*PCCB*	58,216	AccD/PCCB family	Lipid metabolism	N/A	N/A
296	PK3CG_HUMAN	P48736	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform (PI3-kinase subunit gamma) (PI3K-gamma) (PI3Kgamma) (PtdIns-3-kinase subunit gamma) (EC 2.7.1.137) (EC 2.7.1.153) (EC 2.7.1.154) (Phosphatidylinositol 4,5-bisphosphate 3-kinase 110 kDa catalytic subunit gamma) (PtdIns-3-kinase subunit p110-gamma) (p110gamma) (Phosphoinositide-3-kinase catalytic gamma polypeptide) (Serine/threonine protein kinase PIK3CG) (EC 2.7.11.1) (p120-PI3K)	*PIK3CG*	126,454	PI3/PI4-kinase family	Immune response	N/A	N/A
297	HEM1_HUMAN	P13196	5-aminolevulinate synthase, nonspecific, mitochondrial (ALAS-H) (EC 2.3.1.37) (5-aminolevulinic acid synthase 1) (Delta-ALA synthase 1) (Delta-aminolevulinate synthase 1)	*ALAS1*	70,581	Class-II pyridoxal-phosphate-dependent aminotransferase family	Cellular process (cell proliferation and differentiation)	N/A	N/A
298	MAP4_HUMAN	P27816	Microtubule-associated protein 4 (MAP-4)	*MAP4*	121,005	Unknown	Cellular process (cell division)	N/A	N/A
299	ANR17_HUMAN	O75179	Ankyrin repeat domain-containing protein 17 (Gene trap ankyrin repeat protein) (Serologically defined breast cancer antigen NY-BR-16)	*ANKRD17*	274,258	Unknown	Immune response	N/A	N/A
300	YB046_HUMAN	A6NES4	Maestro heat-like repeat-containing protein family member 2A (HEAT repeat-containing protein 7B1)	*MROH2A*	189,561	Unknown	Unknown	N/A	N/A
301	ZN101_HUMAN	Q8IZC7	Zinc finger protein 101 (Zinc finger protein HZF12)	*ZNF101*	50,339	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
302	SREC_HUMAN	Q14162	Scavenger receptor class F member 1 (Acetyl LDL receptor) (Scavenger receptor expressed by endothelial cells 1) (SREC-I)	*SCARF1*	87,387	Unknown	Cell interaction and communication (cell adhesion)	N/A	N/A
303	WDR17_HUMAN	Q8IZU2	WD repeat-containing protein 17	*WDR17*	147,703	Unknown	Unknown	N/A	N/A
304	CYFP1_HUMAN	Q7L576	Cytoplasmic FMR1-interacting protein 1 (Specifically Rac1-associated protein 1) (Sra-1) (p140sra-1)	*CYFIP1*	145,182	CYFIP family	Cellular process (organ development)	N/A	N/A

305	GP123_HUMAN	Q86SQ6	Adhesion G protein-coupled receptor A1 (G-protein coupled receptor 123)	*ADGRA1*	60,885	G-protein coupled receptor 2 family, Adhesion G-protein coupled receptor (ADGR) subfamily	Cell interaction and communication (signal transduction)	N/A	N/A
306	ZN281_HUMAN	Q9Y2X9	Zinc finger protein 281 (GC-box-binding zinc finger protein 1) (Transcription factor ZBP-99) (Zinc finger DNA-binding protein 99)	*ZNF281*	96,915	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
307	NOD2_HUMAN	Q9HC29	Nucleotide-binding oligomerization domain-containing protein 2 (Caspase recruitment domain-containing protein 15) (Inflammatory bowel disease protein 1)	*NOD2*	115,283	Unknown	Immune response	N/A	N/A
308	RNC_HUMAN	Q9NRR4	Ribonuclease 3 (EC 3.1.26.3) (Protein Drosha) (Ribonuclease III) (RNase III) (p241)	*DROSHA*	159,316	Ribonuclease III family	Immune response	N/A	N/A
309	CL063_HUMAN	Q96N23	Cilia- and flagella-associated protein 54	*CFAP54*	351,970	CFAP54 family	Cellular process (cell proliferation and differentiation)	N/A	N/A
310	LETM1_HUMAN	O95202	Mitochondrial proton/calcium exchanger protein (Leucine zipper-EF-hand-containing transmembrane protein 1)	*LETM1*	83,354	LETM1 family	Cell interaction and communication	N/A	N/A
311	K0226_HUMAN	Q92622	Run domain Beclin-1-interacting and cysteine-rich domain-containing protein (Rubicon) (Beclin-1 associated RUN domain containing protein) (Baron)	*RUBCN*	108,622	Unknown	Cellular process (autophagy)	N/A	N/A
312	AFF2_HUMAN	P51816	AF4/FMR2 family member 2 OS=Homo sapiens GN=AFF2 PE=1 SV=4	*AFF2*	144,771	AF4 family	Cellular process (organ development)	N/A	N/A
313	ZN292_HUMAN	O60281	Zinc finger protein 292 OS=Homo sapiens GN=ZNF292 PE=1 SV=2	*ZNF292*	304,816	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
314	ABCA3_HUMAN	Q99758	ATP-binding cassette sub-family A member 3 OS=Homo sapiens GN=ABCA3 PE=1 SV=2	*ABCA3*	191,362	ABC transporter superfamily, ABCA family	Cell interaction and communication	N/A	N/A
315	GP171_HUMAN	O14626	Probable G-protein coupled receptor 171 OS=Homo sapiens GN=GPR171 PE=2 SV=1	*GPR171*	36,754	G-protein coupled receptor 1 family	Signaling pathway (G protein-coupled receptor)	N/A	N/A

316	COJA1_HUMAN	Q14993	Collagen alpha-1(XIX) chain OS=Homo sapiens GN=COL19A1 PE=1 SV=3	*COL19A1*	115,221	Fibril-associated collagens with interrupted helices (FACIT) family	Structural constituents (extracellular matrix)	N/A	N/A
317	RO60_HUMAN	P10155	60 kDa SS-A/Ro ribonucleoprotein OS=Homo sapiens GN=TROVE2 PE=1 SV=2	*RO60*	60,671	Ro 60 kDa family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
318	KV401_HUMAN	P06312	Ig kappa chain V-IV region (Fragment) OS=Homo sapiens GN=IGKV4-1 PE=4 SV=1	*IGKV4-1*	13,380	Unknown	Immune response	N/A	N/A
319	KDM6A_HUMAN	O15550	Lysine-specific demethylase 6A OS=Homo sapiens GN=KDM6A PE=1 SV=2	*KDM6A*	154,177	UTX family	Cellular process (cell proliferation and differentiation)	N/A	N/A
320	TNPO2_HUMAN	O14787	Transportin-2 OS=Homo sapiens GN=TNPO2 PE=1 SV=3	*TNPO2*	101,388	Importin beta family, Importin beta-2 subfamily	Cell interaction and communication	N/A	N/A
321	VGLL4_HUMAN	Q14135	Transcription cofactor vestigial-like protein 4 OS=Homo sapiens GN=VGLL4 PE=1 SV=4	*VGLL4*	30,948	Vestigial family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
322	FMNL3_HUMAN	Q8IVF7	Formin-like protein 3 OS=Homo sapiens GN=FMNL3 PE=1 SV=3	*FMNL3*	117,213	Formin homology family	Structural constituents (actomyosin structure)	N/A	N/A
323	ZO1_HUMAN	Q07157	Tight junction protein ZO-1 OS=Homo sapiens GN=TJP1 PE=1 SV=3	*TJP1*	195,459	MAGUK family	Cell interaction and communication (cell adhesion)	N/A	N/A
324	LPHN1_HUMAN	O94910	Adhesion G protein-coupled receptor L1 (Calcium-independent alpha-latrotoxin receptor 1) (CIRL-1) (Latrophilin-1) (Lectomedin-2)	*ADGRL1*	162,717	G-protein coupled receptor 2 family, Adhesion G-protein coupled receptor (ADGR) subfamily	Signaling pathway (G protein-coupled receptor)	N/A	N/A
325	SART3_HUMAN	Q15020	Squamous cell carcinoma antigen recognized by T-cells 3 OS=Homo sapiens GN=SART3 PE=1 SV=1	*SART3*	109,935	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
326	GNTK_HUMAN	Q5T6J7	Probable gluconokinase OS=Homo sapiens GN=C9orf103 PE=2 SV=1	*IDNK*	20,578	Gluconokinase GntK/GntV family	Others (D-gluconate catabolic process)	N/A	N/A
327	ITA9_HUMAN	Q13797	Integrin alpha-9 OS=Homo sapiens GN=ITGA9 PE=1 SV=2	*ITGA9*	114,489	Integrin alpha chain family	Structural constituents (extracellular matrix)	N/A	N/A
328	LMNB2_HUMAN	Q03252	Lamin-B2 OS=Homo sapiens GN=LMNB2 PE=1 SV=3	*LMNB2*	69,948	Intermediate filament family	Structural constituents (lamin filament structure)	N/A	N/A
329	APBA2_HUMAN	Q99767	Amyloid beta A4 precursor protein-binding family A member 2 OS=Homo sapiens GN=APBA2 PE=1 SV=3	*APBA2*	82,512	Unknown	Cellular process (organ development)	N/A	N/A

330	FBF1_HUMAN	Q8TES7	Fas-binding factor 1 OS=Homo sapiens GN=FBF1 PE=1 SV=2	*FBF1*	125,446	Unknown	Structural constituents (cilia structure)	N/A	N/A
331	K1731_HUMAN	Q9C0D2	Centrosomal protein of 295 kDa	*CEP295*	295,176	Unknown	Cellular process (regulation of centriole replication)	N/A	N/A
332	PKHG1_HUMAN	Q9ULL1	Pleckstrin homology domain-containing family G member 1 OS=Homo sapiens GN=PLEKHG1 PE=1 SV=2	*PLEKHG1*	155,439	Unknown	Others (Rho guanyl-nucleotide exchange factor activity)	N/A	N/A
333	TEX15_HUMAN	Q9BXT5	Testis-expressed sequence 15 protein OS=Homo sapiens GN=TEX15 PE=1 SV=1	*TEX15*	315,336	TEX15 family	Cellular process (cell proliferation and differentiation)	N/A	N/A
334	CA113_HUMAN	A4FU49	SH3 domain-containing protein C1orf113 OS=Homo sapiens GN=C1orf113 PE=1 SV=2	*SH3D21*	70,519	Unknown	Unknown	N/A	N/A
335	ZN646_HUMAN	O15015	Zinc finger protein 646 OS=Homo sapiens GN=ZNF646 PE=2 SV=1	*ZNF646*	200,825	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
336	NAL14_HUMAN	Q86W24	NACHT, LRR and PYD domains-containing protein 14 OS=Homo sapiens GN=NLRP14 PE=2 SV=1	*NLRP14*	124,733	NLRP family	Cellular process (cell proliferation and differentiation)	N/A	N/A
337	CSKP_HUMAN	O14936	Peripheral plasma membrane protein CASK OS=Homo sapiens GN=CASK PE=1 SV=3	*CASK*	105,123	Protein kinase superfamily, CAMK Ser/Thr protein kinase family, CaMK subfamily; MAGUK family	Cell interaction and communication	N/A	N/A
338	MYO3B_HUMAN	Q8WXR4	Myosin-IIIb OS=Homo sapiens GN=MYO3B PE=2 SV=3	*MYO3B*	151,829	TRAFAC class myosin-kinesin ATPase superfamily, Myosin family; Protein kinase superfamily, STE Ser/Thr protein kinase family	Cellular process (cell proliferation and differentiation)	N/A	N/A
339	NMDE1_HUMAN	Q12879	Glutamate [NMDA] receptor subunit epsilon-1 OS=Homo sapiens GN=GRIN2A PE=1 SV=1	*GRIN2A*	165,283	Glutamate-gated ion channel (TC 1.A.10.1) family, NR2A/GRIN2A subfamily	Cell interaction and communication	N/A	N/A
340	CNTP3_HUMAN	Q9BZ76	Contactin-associated protein-like 3 OS=Homo sapiens GN=CNTNAP3 PE=2 SV=3	*CNTNAP3*	140,690	Neurexin family	Cell interaction and communication (cell adhesion)	N/A	N/A
341	CJ092_HUMAN	Q8IYW2	Cilia- and flagella-associated protein 46 (Tetratricopeptide repeat protein 40)	*CFAP46*	303,500	CFAP46 family	Structural constituents (cilia structure)	N/A	N/A
342	SI1L2_HUMAN	Q9P2F8	Signal-induced proliferation-associated 1-like protein 2 OS=Homo sapiens GN=SIPA1L2 PE=1 SV=2	*SIPA1L2*	190,438	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A

343	IREB2_HUMAN	P48200	Iron-responsive element-binding protein 2 OS=Homo sapiens GN=IREB2 PE=1 SV=2	*IREB2*	105,059	Aconitase/IPM isomerase family	Ion binding and transport (iron)	N/A	N/A
344	PZRN4_HUMAN	Q6ZMN7	PDZ domain-containing RING finger protein 4 OS=Homo sapiens GN=PDZRN4 PE=1 SV=3	*PDZRN4*	117,103	Unknown	Ion binding and transport (metal)	N/A	N/A
345	EXOC1_HUMAN	Q9NV70	Exocyst complex component 1 OS=Homo sapiens GN=EXOC1 PE=1 SV=4	*EXOC1*	101,982	SEC3 family	Immune response (response to virus)	N/A	N/A
346	ANS1B_HUMAN	Q7Z6G8	Ankyrin repeat and sterile alpha motif domain-containing protein 1B OS=Homo sapiens GN=ANKS1B PE=1 SV=1	*ANKS1B*	138,066	Unknown	Signaling pathway (ephrin receptor)	N/A	N/A
347	PLAK_HUMAN	P14923	Junction plakoglobin OS=Homo sapiens GN=JUP PE=1 SV=3	*JUP*	81,745	Beta-catenin family	Cell interaction and communication (cell adhesion)	N/A	N/A
348	FCN3_HUMAN	O75636	Ficolin-3 OS=Homo sapiens GN=FCN3 PE=1 SV=2	*FCN3*	32,903	Ficolin lectin family	Immune response (response to virus)	N/A	N/A
349	RIMS2_HUMAN	Q9UQ26	Regulating synaptic membrane exocytosis protein 2 OS=Homo sapiens GN=RIMS2 PE=1 SV=2	*RIMS2*	160,403	Unknown	Cell interaction and communication (exocytosis)	N/A	N/A
350	SC16B_HUMAN	Q96JE7	Protein transport protein Sec16B OS=Homo sapiens GN=SEC16B PE=1 SV=2	*SEC16B*	116,604	SEC16 family	Cellular process (autophagy)	N/A	N/A
351	K1239_HUMAN	Q9ULI1	NACHT and WD repeat domain-containing protein 2 (Leucine-rich repeat and WD repeat-containing protein KIAA1239)	*NWD2*	197,466	Unknown	Unknown	N/A	N/A
352	BSN_HUMAN	Q9UPA5	Protein bassoon OS=Homo sapiens GN=BSN PE=1 SV=4	*BSN*	416,469	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A
353	NPHN_HUMAN	O60500	Nephrin OS=Homo sapiens GN=NPHS1 PE=1 SV=1	*NPHS1*	134,742	Immunoglobulin superfamily	Cell interaction and communication (cell adhesion)	N/A	N/A
354	GLYR1_HUMAN	Q49A26	Putative oxidoreductase GLYR1 OS=Homo sapiens GN=GLYR1 PE=1 SV=3	*GLYR1*	60,547	HIBADH-related family, NP60 subfamily	Cellular process (regulation of gene transcription and translation)	N/A	N/A

355	NEMO_HUMAN	Q9Y6K9	NF-kappa-B essential modulator OS=Homo sapiens GN=IKBKG PE=1 SV=2	*IKBKG*	48,198	Unknown	Inflammatory response	N/A	N/A
356	GFPT2_HUMAN	O94808	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 2 OS=Homo sapiens GN=GFPT2 PE=1 SV=3	*GFPT2*	76,931	Unknown	Others (UDP-N-acetylglucosamine metabolic process)	N/A	N/A
357	SRGP1_HUMAN	Q7Z6B7	SLIT-ROBO Rho GTPase-activating protein 1 OS=Homo sapiens GN=SRGAP1 PE=1 SV=1	*SRGAP1*	124,264	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A
358	DACT1_HUMAN	Q9NYF0	Dapper homolog 1 OS=Homo sapiens GN=DACT1 PE=2 SV=2	*DACT1*	90,174	Dapper family	Signaling pathway	N/A	N/A
359	PRAX_HUMAN	Q9BXM0	Periaxin OS=Homo sapiens GN=PRX PE=1 SV=1	*PRX*	154,905	Periaxin family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
360	EMAL6_HUMAN	Q6ZMW3	Echinoderm microtubule-associated protein-like 6 OS=Homo sapiens GN=EML6 PE=2 SV=2	*EML6*	217,899	WD repeat EMAP family	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
361	CLCN3_HUMAN	P51790	H(+)/Cl(-) exchange transporter 3 OS=Homo sapiens GN=CLCN3 PE=1 SV=2	*CLCN3*	90,966	Chloride channel (TC 2.A.49) family, ClC-3/CLCN3 subfamily	Cell interaction and communication	N/A	N/A
362	GP112_HUMAN	Q8IZF6	Adhesion G-protein coupled receptor G4 (G-protein coupled receptor 112)	*ADGRG4*	333,368	G-protein coupled receptor 2 family, Adhesion G-protein coupled receptor (ADGR) subfamily	Signaling pathway (G protein-coupled receptor)	N/A	N/A
363	FHL19_HUMAN	P0C7X4	Putative ferritin heavy polypeptide-like 19 OS=Homo sapiens GN=FTHL19 PE=5 SV=1	*FTH1P19*	22,644	Ferritin family	Ion binding and transport (iron)	N/A	N/A
364	SDCG8_HUMAN	Q86SQ7	Serologically defined colon cancer antigen 8 OS=Homo sapiens GN=SDCCAG8 PE=1 SV=1	*SDCCAG8*	82,682	Unknown	Cell interaction and communication (cell junction)	N/A	N/A
365	MRCKA_HUMAN	Q5VT25	Serine/threonine-protein kinase MRCK alpha OS=Homo sapiens GN=CDC42BPA PE=1 SV=1	*CDC42BPA*	197,307	Protein kinase superfamily, AGC Ser/Thr protein kinase family, DMPK subfamily	Structural constituents (actin cytoskeletal structure)	N/A	N/A
366	BTBD3_HUMAN	Q9Y2F9	BTB/POZ domain-containing protein 3 OS=Homo sapiens GN=BTBD3 PE=2 SV=1	*BTBD3*	58,420	Unknown	Cellular process (organ development)	N/A	N/A
367	SCAR5_HUMAN	Q6ZMJ2	Scavenger receptor class A member 5 OS=Homo sapiens GN=SCARA5 PE=2 SV=1	*SCARA5*	53,994	SCARA5 family	Cell interaction and communication	N/A	N/A

368	DP13A_HUMAN	Q9UKG1	DCC-interacting protein 13-alpha OS=Homo sapiens GN=APPL1 PE=1 SV=1	*APPL1*	79,663	Unknown	Immune response	N/A	N/A
369	ERF3B_HUMAN	Q8IYD1	Eukaryotic peptide chain release factor GTP-binding subunit ERF3B OS=Homo sapiens GN=GSPT2 PE=1 SV=2	*GSPT2*	68,883	TRAFAC class translation factor GTPase superfamily, Classic translation factor GTPase family, ERF3 subfamily	Cellular process (regulation of gene transcription and translation)	N/A	N/A
370	EMIL2_HUMAN	Q9BXX0	EMILIN-2 OS=Homo sapiens GN=EMILIN2 PE=1 SV=2	*EMILIN2*	115,687	Unknown	Cell interaction and communication (cell adhesion)	N/A	N/A
371	K0100_HUMAN	Q14667	Protein KIAA0100 (Antigen MLAA-22) (Breast cancer-overexpressed gene 1 protein)	*KIAA0100*	253,700	SABRE family	Unknown	N/A	N/A
372	CUL9_HUMAN	Q8IWT3	Cullin-9 OS=Homo sapiens GN=CUL9 PE=1 SV=2	*CUL9*	281,229	Cullin family	Protein metabolism (ubiquitin-dependent protein catabolic process)	N/A	N/A
373	KINH_HUMAN	P33176	Kinesin-1 heavy chain OS=Homo sapiens GN=KIF5B PE=1 SV=1	*KIF5B*	109,685	TRAFAC class myosin-kinesin ATPase superfamily, Kinesin family, Kinesin subfamily	Cell interaction and communication	N/A	N/A
374	ZN587_HUMAN	Q96SQ5	Zinc finger protein 587 OS=Homo sapiens GN=ZNF587 PE=2 SV=1	*ZNF587*	65,622	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
375	EVL_HUMAN	Q9UI08	Ena/VASP-like protein OS=Homo sapiens GN=EVL PE=1 SV=2	*EVL*	44,620	Ena/VASP family	Structural constituents (actin cytoskeletal structure)	N/A	N/A
376	PPARD_HUMAN	Q03181	Peroxisome proliferator-activated receptor delta OS=Homo sapiens GN=PPARD PE=1 SV=1	*PPARD*	49,903	Nuclear hormone receptor family, NR1 subfamily	Lipid metabolism	N/A	N/A
377	RUFY3_HUMAN	Q7L099	Protein RUFY3 OS=Homo sapiens GN=RUFY3 PE=1 SV=1	*RUFY3*	52,965	Unknown	Cell interaction and communication	N/A	N/A
378	ATP9B_HUMAN	O43861	Probable phospholipid-transporting ATPase IIB OS=Homo sapiens GN=ATP9B PE=2 SV=4	*ATP9B*	129,304	Cation transport ATPase (P-type) (TC 3.A.3) family, Type IV subfamily	Cell interaction and communication (endocytosis)	N/A	N/A
379	ITCH_HUMAN	Q96J02	E3 ubiquitin-protein ligase Itchy homolog OS=Homo sapiens GN=ITCH PE=1 SV=2	*ITCH*	102,803	Unknown	Cellular process (cell apoptosis)	N/A	N/A
380	TRFL_HUMAN	P02788	Lactotransferrin OS=Homo sapiens GN=LTF PE=1 SV=6	*LTF*	78,182	Transferrin family	Immune response (response to bacteria, fungi)	N/A	N/A
381	PTPA_HUMAN	Q15257	Serine/threonine-protein phosphatase 2A regulatory subunit B~ OS=Homo sapiens GN=PPP2R4 PE=1 SV=3	*PTPA*	40,668	PTPA-type PPIase family	Cellular process (cell apoptosis)	N/A	N/A

382	LOXL2_HUMAN	Q9Y4K0	Lysyl oxidase homolog 2 OS=Homo sapiens GN=LOXL2 PE=1 SV=1	*LOXL2*	86,725	Lysyl oxidase family	Cell interaction and communication (cell adhesion)	N/A	N/A
383	NR2F6_HUMAN	P10588	Nuclear receptor subfamily 2 group F member 6 OS=Homo sapiens GN=NR2F6 PE=1 SV=2	*NR2F6*	42,979	Nuclear hormone receptor family, NR2 subfamily	Cellular process (organ development)	N/A	N/A
384	GRID1_HUMAN	Q9ULK0	Glutamate receptor delta-1 subunit OS=Homo sapiens GN=GRID1 PE=2 SV=2	*GRID1*	112,131	Glutamate-gated ion channel (TC 1.A.10.1) family, GRID1 subfamily	Cell interaction and communication (Cell interaction and communication (synaptic transmission))	N/A	N/A
385	FANCG_HUMAN	O15287	Fanconi anemia group G protein OS=Homo sapiens GN=FANCG PE=1 SV=1	*FANCG*	68,554	Unknown	Others (response to stimuli: DNA damage stimulus)	N/A	N/A
386	PRP31_HUMAN	Q8WWY3	U4/U6 small nuclear ribonucleoprotein Prp31 OS=Homo sapiens GN=PRPF31 PE=1 SV=2	*PRPF31*	55,456	PRP31 family	Cellular process (mRNA splicing)	N/A	N/A
387	CO2A1_HUMAN	Q9NZJ5	Collagen alpha-1(II) chain OS=Homo sapiens GN=COL2A1 PE=1 SV=3	*COL2A1*	141,785	Fibrillar collagen family	Structural constituents (extracellular matrix)	N/A	N/A
388	CSKI1_HUMAN	Q8WXD9	Caskin-1 OS=Homo sapiens GN=CASKIN1 PE=1 SV=1	*CASKIN1*	149,814	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A
389	AF1L2_HUMAN	Q8N4X5	Actin filament-associated protein 1-like 2 OS=Homo sapiens GN=AFAP1L2 PE=1 SV=1	*AFAP1L2*	91,300	Unknown	Inflammatory response	N/A	N/A
390	FAT1_HUMAN	Q14517	Protocadherin Fat 1 (Cadherin family member 7) (Cadherin-related tumor suppressor homolog) (Protein fat homolog) [Cleaved into: Protocadherin Fat 1, nuclear form]	*FAT1*	506,273	Unknown	Cell interaction and communication (cell adhesion)	N/A	N/A
391	SAM9L_HUMAN	Q8IVG5	Sterile alpha motif domain-containing protein 9-like (SAM domain-containing protein 9-like)	*SAMD9L*	184,533	Unknown	Unknown	N/A	N/A
392	ZNF70_HUMAN	Q9UC06	Zinc finger protein 70 OS=Homo sapiens GN=ZNF70 PE=2 SV=2	*ZNF70*	50,802	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
393	GRM1A_HUMAN	Q96CP6	GRAM domain-containing protein 1A OS=Homo sapiens GN=GRAMD1A PE=1 SV=2	*GRAMD1A*	80,680	Unknown	Cellular process (autophagy)	N/A	N/A
394	CD20B_HUMAN	Q86Y33	Cell division cycle protein 20 homolog B OS=Homo sapiens GN=CDC20B PE=2 SV=2	*CDC20B*	57,335	WD repeat CDC20/Fizzy family	Cellular process (cell division)	N/A	N/A

395	QRIC1_HUMAN	Q2TAL8	Glutamine-rich protein 1 OS=Homo sapiens GN=QRICH1 PE=1 SV=1	*QRICH1*	86,436	Unknown	Unknown	N/A	N/A
396	COMP_HUMAN	P11474	Cartilage oligomeric matrix protein OS=Homo sapiens GN=COMP PE=1 SV=2	*COMP*	82,860	Thrombospondin family	Inflammatory response (unfolded protein response)	N/A	N/A
397	RXFP2_HUMAN	Q8WXD0	Relaxin receptor 2 OS=Homo sapiens GN=RXFP2 PE=1 SV=1	*RXFP2*	86,453	G-protein coupled receptor 1 family	Signaling pathway (G protein-coupled receptor)	N/A	N/A
398	CTNL1_HUMAN	Q9UBT7	Alpha-catulin OS=Homo sapiens GN=CTNNAL1 PE=1 SV=2	*CTNNAL1*	81,896	Vinculin/alpha-catenin family	Cell interaction and communication (cell adhesion)	N/A	N/A
399	ECT2L_HUMAN	Q008S8	Epithelial cell-transforming sequence 2 oncogene-like OS=Homo sapiens GN=ECT2L PE=2 SV=2	*ECT2L*	104,880	Unknown	Unknown	N/A	N/A
400	ATP7A_HUMAN	Q04656	Copper-transporting ATPase 1 OS=Homo sapiens GN=ATP7A PE=1 SV=3	*ATP7A*	163,373	Cation transport ATPase (P-type) (TC 3.A.3) family, Type IB subfamily	Others (copper ion metabolism)	N/A	N/A
401	COG5_HUMAN	Q9UP83	Conserved oligomeric Golgi complex subunit 5 OS=Homo sapiens GN=COG5 PE=1 SV=2	*COG5*	92,743	COG5 family	Cell interaction and communication	N/A	N/A
402	SYT1_HUMAN	P21579	Synaptotagmin-1 OS=Homo sapiens GN=SYT1 PE=1 SV=1	*SYT1*	47,573	Synaptotagmin family	Cell interaction and communication	N/A	N/A
403	PABP1_HUMAN	P11940	Polyadenylate-binding protein 1 OS=Homo sapiens GN=PABPC1 PE=1 SV=2	*PABPC1*	70,671	Polyadenylate-binding protein type-1 family	Cellular process (mRNA splicing)	N/A	N/A
404	IQEC1_HUMAN	Q6DN90	IQ motif and SEC7 domain-containing protein 1 OS=Homo sapiens GN=IQSEC1 PE=1 SV=1	*IQSEC1*	108,314	BRAG family	Structural constituents (actin cytoskeletal structure)	N/A	N/A
405	MP2K6_HUMAN	P52564	Dual specificity mitogen-activated protein kinase kinase 6 OS=Homo sapiens GN=MAP2K6 PE=1 SV=1	*MAP2K6*	37,492	Protein kinase superfamily, STE Ser/Thr protein kinase family, MAP kinase kinase subfamily	Cellular process (cell apoptosis)	N/A	N/A
406	HPTR_HUMAN	P00739	Haptoglobin-related protein OS=Homo sapiens GN=HPR PE=1 SV=2	*HPR*	39,030	Peptidase S1 family	Inflammatory response	N/A	N/A
407	POTEF_HUMAN	A5A3E0	POTE ankyrin domain family member F OS=Homo sapiens GN=POTEF PE=1 SV=2	*POTEF*	121,445	POTE family; Actin family	Cellular process (organ development)	N/A	N/A
408	FETUA_HUMAN	P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1	*AHSG*	39,341	Fetuin family	Inflammatory response	N/A	N/A

409	ZDBF2_HUMAN	Q9HCK1	DBF4-type zinc finger-containing protein 2 OS=Homo sapiens GN=ZDBF2 PE=1 SV=3	*ZDBF2*	265,618	Unknown	Ion binding and transport (zinc)	N/A	N/A
410	APOA_HUMAN	P08519	Apolipoprotein(a) OS=Homo sapiens GN=LPA PE=1 SV=1	*LPA*	501,319	Peptidase S1 family, Plasminogen subfamily	Lipid metabolism	N/A	N/A
411	APC2_HUMAN	P02655	Apolipoprotein C-II (Apo-CII) (ApoC-II) (Apolipoprotein C2) [Cleaved into: Proapolipoprotein C-II (ProapoC-II)]	*APOC2*	11,284	Apolipoprotein C2 family	Lipid metabolism	N/A	N/A
412	PK1L2_HUMAN	Q7Z442	Polycystic kidney disease protein 1-like 2 OS=Homo sapiens GN=PKD1L2 PE=1 SV=3	*PKD1L2*	272,514	Polycystin family	Others (response to stimuli: mechanical stimulus)	N/A	N/A
413	YLPM1_HUMAN	P49750	YLP motif-containing protein 1 OS=Homo sapiens GN=YLPM1 PE=1 SV=3	*YLPM1*	241,645	Unknown	Cellular process (regulation of telomere maintenance)	N/A	N/A
414	CHSS1_HUMAN	Q86X52	Chondroitin sulfate synthase 1 OS=Homo sapiens GN=CHSY1 PE=1 SV=3	*CHSY1*	91,784	Chondroitin N-acetylgalactosaminyltransferase family	Cellular process (organ development)	N/A	N/A
415	KDM6B_HUMAN	O15054	Lysine-specific demethylase 6B OS=Homo sapiens GN=KDM6B PE=1 SV=3	*KDM6B*	176,632	UTX family	Cellular process (organ development)	N/A	N/A
416	LTBP1_HUMAN	Q14766	Latent-transforming growth factor beta-binding protein 1 OS=Homo sapiens GN=LTBP1 PE=1 SV=4	*LTBP1*	186,796	LTBP family	Cellular process (organ development)	N/A	N/A
417	UACA_HUMAN	Q9BZF9	Uveal autoantigen with coiled-coil domains and ankyrin repeats OS=Homo sapiens GN=UACA PE=1 SV=2	*UACA*	162,505	Unknown	Cellular process (cell apoptosis)	N/A	N/A
418	WFDC8_HUMAN	Q8IUA0	WAP four-disulfide core domain protein 8 OS=Homo sapiens GN=WFDC8 PE=2 SV=2	*WFDC8*	27,824	Unknown	Others (serine-type endopeptidase inhibitor activity)	N/A	N/A
419	DEN4C_HUMAN	Q5VZ89	DENN domain-containing protein 4C OS=Homo sapiens GN=DENND4C PE=1 SV=2	*DENND4C*	212,711	Unknown	Cell interaction and communication (signal tranduction)	N/A	N/A
420	IWS1_HUMAN	Q96ST2	Protein IWS1 homolog OS=Homo sapiens GN=IWS1 PE=1 SV=2	*IWS1*	91,955	IWS1 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
421	MBTD1_HUMAN	Q05BQ5	MBT domain-containing protein 1 OS=Homo sapiens GN=MBTD1 PE=1 SV=2	*MBTD1*	70,547	Unknown	Cellular process (organ development)	N/A	N/A

422	RGS9_HUMAN	O75916	Regulator of G-protein signaling 9 OS=Homo sapiens GN=RGS9 PE=1 SV=1	*RGS9*	76,966	Unknown	Signaling pathway (G protein-coupled receptor)	N/A	N/A
423	WWP1_HUMAN	Q9H0M0	NEDD4-like E3 ubiquitin-protein ligase WWP1 OS=Homo sapiens GN=WWP1 PE=1 SV=1	*WWP1*	105,202	Unknown	Cell interaction and communication	N/A	N/A
424	UBP32_HUMAN	Q8NFA0	Ubiquitin carboxyl-terminal hydrolase 32 OS=Homo sapiens GN=USP32 PE=1 SV=1	*USP32*	181,656	Peptidase C19 family	Protein metabolism (ubiquitin-dependent protein catabolic process)	N/A	N/A
425	4ET_HUMAN	Q9NRA8	Eukaryotic translation initiation factor 4E transporter OS=Homo sapiens GN=EIF4ENIF1 PE=1 SV=2	*EIF4ENIF1*	108,201	4E-T/EIF4E-T family	Cellular process (mRNA splicing)	N/A	N/A
426	UBR5_HUMAN	O95071	E3 ubiquitin-protein ligase UBR5 OS=Homo sapiens GN=UBR5 PE=1 SV=2	*UBR5*	309,352	Unknown	Signaling pathway	N/A	N/A
427	BCORL_HUMAN	Q5H9F3	BCL-6 corepressor-like protein 1 OS=Homo sapiens GN=BCORL1 PE=1 SV=1	*BCORL1*	182,526	BCOR family	Cellular process (cell division)	N/A	N/A
428	NCKP1_HUMAN	Q9Y2A7	Nck-associated protein 1 OS=Homo sapiens GN=NCKAP1 PE=1 SV=1	*NCKAP1*	128,790	HEM-1/HEM-2 family	Cellular process (organ development)	N/A	N/A
429	DOP1_HUMAN	Q5JWR5	Protein dopey-1 OS=Homo sapiens GN=DOPEY1 PE=1 SV=1	*DOP1A*	277,355	Dopey family	Cell interaction and communication	N/A	N/A
430	NU205_HUMAN	Q92621	Nuclear pore complex protein Nup205 OS=Homo sapiens GN=NUP205 PE=1 SV=2	*NUP205*	227,922	NUP186/NUP192/NUP205 family	Cell interaction and communication	N/A	N/A
431	KI13A_HUMAN	Q9H1H9	Kinesin-like protein KIF13A OS=Homo sapiens GN=KIF13A PE=1 SV=2	*KIF13A*	202,308	TRAFAC class myosin-kinesin ATPase superfamily, Kinesin family	Cellular process (cell division)	N/A	N/A
432	TEN1_HUMAN	Q9UKZ4	Teneurin-1 OS=Homo sapiens GN=ODZ1 PE=1 SV=2	*TENM1*	305,011	Tenascin family, Teneurin subfamily	Cellular process (organ development)	N/A	N/A
433	FIL1L_HUMAN	Q4L180	Filamin A-interacting protein 1-like OS=Homo sapiens GN=FILIP1L PE=1 SV=2	*FILIP1L*	130,382	FILIP1 family	Unknown	N/A	N/A
434	COBA1_HUMAN	P12107	Collagen alpha-1(XI) chain OS=Homo sapiens GN=COL11A1 PE=1 SV=4	*COL11A1*	181,065	Fibrillar collagen family	Structural constituents (extracellular matrix)	N/A	N/A
435	MYT1_HUMAN	Q01538	Myelin transcription factor 1 OS=Homo sapiens GN=MYT1 PE=1 SV=2	*MYT1*	122,329	MYT1 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A

436	KIF7_HUMAN	Q2M1P5	Kinesin-like protein KIF7 OS=Homo sapiens GN=KIF7 PE=1 SV=2	*KIF7*	150,587	TRAFAC class myosin-kinesin ATPase superfamily, Kinesin family, KIF27 subfamily	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
437	SI1L1_HUMAN	O43166	Signal-induced proliferation-associated 1-like protein 1 OS=Homo sapiens GN=SIPA1L1 PE=1 SV=4	*SIPA1L1*	200,029	Unknown	Others (regulation of GTPase activity)	N/A	N/A
438	CG063_HUMAN	A5D8W1	Uncharacterized protein C7orf63 OS=Homo sapiens GN=C7orf63 PE=2 SV=3	*CFAP69*	105,883	Unknown	Others (flagellated sperm motility)	N/A	N/A
439	PKHH2_HUMAN	Q8IVE3	Pleckstrin homology domain-containing family H member 2 OS=Homo sapiens GN=PLEKHH2 PE=2 SV=2	*PLEKHH2*	168,229	Unknown	Unknown	N/A	N/A
440	CCD66_HUMAN	A2RUB6	Coiled-coil domain-containing protein 66 OS=Homo sapiens GN=CCDC66 PE=1 SV=4	*CCDC66*	109,411	Unknown	Cellular process (organ development)	N/A	N/A
441	TOPB1_HUMAN	Q92547	DNA topoisomerase 2-binding protein 1 OS=Homo sapiens GN=TOPBP1 PE=1 SV=2	*TOPBP1*	170,679	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A
442	ATS12_HUMAN	P58397	A disintegrin and metalloproteinase with thrombospondin motifs 12 OS=Homo sapiens GN=ADAMTS12 PE=1 SV=2	*ADAMTS12*	177,676	Unknown	Cell interaction and communication (cell adhesion)	N/A	N/A
443	UCKL1_HUMAN	Q9NWZ5	Uridine-cytidine kinase-like 1 OS=Homo sapiens GN=UCKL1 PE=1 SV=2	*UCKL1*	61,141	Uridine kinase family	Unknown	N/A	N/A
444	FREM3_HUMAN	P0C091	FRAS1-related extracellular matrix protein 3 OS=Homo sapiens GN=FREM3 PE=2 SV=1	*FREM3*	238,179	FRAS1 family	Cell interaction and communication (cell adhesion)	N/A	N/A
445	KGP1_HUMAN	Q13976	cGMP-dependent protein kinase 1 OS=Homo sapiens GN=PRKG1 PE=1 SV=3	*PRKG1*	76,364	Protein kinase superfamily, AGC Ser/Thr protein kinase family, cGMP subfamily	Signaling pathway	N/A	N/A
446	TRI46_HUMAN	Q7Z4K8	Tripartite motif-containing protein 46 OS=Homo sapiens GN=TRIM46 PE=2 SV=2	*TRIM46*	83,424	TRIM/RBCC family	Cell interaction and communication (synaptic transmission)	N/A	N/A
447	ZN318_HUMAN	Q5VUA4	Zinc finger protein 318 OS=Homo sapiens GN=ZNF318 PE=1 SV=2	*ZNF318*	251,112	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
448	INSR_HUMAN	P06213	Insulin receptor OS=Homo sapiens GN=INSR PE=1 SV=3	*INSR*	156,333	Protein kinase superfamily, Tyr protein kinase family, Insulin receptor subfamily	Signaling pathway (MAPK)	N/A	N/A

449	TET2_HUMAN	Q6N021	Probable methylcytosine dioxygenase TET2 OS=Homo sapiens GN=TET2 PE=1 SV=3	*TET2*	223,811	TET family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
450	ARI4A_HUMAN	P29374	AT-rich interactive domain-containing protein 4A OS=Homo sapiens GN=ARID4A PE=1 SV=3	*ARID4A*	142,752	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A
451	PTC2_HUMAN	Q9Y6C5	Protein patched homolog 2 OS=Homo sapiens GN=PTCH2 PE=2 SV=2	*PTCH2*	130,544	Patched family	Cellular process (cell proliferation and differentiation)	N/A	N/A
452	WDR7_HUMAN	Q9Y4E6	WD repeat-containing protein 7 OS=Homo sapiens GN=WDR7 PE=2 SV=2	*WDR7*	163,810	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
453	NOX5_HUMAN	Q96PH1	NADPH oxidase 5 OS=Homo sapiens GN=NOX5 PE=1 SV=1	*NOX5*	86,439	Unknown	Cellular process (cell apoptosis)	N/A	N/A
454	PLPL8_HUMAN	Q9NP80	Calcium-independent phospholipase A2-gamma OS=Homo sapiens GN=PNPLA8 PE=1 SV=1	*PNPLA8*	88,477	Unknown	Lipid metabolism	N/A	N/A
455	TTC28_HUMAN	Q96AY4	Tetratricopeptide repeat protein 28 OS=Homo sapiens GN=TTC28 PE=1 SV=4	*TTC28*	270,884	Unknown	Cellular process (cell division)	N/A	N/A
456	CUL3_HUMAN	Q13618	Cullin-3 OS=Homo sapiens GN=CUL3 PE=1 SV=2	*CUL3*	88,930	Cullin family	Cellular process (cell division)	N/A	N/A
457	CBLB_HUMAN	Q13191	E3 ubiquitin-protein ligase CBL-B OS=Homo sapiens GN=CBLB PE=1 SV=2	*CBLB*	109,450	Unknown	Immune response (cell surface receptor)	N/A	N/A
458	CQ066_HUMAN	A2RTY3	Uncharacterized protein C17orf66 OS=Homo sapiens GN=C17orf66 PE=2 SV=2	*HEATR9*	65,681	Unknown	Cellular process (cell proliferation and differentiation)	N/A	N/A
459	ZZZ3_HUMAN	Q8IYH5	ZZ-type zinc finger-containing protein 3 OS=Homo sapiens GN=ZZZ3 PE=1 SV=1	*ZZZ3*	102,023	Unknown	Protein metabolism (histone H4 acetylation)	N/A	N/A
460	P85B_HUMAN	O00459	Phosphatidylinositol 3-kinase regulatory subunit beta OS=Homo sapiens GN=PIK3R2 PE=1 SV=1	*PIK3R2*	81,545	PI3K p85 subunit family	Glucose metabolism	N/A	N/A
461	F161B_HUMAN	Q96MY7	Protein FAM161B OS=Homo sapiens GN=FAM161B PE=2 SV=2	*FAM161B*	73,647	FAM161 family	Structural constituents (cilia structure)	N/A	N/A
462	ARI5A_HUMAN	Q03989	AT-rich interactive domain-containing protein 5A OS=Homo sapiens GN=ARID5A PE=2 SV=2	*ARID5A*	64,074	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A

463	ANKZ1_HUMAN	Q9H8Y5	Ankyrin repeat and zinc finger domain-containing protein 1 OS=Homo sapiens GN=ANKZF1 PE=1 SV=1	*ANKZF1*	80,927	ANKZF1/VMS1 family	Protein metabolism (ubiquitin-dependent protein catabolic process)	N/A	N/A
464	TXLNG_HUMAN	Q9NUQ3	Gamma-taxilin OS=Homo sapiens GN=TXLNG PE=1 SV=2	*TXLNG*	60,586	Taxilin family	Cellular process (organ development)	N/A	N/A
465	YD026_HUMAN	Q6ZMT9	Putative death domain-containing protein FLJ16686 OS=Homo sapiens PE=2 SV=2	*DTHD1*	88,329	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A
466	US6NL_HUMAN	Q92738	USP6 N-terminal-like protein OS=Homo sapiens GN=USP6NL PE=1 SV=3	*USP6NL*	94,104	Unknown	Cell interaction and communication	N/A	N/A
467	RLF_HUMAN	Q13129	Zinc finger protein Rlf OS=Homo sapiens GN=RLF PE=1 SV=2	*RLF*	217,953	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
468	PTCD3_HUMAN	Q96EY7	Pentatricopeptide repeat-containing protein 3, mitochondrial OS=Homo sapiens GN=PTCD3 PE=1 SV=3	*PTCD3*	78,550	Mitochondrion-specific ribosomal protein mS39 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
469	CB016_HUMAN	Q68DN1	Uncharacterized protein C2orf16 OS=Homo sapiens GN=C2orf16 PE=1 SV=2	*C2orf16*	224,321	Unknown	Unknown	N/A	N/A
470	SHAN3_HUMAN	Q9BYB0	SH3 and multiple ankyrin repeat domains protein 3 OS=Homo sapiens GN=SHANK3 PE=1 SV=2	*SHANK3*	184,667	Unknown	Cellular process (organ development)	N/A	N/A
471	IKKA_HUMAN	O15111	Inhibitor of nuclear factor kappa-B kinase subunit alpha OS=Homo sapiens GN=CHUK PE=1 SV=1	*CHUK*	84,640	Protein kinase superfamily, Ser/Thr protein kinase family, I-kappa-B kinase subfamily	Inflammatory response	N/A	N/A
472	CAMP1_HUMAN	Q5T5Y3	Calmodulin-regulated spectrin-associated protein 1 OS=Homo sapiens GN=CAMSAP1 PE=1 SV=2	*CAMSAP1*	177,972	CAMSAP1 family	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
473	TBCEL_HUMAN	Q5QJ74	Tubulin-specific chaperone cofactor E-like protein OS=Homo sapiens GN=TBCEL PE=2 SV=2	*TBCEL*	48,195	Unknown	Structural constituents (microtubule cytoskeletal structure)	N/A	N/A
474	FRM4A_HUMAN	Q9P2Q2	FERM domain-containing protein 4A OS=Homo sapiens GN=FRMD4A PE=1 SV=3	*FRMD4A*	115,458	Unknown	Protein metabolism (regulation of protein secretion)	N/A	N/A
475	JARD2_HUMAN	Q92833	Protein Jumonji OS=Homo sapiens GN=JARID2 PE=1 SV=2	*JARID2*	138,734	Unknown	Cellular process (organ development)	N/A	N/A

476	SC11B_HUMAN	P0C7V7	Putative signal peptidase complex catalytic subunit SEC11B OS=Homo sapiens GN=SEC11B PE=5 SV=1	*SEC11B*	19,160	Peptidase S26B family	Cell interaction and communication (signal transduction)	N/A	N/A
477	CERK1_HUMAN	Q8TCT0	Ceramide kinase OS=Homo sapiens GN=CERK PE=1 SV=1	*CERK*	59,977	Unknown	Inflammatory response (ceramide/glycosphingolipid metabolic process)	N/A	N/A
478	GP177_HUMAN	Q5T9L3	Integral membrane protein GPR177 OS=Homo sapiens GN=GPR177 PE=2 SV=2	*WLS*	62,253	Wntless family	Cellular process (organ development)	N/A	N/A
479	PB1_HUMAN	Q86U86	Protein polybromo-1 OS=Homo sapiens GN=PBRM1 PE=1 SV=1	*PBRM1*	192,948	Unknown	Cellular process (cell division)	N/A	N/A
480	TNR1A_HUMAN	P19438	Tumor necrosis factor receptor superfamily member 1A OS=Homo sapiens GN=TNFRSF1A PE=1 SV=1	*TNFRSF1A*	50,495	Unknown	Inflammatory response	N/A	N/A
481	GLIS2_HUMAN	Q9BZE0	Zinc finger protein GLIS2 OS=Homo sapiens GN=GLIS2 PE=1 SV=1	*GLIS2*	55,689	GLI C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
482	TRXR3_HUMAN	Q86VQ6	Thioredoxin reductase 3 OS=Homo sapiens GN=TXNRD3 PE=1 SV=2	*TXNRD3*	70,683	Class-I pyridine nucleotide-disulfide oxidoreductase family	Cellular process (cell proliferation and differentiation)	N/A	N/A
483	RCCD1_HUMAN	A6NED2	RCC1 domain-containing protein 1 OS=Homo sapiens GN=RCCD1 PE=1 SV=1	*RCCD1*	40,079	Unknown	Cellular process (cell division)	N/A	N/A
484	E41L5_HUMAN	Q9HCM4	Band 4.1-like protein 5 OS=Homo sapiens GN=EPB41L5 PE=1 SV=3	*EPB41L5*	81,856	Unknown	Structural constituents (actomyosin structure)	N/A	N/A
485	TTC4_HUMAN	O95801	Tetratricopeptide repeat protein 4 OS=Homo sapiens GN=TTC4 PE=1 SV=3	*TTC4*	44,679	TTC4 family	Immune response (response to virus)	N/A	N/A
486	HEAT4_HUMAN	Q86WZ0	HEAT repeat-containing protein 4 OS=Homo sapiens GN=HEATR4 PE=1 SV=1	*HEATR4*	117,175	Unknown	Protein metabolism (peptidyl-lysine modification to peptidyl-hypusine)	N/A	N/A
487	MCM4_HUMAN	P33991	DNA replication licensing factor MCM4 OS=Homo sapiens GN=MCM4 PE=1 SV=5	*MCM4*	96,558	MCM family	Cellular process (cell division)	N/A	N/A
488	ZBT48_HUMAN	P10074	Zinc finger and BTB domain-containing protein 48 OS=Homo sapiens GN=ZBTB48 PE=1 SV=2	*ZBTB48*	77,054	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
489	DD11L_HUMAN	A8MPP1	Putative ATP-dependent RNA helicase DDX11-like protein OS=Homo sapiens PE=1 SV=1	*DDX11L8*	101,811	DEAD box helicase family, DEAH subfamily, DDX11/CHL1 sub-subfamily	Others (nucleobase-containing compound metabolic process)	N/A	N/A

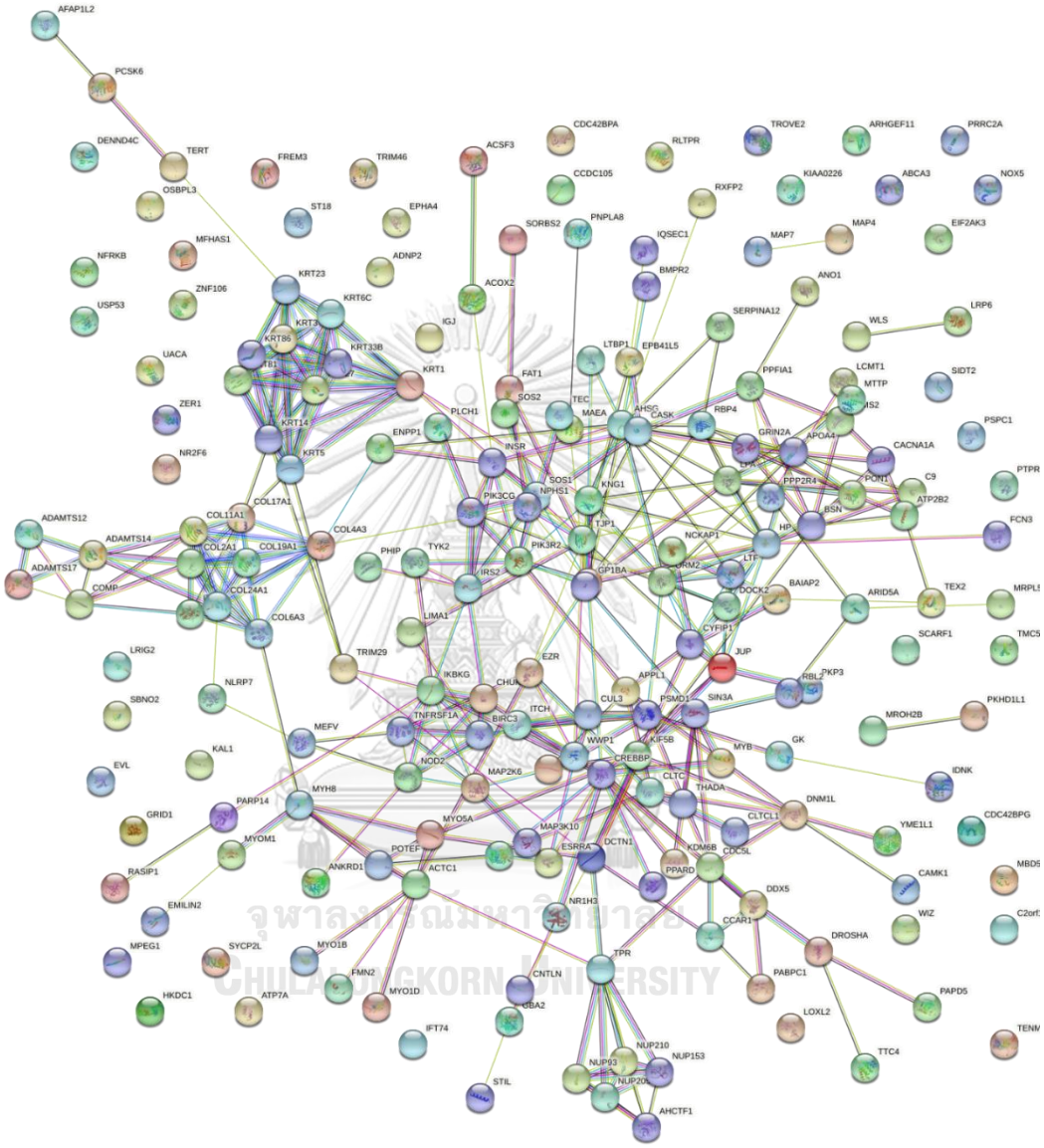
490	SIM1_HUMAN	P81133	Single-minded homolog 1 OS=Homo sapiens GN=SIM1 PE=2 SV=2	*SIM1*	85,515	Unknown	Cellular process (cell division)	N/A	N/A
491	TPR_HUMAN	P12270	Nucleoprotein TPR OS=Homo sapiens GN=TPR PE=1 SV=3	*TPR*	267,293	TPR family	Cellular process (cell division)	N/A	N/A
492	Z585A_HUMAN	Q6P3V2	Zinc finger protein 585A OS=Homo sapiens GN=ZNF585A PE=1 SV=2	*ZNF585A*	87,974	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
493	RBM20_HUMAN	Q5T481	Probable RNA-binding protein 20 OS=Homo sapiens GN=RBM20 PE=1 SV=2	*RBM20*	134,357	Unknown	Cellular process (mRNA splicing)	N/A	N/A
494	DEP1A_HUMAN	Q5TB30	DEP domain-containing protein 1A OS=Homo sapiens GN=DEPDC1A PE=1 SV=1	*DEPDC1*	92,960	Unknown	Cell interaction and communication	N/A	N/A
495	ZN827_HUMAN	Q17R98	Zinc finger protein 827 OS=Homo sapiens GN=ZNF827 PE=2 SV=1	*ZNF827*	119,165	Krueppel C2H2-type zinc-finger protein family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
496	PKP3_HUMAN	Q9Y446	Plakophilin-3 OS=Homo sapiens GN=PKP3 PE=1 SV=1	*PKP3*	87,082	Beta-catenin family	Cell interaction and communication (cell adhesion)	N/A	N/A
497	DUS8_HUMAN	Q13202	Dual specificity protein phosphatase 8 OS=Homo sapiens GN=DUSP8 PE=2 SV=2	*DUSP8*	65,827	Protein-tyrosine phosphatase family, Non-receptor class dual specificity subfamily	Signaling pathway (MAPK)	N/A	N/A
498	MFHA1_HUMAN	Q9Y4C4	Malignant fibrous histiocytoma-amplified sequence 1 OS=Homo sapiens GN=MFHAS1 PE=1 SV=1	*MFHAS1*	116,950	Unknown	Inflammatory response	N/A	N/A
499	CRDL2_HUMAN	Q6WN34	Chordin-like protein 2 OS=Homo sapiens GN=CHRDL2 PE=1 SV=1	*CHRDL2*	47,495	Unknown	Cellular process (organ development)	N/A	N/A
500	WDR81_HUMAN	Q562E7	WD repeat-containing protein 81 OS=Homo sapiens GN=WDR81 PE=2 SV=1	*WDR81*	211,697	WD repeat WDR81 family	Cell interaction and communication	N/A	N/A
501	AT10D_HUMAN	Q9P241	Probable phospholipid-transporting ATPase VD OS=Homo sapiens GN=ATP10D PE=2 SV=2	*ATP10D*	160,274	Cation transport ATPase (P-type) (TC 3.A.3) family, Type IV subfamily	Cell interaction and communication	N/A	N/A
502	GORL_HUMAN	A0PJM3	Putative exonuclease GOR-like protein OS=Homo sapiens GN=REXO1L2P PE=5 SV=2			Unknown	Unknown	N/A	N/A
503	GLE1_HUMAN	Q53GS7	Nucleoporin GLE1 OS=Homo sapiens GN=GLE1 PE=1 SV=2	*GLE1*	79,836	GLE1 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
504	F48B2_HUMAN	P0C7V6	Putative protein FAM48B2 OS=Homo sapiens GN=FAM48B2 PE=5 SV=1	*SUPT20HL2*	87,541	SPT20 family	Cellular process (regulation of gene transcription and translation)	N/A	N/A

505	SNUT1_HUMAN	O43290	U4/U6.U5 tri-snRNP-associated protein 1 OS=Homo sapiens GN=SART1 PE=1 SV=1	*SART1*	90,255	SNU66/SART1 family	Cellular process (mRNA splicing)	N/A	N/A
506	ARAP2_HUMAN	Q8WZ64	Arf-GAP with Rho-GAP domain, ANK repeat and PH domain-containing protein 2 OS=Homo sapiens GN=ARAP2 PE=1 SV=2	*ARAP2*	193,452	Unknown	Cell interaction and communication (signal transduction)	N/A	N/A
507	RINT1_HUMAN	Q6NUQ1	RAD50-interacting protein 1 OS=Homo sapiens GN=RINT1 PE=1 SV=1	*RINT1*	90,632	RINT1 family	Cell interaction and communication (signal transduction)	N/A	N/A
508	IGS22_HUMAN	Q8N9C0	Immunoglobulin superfamily member 22 OS=Homo sapiens GN=IGSF22 PE=2 SV=2	*IGSF22*	100,400	Unknown	Unknown	N/A	N/A
509	PMS2_HUMAN	P54278	Mismatch repair endonuclease PMS2 OS=Homo sapiens GN=PMS2 PE=1 SV=1	*PMS2*	95,797	DNA mismatch repair MutL/HexB family	Others (response to stimuli: drug)	N/A	N/A
510	MILK2_HUMAN	Q8IY33	MICAL-like protein 2 OS=Homo sapiens GN=MICALL2 PE=1 SV=1	*MICALL2*	97,502	Unknown	Cell interaction and communication (cell junction)	N/A	N/A
511	BUB3_HUMAN	O43684	Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=1 SV=1	*BUB3*	37,155	WD repeat BUB3 family	Others (nucleobase-containing compound metabolic process)	N/A	N/A
512	NSMA3_HUMAN	Q9NXE4	Sphingomyelin phosphodiesterase 4 OS=Homo sapiens GN=SMPD4 PE=1 SV=2	*SMPD4*	97,810	Unknown	Inflammatory response (ceramide/glycosphingolipid metabolic process)	N/A	N/A
513	TEX2_HUMAN	Q8IWB9	Testis-expressed sequence 2 protein OS=Homo sapiens GN=TEX2 PE=1 SV=2	*TEX2*	125,303	Unknown	Inflammatory response (ceramide/glycosphingolipid metabolic process)	N/A	N/A
514	HKDC1_HUMAN	Q2TB90	Putative hexokinase HKDC1 OS=Homo sapiens GN=HKDC1 PE=2 SV=2	*HKDC1*	102,545	Hexokinase family	Glucose metabolism	N/A	N/A
515	MCM8_HUMAN	Q9UJA3	DNA replication licensing factor MCM8 OS=Homo sapiens GN=MCM8 PE=1 SV=2	*MCM8*	93,697	MCM family	Cellular process (cell division)	N/A	N/A
516	TTBK1_HUMAN	Q5TCY1	Tau-tubulin kinase 1 OS=Homo sapiens GN=TTBK1 PE=1 SV=2	*TTBK1*	142,737	Protein kinase superfamily, CK1 Ser/Thr protein kinase family	Cellular process (regulation of gene transcription and translation)	N/A	N/A
517	KBTB7_HUMAN	Q8WVZ9	Kelch repeat and BTB domain-containing protein 7 OS=Homo sapiens GN=KBTBD7 PE=2 SV=1	*KBTBD7*	77,163	Unknown	Cellular process (regulation of gene transcription and translation)	N/A	N/A

518	GBA2_HUMAN	Q9HCG7	Non-lysosomal glucosylceramidase OS=Homo sapiens GN=GBA2 PE=1 SV=2	*GBA2*	104,649	Non-lysosomal glucosylceramidase family	Lipid metabolism	N/A	N/A
519	DGKA_HUMAN	P23743	Diacylglycerol kinase alpha OS=Homo sapiens GN=DGKA PE=1 SV=3	*DGKA*	82,630	Eukaryotic diacylglycerol kinase family	Inflammatory response (diacylglycerol metabolic process)	N/A	N/A
520	WDR67_HUMAN	Q96DN5	WD repeat-containing protein 67 OS=Homo sapiens GN=WDR67 PE=2 SV=2	*TBC1D31*	124,189	Unknown	Unknown	N/A	N/A
521	CA167_HUMAN	Q5SNV9	Uncharacterized protein C1orf167 OS=Homo sapiens GN=C1orf167 PE=2 SV=2	*C1orf167*	162,423	Unknown	Unknown	N/A	N/A
522	REXO1_HUMAN	Q8N1G1	RNA exonuclease 1 homolog OS=Homo sapiens GN=REXO1 PE=1 SV=2	*REXO1*	131,510	REXO1/REXO3 family	Unknown	N/A	N/A
523	GP125_HUMAN	Q8IWK6	Adhesion G protein-coupled receptor A3 (G-protein coupled receptor 125)	*ADGRA3*	146,151	G-protein coupled receptor 2 family, Adhesion G-protein coupled receptor (ADGR) subfamily	Signaling pathway (G protein-coupled receptor)	N/A	N/A

Appendix I

Protein-protein interaction networks (Study III)



The protein-protein interaction networks of 212 proteins were investigated using the STRING database limited to *Homo sapiens* (https://string-db.org/cgi/network?taskId=btEmDwQaxSlQ&sessionId=blMUWdLekXNg). The interacting proteins are represented as nodes and their biological relationships as line.

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PUBLICATION	Thaipitakwong T. and Aramwit P. A review of the efficacy, safety, and clinical implications of naturally derived dietary supplements for dyslipidemia. Am J Cardiovasc Drugs. 2017;17(1):27-35. Thaipitakwong T, Numhom S, Aramwit P. Mulberry leaves and their potential effects against cardiometabolic risks: a review of chemical compositions, biological properties and clinical efficacy. Pharm Biol. 2018;56(1):109-118. Thaipitakwong T, Supasyndh O, Rasmi Y, Aramwit P. A randomized controlled study of dose-finding, efficacy, and safety of mulberry leaves on glycemic profiles in obese persons with borderline diabetes. Complement Ther Med. 2020;49:102292. Thaipitakwong T, Hussain S, Filipek PA, Leitner PD, Valovka T, Jakschitz T, Gstir R, Bonn G, Aramwit P. Mulberry (Morus alba L.) leaf extracts: chemical compositions and antioxidative properties regarding drying processes. Nat Prod Res. (In review).
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