Green Tea Polyphenols: a Natural Therapeutic Approach for Metabolic Syndrome and Diabetes Prevention

A thesis submitted for the Degree of Doctor of Philosophy

by

Jane Jung Yeon Kim

School of Medical and Molecular Biosciences

Faculty of Science

University of Technology Sydney, Australia

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Declaration

This thesis titled “Green Tea Polyphenols: a Natural Therapeutic Approach for Metabolic Syndrome and Diabetes Prevention” is of original work. The work presented in this thesis was carried out under the supervision of A/Prof Xianqin Qu and A/Prof Chris Zaslawski at University of Technology, Sydney. This thesis is of original work and has not been submitted by the candidate for the award of any other degree.

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Publications

Publications in Peer Reviewed Journals

Kim, J.J.Y., Tan, Y., Xiao, L., Sun, Y. & Qu, X. (2013). Green Tea Polyphenol Epigallocatechin-3-Gallate Enhance Glycogen Synthesis and Inhibit Lipogenesis in Hepatocytes. *Biomed Res Int,* 2013,920128.

Lao, W.G., **Kim, J.J.Y.,** Tan, Y., Jin, X., Xiao, L. & Qu, X (2014). Green Tea Polyphenols Inhibit Adipogenesis in 3T3-L1 Preadipocytes Through Down-regulation of SREBP-1c Expression and C/EBPα -PPARγ Pathway. *J. Nutr. Biochem.* (submitted and under review).

Kim, J.J.Y., Lao, W.G., Tan, Y., Xiao, L. & Qu, X. (2014). Green Tea Polyphenols Improves Insulin Resistance in High Fat Fed Obese Zucker Rats (manuscript in preparation).

Kim, J.J.Y., Lao, W.G., Tan, Y., Xiao, L. & Qu, X. (2014). Effects of Green Tea Polyphenols on Non-alcoholic Fatty Liver Disease Induced by High-fat Diet in Zucker Fatty Rats (manuscript in preparation).

Papers and Posters Presented at Scientific Conferences

Kim, J.J.Y., Xiao, L., Tan, Y. & Qu, X. (2010). An Investigation into the Effects of Green Tea Polyphenols on Glucose Metabolism in 3T3-L1 Cells. *RNSH/UTS Scientific Research Meeting, Sydney* (Poster).

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Table of Contents

List of Figures

List of Tables

Abbreviations

2DOG	2-deoxyglucose
ACC	acetyl-CoA carboxylase
AGI	Alpha-glucosidase inhibitors
AMPK	AMP-activated protein kinase
ANOVA	one-way analysis of variance
ALT	alanine transaminase
AST	aspartate transaminase
ATCC	American Type Culture Collection
ATPIII	Adult Treatment Panel III
BBR	berberine
BMI	Body mass index
BSA	bovine serum albumin
BW	body weight
cAMP	Cyclic adenosine monophosphate
CEBPα	CCAAT-enhancer-binding protein-α
CHM	Chinese herbal medicine
ChREBP	carbohydrate-responsive element-binding protein
CM	complete media
CREB	cAMP response element-binding protein
CRP	C-reactive protein

CVD	cardiovascular disease
DAG	diacylglycerol
DBP	diastolic blood pressure
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulfoxide
EC	epicatechin
ECG	epicatechin gallate
EGC	epigallocatechin
EGCG	epigallocatechin gallate
EGIR	European Group for the Study of Insulin Resistance
fatty acyl-CoA	fatty acyl-coenzyme A
FBS	fetal bovine serum
FFA	free fatty acids
FAS	fatty acid synthase
G-6-Pase	glucose-6-phosphatase
GDH	glutamate dehydrogenase
GLUT1	glucose transporter 1
GLUT2	glucose transporter 2
GLUT3	glucose transporter 3
GLUT4	glucose transporter 4
GOD-PAP	glucose oxidase-peroxidase
GS	glycogen synthesis

GSK3β	glycogen synthase kinase 3β
GTP	green tea polyphenols
HOMA-IR	homeostasis model assessment of insulin resistance
HDL	high-density lipoprotein
HE	haematoxylin and eosin
HFD	high-fat diet
FPG	fasting plasma glucose
HSL	hormone-sensitive lipase
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IFN	interferon
IGT	impaired glucose tolerance
IL	interleukin
IRS	insulin receptor substrate
ISDN	isosorbide dinitrate
LC-MS	liquid chromatography-mass spectrometry
LDL	low-density lipoprotein
LKB1	liver kinase B1
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemotactic protein-1
MetS	metabolic syndrome
MI	myocardial infarction

MLC	myosin light chain
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NAD	nicotinamide adenine dinucleotide
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NCEP	National Cholesterol Education Program
NEFA	non-esterified free fatty acids
NIDDM	non-insulin dependent diabetes
NO	nitric oxide
OGTT	oral glucose tolerance test
ORO	oil red O
PAI-1	plasminogen activator inhibitor-1
PCOS	polycystic ovarian syndrome
PDE3b	phosphodiesterase 3b
PDH	pyruvate dehydrogenase
PDK1	PI-dependent kinase 1
PEPCK	Phosphoenolpyruvate carboxykinase
PI3-K	phosphoinositide 3-kinase
PKA	protein kinase A
PKB/Akt	protein kinase B
PKC	protein kinase C
PPARγ	peroxisome proliferator activated receptor-γ

PVD	peripheral vascular disease
PVDF	polyvinyldenedifluoride
RBP4	retinol binding protein-4
SBP	systolic blood pressure
SM	starving media
SREBP-1c	sterol regulatory element binding protein-1c
STZ	streptozotocin
T2D	type 2 diabetes
TAG	triacylglycerol
TG	triglycerides
TNF-α	tumour necrosis factor-α
TZD	thiazolidinedione
VLDL-C	very low-density lipoprotein-cholesterol
WHO	World Health Organization
WTH	Waist-to-Hip
ZF	Zucker fatty (fa/fa)

Abstract

Metabolic syndrome (MetS) is a collection of interrelated disorders that increase the risk of type 2 diabetes (T2D) and cardiovascular disease. Abnormalities associated with MetS include, but are not limited to, central obesity, insulin resistance, glucose intolerance, hyperglycaemia, hyperlipidaemia and hypertension. MetS has caused an encumbrance to public health globally. Due to the complex nature of MetS and the lack of availability of effective medications, there is an urgent need for the implementation of novel oral agents to manage Mets and prevent T2D and cardiovascular complications.

The beneficial effects of green tea polyphenols (GTP) for MetS have been recently reported. However, the direct effects and mechanisms of GTP on abnormalities of glucose and lipid metabolism are not fully understood. This thesis investigated effects and mechanisms of GTP in obesity, insulin resistant and metabolic dysfunctions through *in vitro* and *in vivo* studies. The effects of GTP on biochemical parameters and its actions in major organs involved in glucose homeostasis, fat metabolism and insulin sensitivity such as skeletal muscle, liver and adipose tissue were elucidated. The study was further extended to investigate the effect of GTP on a model of non-alcoholic fatty liver disease (NAFLD).

In 3T3-L1 adipocytes, GTP-EGCG (epigallocatechin gallate) improved glucose uptake and inhibited lipolysis significantly. It was revealed that GTP-EGCG significantly increased glucose uptake by up-regulating expressions of IRS-1, PKB/Akt and GLUT4. The probable mechanism for decreased lipolysis with GTP-EGCG may be through down-regulation of PKA, as observed in this thesis. GTP-EGCG also enhanced glycogen synthesis in a dose-dependent manner and significantly increased glycogen synthesis two-fold compared with insulin alone in HepG2 cells. Western blotting revealed that phosphorylation of GSK3β and

GS was significantly increased in GTP-EGCG treated HepG2 cells compared to non-treated cells. GTP-EGCG also significantly inhibited lipogenesis and the likely mechanism behind this inhibition involved enhanced expression of phosphorylated AMPKα and ACC in HepG2 cells.

A marked state of insulin resistance was observed in high fat diet (HFD) fed obese Zuker fatty (ZF) rats compared to their lean littermates, which is associated with significant defects in the insulin-signalling/glucose transport system. GTP significantly improved fasting metabolic parameters, including glucose, insulin, TG, NEFA and cholesterol, and improved insulin sensitivity and glucose intolerance in HFD ZF rats. The likely molecular mechanisms involved are regulation of the elements of the insulin-signalling pathway such as PKB/Akt, GLUT4 translocation and regulation of PKC translocation in skeletal muscle of HFD ZF rats. Data from this thesis also strongly supports that GTP reduces fat accumulation in the liver and hepatic insulin resistance. This is evidenced by significant reduction in serum levels of ALT and AST hepatic TG and lipid content in HFD ZF rats administered GTP. Mechanisms are likely to up regulate GSK3β and GS expressions thereby enhancing glycogen synthesis and down-regulating *de novo* lipogenesis through regulation of AMPKα, ACC and PEPCK expressions.

Overall, the results presented in this thesis provide insight into GTP as a potential therapeutic agent for MetS and related disorders such as T2D, obesity and NAFLD. GTP may be a valuable natural and cost-effective therapy for the treatment and prevention of metabolic disorders.