A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED HUMAN CLINICAL TRIAL ON THE EFFECTS OF SACHA INCHI (*PLUKENETIA VOLUBILIS L.*) OIL SUPPLEMENTATION IN HYPERGLYCAEMIA, HYPERTENSION AND HYPERLIPIDAEMIA PATIENTS

NUR ANIS RAIHANA BINTI MHD RODZI

UNIVERSITI SAINS MALAYSIA

2024

A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED HUMAN CLINICAL TRIAL ON THE EFFECTS OF SACHA INCHI (*PLUKENETIA VOLUBILIS L.*) OIL SUPPLEMENTATION IN HYPERGLYCAEMIA, HYPERTENSION AND HYPERLIPIDAEMIA PATIENTS

by

NUR ANIS RAIHANA BINTI MHD RODZI

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2024

ACKNOWLEDGEMENT

First and foremost, I thank Almighty Allah for His blessings and opportunities, guidance and patience that was given to me during the whole study duration and thesis writing process. A very special gratitude goes to my beloved research supervisor, Dr. Lee Lai Kuan, from the School of Industrial Technology, Universiti Sains Malaysia, for her endless research supervision, encouragement, and advice. I also would like to thank my co-supervisor, Dr. Mastura Sopian from Bertam Medical Centre, Universiti Sains Malaysia, who have offered continuous reassurances. They have offered me continuous support, knowledge, and valuable experiences throughout my field work and during writing process. Furthermore, I would like to thank the staffs of Specialist Day Clinic from Bertam Medical Centre and the School of Industrial Technology for their assistance in conducting the research. To my dearest parents, Mr. Mhd Rodzi bin Abd Hamid and Mrs Arishah Binti Ahmad, thank you for your beliefs, inspirations and encouragements. I still remembered how both of you forgone your dreams of furthering your education pathways, only to raise us, when we were children. To my husband, Amir Aiman, thank you for the constant drive and motivation that pushes my limit to complete the study. Lastly, I would like to thank my cats, Zaiton and Oyster, whose quiet comforting presence throughout the challenging phases of my life that occurred during my PhD journey, especially when I was feeling unmotivated, isolated, and alone, a little bit easier. Thank you to everyone, from the bottom of my heart.

Nur Anis Raihana binti Mhd Rodzi, June 2024.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

°C	Celsius
%	Percentage
µg/mL	Microgram per milliliter
µL	Micro liter
µM	Micro meter
µmol	Micromole
cm	Centimeter
EAA/g	Ascorbic acid equivalent per gram
g	Gram
g/dL	Gram per deciliter
g/kg	Gram per kilogram
g/L	Gram per liter
kcal	Kilocalories
kg	Kilogram
kg/m^2	Kilograms per meter square
mcg	Microgram
mg	Milligram
mg GAE	Milligrams of gallic acid equivalent
mg/100g	Milligram per 100 grams
mg/L	Milligram per liter
min	Minute
microIU/mL	Micro International Units per milliliter
ml	Milliliter

mm	Millimetre
mmol	Millimole
mmol/L	Millimole per liter
mmHg	Milliliters of Mercury
mg/dL	Milligrams per deciliter
mU/L	Milliunits per liter
ng/mL	Nanograms per milliliter
nmol/L	Nanomole per liter
pg/mL	Picograms per milliliter
pmol/mL	Picomole per milliliter
rpm	Revolutions per minute
TE/g	Trolox equivalent per gram
U/L	Unit per liter
U/ml	Unit per milliliter

LIST OF ABBREVIATIONS

3Hs	Hyperglycaemia, Hypertension, Hyperlipidaemia
8-iso-PGF2α	8-iso-prostaglandin F2 alpha
8-OHdG	8-hydroxy-2'-deoxyguanosine
AA	Arachidonic acid
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
ACC/AHA	Americal College of Cardiology and American Heart Association
ADA	American Diabetes Association
ALA	Alpha linolenic acid
AMDI	Advanced medical and dental institute
AMPK	5' AMP-activated protein kinase
AOX	antioxidant activity
ApoB100	Apolipoprotein B100
ApoC-II	Apolipoprotein CII
ApoC-III	Apolipoprotein C-III
ApoE	apolipoprotein E
BIA	Biometric impedance analysis
BMI	Body mass index
BMR	Basal metabolism
CAD	Coronary artery disease
CC	Calf circumference
CDC	Centres for Disease Control and Prevention
CE	Cholesteryl ester
CETP	Cholesteryl ester transfer protein
COX	Cyclooxygenase

CRP	C-reactive protein
CVDs	Cardiovascular diseases
DASS-21	Depression, Anxiety and Stress Scale
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPP-4	Dipeptidyl Peptidase-4
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
EPIC	European Prospective Investigation into Cancer and Nutrition Study
ESH/ESC	European Society of Hypertension-European Society of Cardiology
FA	Fatty acids
FBG	Fasting blood glucose
FFAs	Free fatty acids
FFQ	Food frequency questionnaire
FSI	Fasting serum insulin
GLP-1	Glucagon-like Peptide-1
GLUT4	Glucose transporter type 4
HbA1c	Glycated haemoglobin
HC	Hip circumference
HDL-C	High density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance

HPT	Hypertension
HRP	Avidin-horseradish peroxidase
HRQoL	Health-related quality of life
HSL	Hormone-sensitive lipase
IAS	International Atherosclerosis Society
IC	Inhibitory concentration
IDF	International Diabetes Foundation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-1β	Interleukin 1-beta
IL-6	Interleukin-6
LA	Linoleic acid
LDL-C	Low density lipoprotein cholesterol
LMICs	Low and lower-income countries
LPL	Lipoprotein lipase
MDG	Malaysian dietary guideline
MTP	Microsomal transfer protein
MUAC	Mid-upper arm circumference
MUFAs	Monounsaturated fatty acids
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NCDs	Non-communicable diseases
NHANES	National Health and Nutrition Examination Surveys
NHMS	National Health Morbidity Survey
NO	Nitric oxide
NOS	Nitric oxide synthases

NR	Not reported
OA	Oleic acid
OGTT	Oral glucose tolerance test
oxLDL	Oxidised low-density lipoprotein
PCSK9	Proprotein Convertase Subtilisin/Kexin Type 9
PEPCK	Phosphoenolpyruvate carboxykinase
PPARs	Peroxisome proliferator-activated receptors
PPUSMB	Pusat Perubatan USM Bertam
PUFAs	Polyunsaturated fatty acids
RCTs	Randomised controlled trials
ROO-	Peroxyl radical
ROS	Reactive oxygen species
SAA	Serum amyloid A
SBP	Systolic blood pressure
SDGs	Sustainable Development Goals
sdLDL	Small-dense low-density lipoprotein
SFAs	Saturated fatty acids
SGLT2	Sodium-glucose transporter 2
SI	Sacha Inchi
SIO	Sacha Inchi oil
SIRT1	Sirtuin-1
SOD	Superoxide dismutase activity
SOST	Sclerostin
SST	Serum separating tubes
sST2	Soluble serum ST2

T2DM	Type 2 Diabetes Mellitus
TAC	Total antioxidant capacity
TC	Total cholesterol
TG	Triglyceride
TNF-α	Tumor necrosis factor-α
UFAs	Unsaturated fatty acids
UN	United Nations
USM	Universiti Sains Malaysia
VAS	Visual analogue scale
VLDL	Very low-density lipoprotein
WC	Waist circumference
WHF	World Heart Federation
WHO	World Health Organization
WHR	Waist hip ratio

LIST OF APPENDICES

KAJIAN KLINIKAL MANUSIA SECARA RAWAK, RABUN DUA PIHAK DAN DIKAWAL PLASEBO TERHADAP KESAN PENGAMBILAN SUPLEMEN MINYAK SACHA INCHI (*PLUKENETIA VOLUBILIS L.*) DALAM KALANGAN PESAKIT HIPERGLISEMIA, HIPERTENSI DAN HIPERLIPIDEMIA

ABSTRAK

Insiden penyakit hiperglisemia, hipertensi dan hiperlipidemia (3H) banyak berlaku dalam banyak negara maju dan membangun. Minyak Sacha Inchi (SIO) merupakan minyak berasaskan tumbuhan yang kaya dengan asid lemak tak tepu omega-3, 6, dan 9 dengan kesan kesihatan yang menjanjikan. Walaubagaimanapun, kajian keberkesanan SIO dalam kalangan pesakit 3Hs sebagai pemakanan kesihatan tambahan kurang dikaji secara terperinci. Kajian klinikal manusia ini meneroka keberkesanan suplemen SIO terhadap (1) kawalan glisemik; (2) tekanan darah; (3) profil lipid; (4) status radang; (5) status oksidatif; (6) penanda kalsifikasi arteri; (7) status antropometri; (8) kualiti hidup; (9) status kesihatan mental; dan (10) kepatuhan dan toleransi dalam kalangan pesakit 3Hs. Lima puluh empat (n=54) pesakit 3Hs direkrut dari Klinik Pakar Rawatan Harian, Pusat Perubatan USM Bertam, dan diagihkan secara rawak kepada dua kumpulan: iaitu kumpulan SIO (n=27) (menerima 1000 mg SIO setiap hari) atau kumpulan plasebo (n=27) (menerima 1000mg minyak jagung) selama 12 minggu. Penilaian klinikal didapati meningkat secara signifikan dalam kumpulan SIO; yang terbukti dengan pengurangan tekanan darah sistolik ($p<0.05$) dan diastolik ($p<0.05$), TC ($p<0.05$) dan LDL-C ($p<0.05$). Selain itu, kumpulan suplimen SIO juga menunjukkan penurunan yang signifikan dalam kepekatan TNF-α ($p<0.05$), IL-6 ($p<0.05$), CRP ($p<0.01$), SAA ($p<0.01$) dan plasma

adiponektin ($p<0.05$). Seterusnya, hasil yang baik juga dapat dikesan dengan pengurangan paras 8-OHdG ($p<0.01$) dan 8-iso-PGF2α ($p<0.0001$, sementara meningkatkan tahap SOD ($p<0.01$) dan GSH ($p<0.05$) dalam kumpulan suplimen SIO. Kesan yang ketara juga diperhatikan pada kesihatan arteri dengan pengurangan SOST ($p<0.01$) bagi kumpulan SIO. Kumpulan suplimen SIO juga menunjukkan status antropometri yang signifikan untuk FMI ($p<0.01$) dan FFMI ($p<0.05$), serta skor indeks kualiti hidup yang lebih baik untuk domain kesakitan dan ketidakselesaan ($p<0.0001$). Kepatuhan terhadap suplimen adalah sangat baik (97%) dengan aduan ketidakselesaan usus yang minimum. Tiada perubahan yang signifikan diperhatikan untuk kawalan glikemik dan status kesihatan mental. Kesimpulannya, kajian ini membuktikan manfaat suplimen SIO yang pelbagai dalam kalangan pesakit 3Hs sebagai intervensi makanan tambahan yang bernilai dalam pengurusan penyakit.

A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED HUMAN CLINICAL TRIAL ON THE EFFECTS OF SACHA INCHI (*PLUKENETIA VOLUBILIS L.*) OIL SUPPLEMENTATION IN HYPERGLYCAEMIA, HYPERTENSION AND HYPERLIPIDAEMIA PATIENTS

ABSTRACT

The incidence of hyperglycaemia, hypertension and hyperlipidaemia (3Hs) have been prevalent in many developed and developing countries. Sacha Inchi oil (SIO) is a rich source of plant-based omega-3, 6, and 9 polyunsaturated fatty acids with promising health effects. However, the effects of SIO as complementary medicine among the 3Hs individuals are narrowly investigated. The present randomised, double-blind, placebo-controlled human clinical trial aimed to explore the effects of SIO supplementation on (1) glycaemic control; (2) BPs; (3) lipid profiles; (4) inflammatory status; (5) oxidative stress; (6) circulating arterial calcification biomarkers; (7) anthropometry measurements; (8) quality of life; (9) mental health status; and (10) compliancy and tolerability among 3Hs patients. Fifty-four (n=54) 3Hs patients were recruited from Specialist Day Care Clinic, Pusat Perubatan USM Bertam, and randomised into two groups: the SIO group (n=27) (received 1000mg of SIO soft gel per day) or placebo group (n=27) (received 1000mg corn oil soft gel for 12 weeks). Fasting blood samples were collected at baseline and post week-12 to assess clinical and laboratory biochemical markers. Clinical assessments were improved significantly in the SIO supplementation group; as evident by reduction in systolic ($p<0.05$) and diastolic BP ($p<0.05$), TC ($p<0.05$) and LDL-C ($p<0.05$). In addition, SIO supplementation group showed substantive improvement in TNF-α ($p<0.05$), IL-6 ($p<0.05$), CRP ($p<0.01$), SAA ($p<0.01$) and plasma adiponectin ($p<0.05$)

concentrations. Furthermore, favourable outcomes were observed with reduced 8-OHdG ($p<0.01$) and 8-iso-PGF2α ($p<0.0001$), while elevated SOD ($p<0.01$) and GSH ($p<0.05$) levels in the SIO supplementation group. A noteworthy effect was also observed on arterial health, as shown by reduced SOST ($p<0.01$) in the SIO group. The supplementation group also showed significant anthropometry measurements for FMI ($p<0.01$) and FFMI ($p<0.05$), and better quality of life index score for pain and discomfort domain ($p<0.0001$). The compliance towards the supplement was excellent (97%) with and tolerable minimal gastrointestinal complaints. No significant changes were observed for glycaemic control and mental health status. These results suggest that SIO may exert modulatory effects upon 3Hs patients by improving BPs, lipid profiles, inflammatory and oxidative stress status, and circulating serum arterial calcification. Conclusively, this clinical trial may shed light on the multifaceted SIO supplementation in 3Hs patients as a valuable complementary intervention in 3Hs disease management.

CHAPTER 1

INTRODUCTION

1.1 Background

Hyperglycaemia, hypertension, and hyperlipidaemia (3Hs) are among the most serious public health issues worldwide, posing significant burdens on both public health and socioeconomic development. This cluster of health threat is the most commonly diagnosed non-communicable disease (NCDs). In 2017, NCDs were responsible for 73.4% of the deaths worldwide (Global Burden of Diseases, 2017). The number of deaths from NCDs continues to increase annually, most of which can be attributed to cardiovascular diseases (CVDs) (Global Burden of Diseases 2017). The Centres for Disease Control and Prevention (CDC) stated that at least 523 million individuals were affected by CVD in 2019 (World Heart Federation, 2022). It has claimed over 18 million lives each year, approximately 85% are due to atherosclerotic CVD; the main complication caused by the diagnosis of 3Hs, the three major modifiable risk factors (World Heart Federation, 2022; Sehestedt et al., 2011; Bloomgarden, 2007).

A few key initiatives and strategies to control and mitigate the impact of NCDs including the Global Action Plan for the Prevention and Control of NCDS by the World Health Organization (WHO) (2013). This comprehensive Global Action Plan, which targets and recommends policy interventions to reduce the burden of NCDs, emphasises healthy lifestyles, improves early detection and treatment, and strengthens health systems. Public health initiative as a concerted effort to diminish the prevalence of NCDs is also reflected in the Sustainable Development Goals (SDGs) Report

(United Nations Publications, 2020). This has spurred global action to address NCDs through various initiatives and partnerships, especially post-COVID-19 pandemic. Although the scale and nature of initiatives vary across countries, the overall goal remains the same: to reduce the burden of NCDs and promote healthier populations.

At present, the management of 3Hs is based on a series of recommendations from various international guidelines, such as the American Diabetes Association (ADA), American College of Cardiology and American Heart Association (ACC/AHA), International Atherosclerosis Society (IAS), and European Society of Hypertension-European Society of Cardiology (ESH/ESC). These international guidelines are focusing on asymptomatic adults, including those who are at risk of CVD events, that the well-planned treatment of 3Hs involves a myriad of medications depending on the degree of the disease and recommendation for lifestyle changes (Escherick et al., 2020). To complement pharmacotherapy, intensive approaches have been outlined to investigate the potential of nutritional agents to manage 3Hs.

Studies on the effects of functional and nutritional ingredients, such as modulation of glycaemic indices, improvement of antioxidant capacity, lipid profile, inflammation, and ultimately enhanced quality of life have been observed in patients with NCDs (Télessy et al., 2023; Maggioni et al., 2022; Herrera-Vielma et al., 2021). For decades, omega-3 polyunsaturated fatty acids (Ω-3 PUFAs), from either marine or fish oil supplementation were broadly referred into the cardiology guidelines (Skulas-Ray et al., 2019; Weylandt et al., 2015; Kris-Etherton et al., 2002). Of late, the use of plant-based PUFAs are gaining recognition and attention among researchers. Typical plant-based PUFAs, particularly the omega-3 fatty acids (Ω-3) and omega-6 (Ω-6), are often found to be beneficial to cardiometabolic health by improving blood pressure

(BP), glucose homeostasis, and lipid profiles (Billingsley et al., 2018; Wang & Hu, 2017).

Preliminary literature has identified a wide range of potentially beneficial physiological outcomes of various plant-based PUFAs, including antioxidant, anti-inflammatory, antidyslipidemic, hypoglycaemic properties, and dermatological properties (Baker et al., 2016). In addition, accumulating evidence suggests that increased plant-based PUFAs might confer benefits to individuals who have been diagnosed with any combination of 3Hs to reduce the risk of disease progression (Wanders et al., 2019; Kurotani et al., 2013). Few studies investigating the effect of plant-based PUFAs found that the compound were effective in regulating inflammatory cytokines, which directly involved in the 3Hs complex dynamic (Tortosa-Caparrós et al., 2017). Current evidence also indicates that dietary plant-based PUFAs are associated with a modestly lower risk of total CVD, hence highly benefit the 3Hs patient pool (Wang & Hu, 2017).

Sacha Inchi oil (SIO), extracted from the Sacha Inchi (SI) plant, is a perennial, oleaginous plant of the Euphorbiaceous family, which grows in the Amazonian forest. The plant, widely cultivated in Peru and northwestern Brazil, also known as “Inca Peanut”, “wild peanut”, “Inca Inchi”, or “mountain peanut”, has a long history among various native tribal groups in the region (del-Castillo et al., 2019). SIO has been classified as an edible plant oil with the highest proportion of healthy omega-3, 6, & 9 linoleic acyl groups (Hanssen, 2011). Because of these promising characteristics, SIO has been proposed to show various pharmacological properties, particularly its impact in modulating NCDs (Kodahl & Sorensen, 2021).

A number of clinical trials have been conducted to investigate the role of PUFAs present in the SIO for the improvement of cardiometabolic markers. A pilot,

experimental open-study among the hypercholesterolaemia patients reported improved total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) in the SIO supplement group (Garmendia et al., 2014). In contrast, studies in healthy adults using SIO reported no difference in terms of lipid profile, but an improvement in plasma insulin levels and some gene expression that improved glucose homeostasis (Alayón et al., 2018; Gonzales et al., 2018). Similarly, SIO supplementation among the impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) patients helped in improving the insulin resistance index (Derosa et al., 2016). Recently, SIO supplementation in metabolically healthy groups helped reduce inflammatory cytokines, the signalling molecules that trigger elevation of lipid profiles (Alayón et al., 2019). 3Hs is the most prevalent form of NCD in Malaysia. There is also a long-standing interest in plant-based, more sustainable, and healthier options to ameliorate 3Hs. A thorough clinical trial is needed to explore the efficacy of PUFAs on 3Hs. Thus, this study examined the effects of SIO among the 3Hs population in Malaysia.

1.2 Research Statement and Study Rationale

The recent 2023 National Health and Morbidity Survey (NHMS) reported that almost 2.3 million of Malaysian adults are living with three NCDs, which 3Hs makes up to 5.1% of the adult population (NHMS, 2023). The report stated that at least 1 in 6 adults in Malaysia have diabetes (equivalent to 15.6%); 1 in 3 adults are living with hypertension (equivalent to 29.2%); and 7.6 million adults (33.3%) are living with high cholesterol. In 2019, the NHMS stated that the prevalence of hyperglycaemia, raised blood pressure, and hypercholesterolaemia was 18.3%, 30.0% and 24.6%, respectively (NHMS, 2019). Both surveys visualise a worrying trend for 3Hs in comparison to the previous NHMS (NHMS, 2015). Concurrently, the incidence of

depression and other mental health disorders in adults aged over 50 increased significantly from 2019 to 2023. Data from the 2023 NHMS indicated that at least 5.5% of adults in this age group were reported to have mental health issues, representing a doubling of the prevalence rate compared to 2019. These growing trends are placing an increased strain on Malaysia's healthcare system through a rising demand for offering a better health services. In 2022, the annual healthcare costs of CVDs and diabetes have exceeded RM 8.6 billion of productivity loss (MOH, 2022). Despite improvements in the development and implementation of guideline-directed medical therapy, the adverse 3Hs-linked events remains substantially high.

The promising cardioprotective effects of PUFAs of SIO (Cárdenas-Sierra et al., 2021) urged for more distinctive intervention of a plant-based PUFAs supplementation trial. Despite the proof-of-concept of the benefit of administering SIO in patients with either hyperglycaemia, hypertension, or hyperlipidaemia, the effects of SIO in patients with 3Hs is yet to be examined.

1.3 Research Questions

(i) Does SIO supplementation for 12 weeks have any effects on glycaemic control, blood pressure and lipid profiles among patients with 3Hs?

(ii) Does SIO supplementation for 12 weeks have any effects on anti/inflammatory biomarkers, oxidative stress levels, and circulating arterial calcification among patients with 3Hs?

(iii) Does SIO supplementation for 12 weeks have any effects on nutritional status, quality of life, and mental health status among patients with 3Hs?

1.4 Research Objectives

1.4.1 General Objective

To investigate the effects of SIO supplementation in patients with hyperglycaemia, hyperlipidaemia, and hypertension (3Hs).

1.4.2 Specific Objectives

1.4.2 (a) Primary Objectives

(i) To explore the effects of SIO supplementation on the glycaemic control among 3Hs patients.

(ii) To explore the effects of SIO supplementation for BP level reduction among 3Hs patients.

(iii) To explore the effects of SIO supplement intervention on the lipid profiles control among the 3Hs patients.

1.4.2 (b) Secondary Objectives

(i) To evaluate the effects of SIO supplementation on anti/inflammatory biomarker level among 3Hs patients.

(ii) To evaluate the effects of SIO supplementation on oxidative stress level among the 3Hs patients.

(iii) To evaluate the effects of SIO supplement intervention on circulating arterial calcification biomarkers among the 3Hs patients.

1.4.2 (c) Tertiary Objectives

(i) To investigate the influence of SIO supplementation among 3Hs patients on nutritional status.

(ii) To investigate the influence of SIO supplementation among the 3Hs patients on quality of life.

(iii) To investigate the influence of SIO supplementation among 3Hs patients on mental health status.

1.5 Hypotheses

(i) SIO supplementation improved glycaemic control in patients with 3Hs in comparison with placebo.

(ii) SIO supplementation improved blood pressure in patients with 3Hs compared to placebo.

(iii) SIO supplementation improved lipid profiles in patients with 3Hs compared to placebo.

(iv) SIO supplementation intervention improved the anti/inflammatory level in 3Hs patients in comparison with placebo.

(v) SIO supplementation improved oxidative stress among 3Hs patients compared to placebo.

(vi) SIO supplementation intervention improved arterial calcification biomarkers among 3Hs patients.

(vii) SIO supplementation improved anthropometric measurement changes in 3Hs patients.

(viii) SIO supplementation influenced the quality of life of 3Hs patients.

(ix) SIO supplementation influenced the mental health status of 3Hs patients.

1.6 Conceptual Framework

The collective effect of SIO on 3Hs presents a promising strategy for addressing metabolic syndrome. By targeting these key components, SIO supplements may contribute to the reduction of the overall risk of metabolic syndrome associated with NCDs. This conceptual framework highlights the multifaceted benefits of SIO in demonstrating the intricate interplay of factors within metabolic syndrome, thereby offering a holistic approach to reducing the risk of NCDs (Figure 1.1).

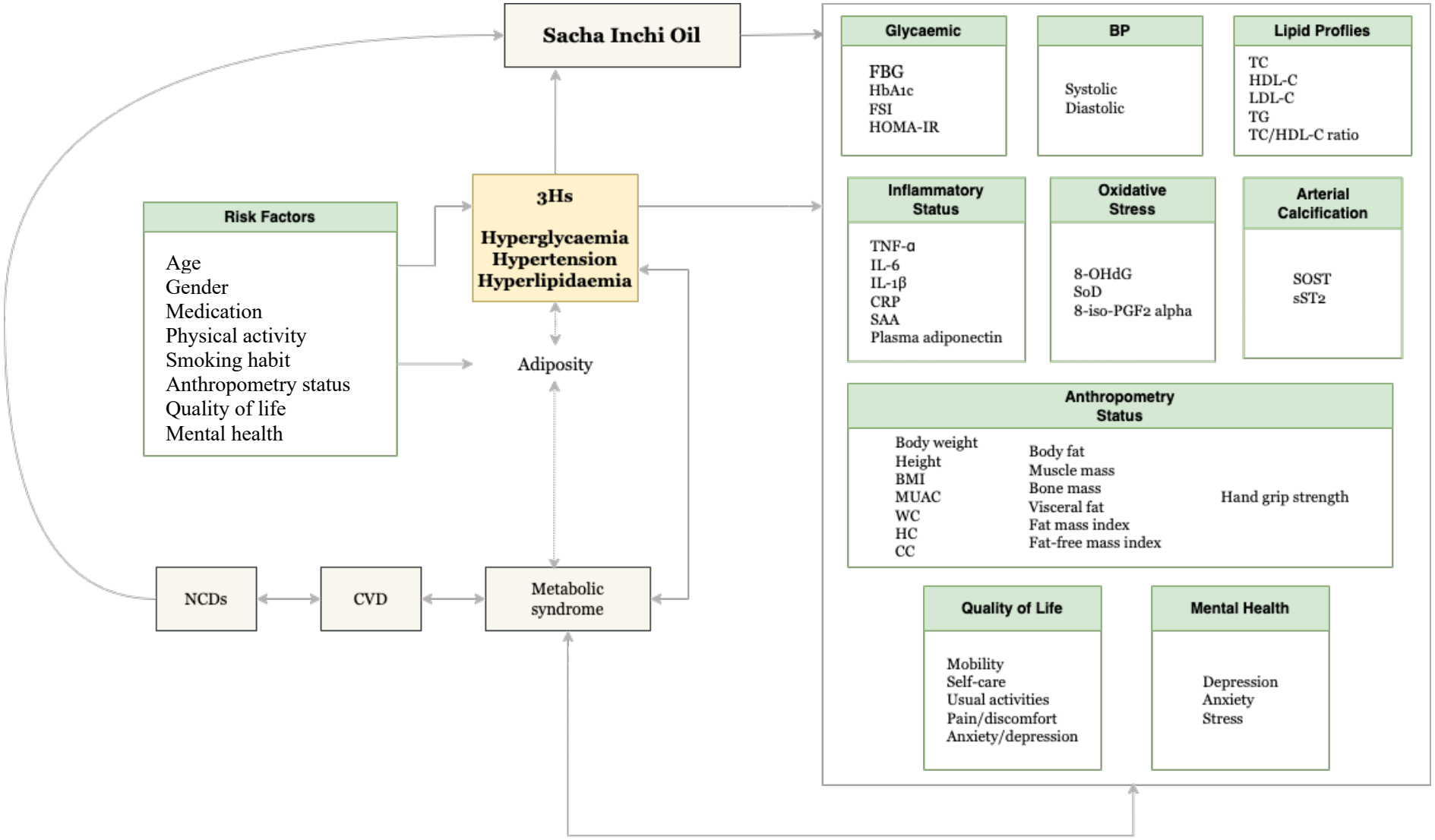

Figure 1.1 Conceptual framework for the clinical trial

CHAPTER 2

LITERATURE REVIEW

2.1 Non-communicable diseases (NCDs)

Non-communicable diseases (NCDs) are by definition describes all chronic diseases that are non-infectious and non-transmissible (Camps, 2014). The most common known NCDs known are obesity, CVDs, T2DM, cancer, chronic respiratory diseases and neurological diseases (Camps, 2014). Obesity and associated metabolic disturbances, for example, are the modifiable risk factors such as unhealthy diets, physical inactivity, tobacco use and the harmful consumption of alcohol (Breda et al., 2019; Saklayen, 2018). It predisposes humans to T2DM, hypertension, atherosclerosis, dyslipidaemia, cancer, and coronary heart disease (Zhao et al., 2023). Metabolic risk factors for NCDs are almost exclusively attributed to raised blood pressures, overweight or obesity, and hyperlipidaemia (Seyedsadjadi & Grant, 2020). Growing evidence links a low-grade, chronic inflammatory state to genetic and environmental factors, nutrition and lifestyle may induce a pro-oxidative and pro-inflammatory state, linked to alterations in mitochondrial structure and functions (Nediani & Dinu, 2022).

The incidence of diseases involving oxidative stress, inflammation, and their related metabolic disturbances is rising, as are age-related diseases due to progressively ageing populations, being increasingly recognised as part of the problem in NCDs (Liu et al., 2020). Currently, 85% people died prematurely from NCDs globally despite many NCDs being highly preventable or modifiable, and technical packages of essential interventions made available, progress is inadequate (WHO, 2018). Although NCDs cause three quarters of all deaths globally, yet only 1-2% of

global financing investment was meant for health. According to The Global NCDs Compact 2020-2030, addressing NCDs is an attractive investment. Cost-effective, high-impact interventions already exist, but they are not being implemented and scaled up in many countries. The estimated gains are tangible and realisable in the short term; as investing as low as USD 0.84 per person, per year in NCDs could save seven million lives in low- and lower-income countries (LMIC) by the end of 2030 (WHO, 2020).

In Malaysia, the Ministry of Health revealed that NCDs have costed Malaysian economy upwards of RM8.9 billion of productivity loss (WHO, 2018). This situation, elucidated by disability and loss of healthy life years, called the burden of disease, costed approximately RM100 billion, equivalent to 7.35% of the national gross domestic product (GDP). In low resource settings, healthcare costs for NCDs quickly drain household resources. The exorbitant costs of NCDs, including treatment, which is often lengthy and expensive, combined with loss of income, forces many household into poverty annually and stifle development. It is both a cause and a consequence of poverty, destroying the economies of families each year. Despite the improvement in the development and implementation of guideline-directed medical therapy, the risk of adverse NCDs-linked events remains substantially high in this country.

NCDs, while representing far more than a health issue, disproportionately burden to vulnerable populations with diseases, disability and death. Countries with healthier populations, where people living with NCDs have timely access to care and diagnosis as well as alternative care to manage their conditions, increases their resilience to future health threats, reduce health costs of severe disease and hospitalisations, thus mitigating productivity losses (WHO SDG Goals, 2022). This threaten the progress towards 2030 agenda for SDG, which target of reducing the

probability of death from any four main NCDs between ages 30 and 70 years by one third, by 2030 (WHO SDG Goals, 2022).

2.2 Hyperglycaemia

The term hyperglycaemia is derived from the Greek language with the following definition; hyper (high) + glykys (sweet/sugar) + haima (blood) (Mouri & Badireddy, 2022). Hyperglycaemia, or raised blood glucose, is the main clinical characteristic attributed to T2DM. It is termed so as plasma blood glucose levels greater than 125 mg/dL during fasting, or greater than 180 mg/dL 2 hours after food ingestion (Magliano & Boyko, 2021). Meanwhile, the diagnostic values for hyperglycaemia, according to the Malaysian Clinical Practice Guidelines for Diabetes (2017) for fasting is > 7.0 mmol/L, and > 11.1 mmol/L during post-prandial. A patient is classified as having an IGT when their plasma blood glucose is measured between 6.1 to 6.9 mmol/L for fasting, and 7.8 to 11.0 mmol/L post-prandial. An individual will inevitably be termed as a T2DM patient should their FBG ≥ 7.0 mmol/L during fasting, and ≥ 11.1 mmol/L during random blood glucose measurement more than 2 hours post prandial. The Malaysian CPG for diabetes stated that for symptomatic individuals, only one abnormal glucose value is diagnostic, meanwhile in asymptomatic individual, 2 abnormal glucose values are required.

2.2.1 Demographic, behavioural and lifestyle factors contributing to hyperglycaemia or T2DM

2.2.1 (a) Age

The National Health and Nutrition Examination Surveys (NHANES) reported that the prevalence of diabetes increases with age (Petersen et al., 2019). In most

populations, the incidence of T2DM is low before 30 years, but increases rapidly and continuously with older age. In Malaysia, this can be seen through the latest NHMS report; as only 5.4% of those with raised blood glucose were in the 20-24 years age group, with a peak of 43.4% of the population with raised blood glucose were among the 65-69 years old (Ministry of Health Malaysia, 2020). Prospective observational studies have generally reported age to be a strong risk factor, independent of major correlated lifestyle risk factors, including obesity. The International Diabetes Foundation (IDF) has projected that 629 millions of people will be diabetic by the year of 2045, with 79% of them attributed to the population ageing (Magliano & Boyko, 2021).

2.2.1 (b) Gender

In the European Prospective Investigation into Cancer and Nutrition Study (EPIC), higher risk of diabetes in men compared with women was observed consistently across different European countries (EPIC Study, 2011). In Malaysia, the NHMS 2019 reported a somewhat similar prevalence of T2DM among males and females with 18.2% and 18.4%, respectively (Ministry of Health Malaysia, 2020).

2.2.1 (c) Race and Ethnicity

The prevalence of diabetes varies widely among countries worldwide. National surveys in the USA for people aged 20 years old and above reported that non-Hispanic whites, Asian Americans, Hispanics/Latinos, and non-Hispanic blacks were among the top races to have been diagnosed with diabetes. Meanwhile, in the Europe, black African, African Caribbean and South Asian (Indian, Pakistani and Bangladeshi) were among the common ethnicity for known diabetes (Narayan & Kanaya, 2020). In

Malaysia, Indians demonstrated the highest prevalence for raised blood glucose and known diabetes at 31.4%, followed by Malays (21.6%), Chinese (15.1%), and Bumiputera Sarawak (12.3%) (NHMS, 2019). These ethnic differences can be explained by differences in the prevalence of obesity, behavioural risk factors, and socioeconomic status (SES).

2.2.1 (d) Genetic

A family history of diabetes has been linked with increased risk for T2DM. Early efforts to identify genetic variants for T2DM heritability in epidemiologic study involved genome-wide linkage and candidate gene approaches. With the studies incorporating high-throughput, parallel genotyping technologies, including genome-wide association studies (GWAS), the field has rapidly advanced, identifying and replicating multiple novel loci associated with T2DM. Many common genetic variants predisposing to T2DM uncovered initially by GWAS have been located near genes implicated in beta cell functions (Shoily et al., 2021). Subsequently, genetic variants implicated in insulin resistance pathways were uncovered by accounting differences in BMI. In addition to genetic risk for T2DM, family members often share non-genetic environmental risk factors that contribute to risk for diabetes (Beulens et al., 2022).

2.2.1 (e) Nutrition

The T2DM diabetes epidemic has been attributed to urbanisation and environmental transition leading to sedentary behaviour and overnutrition. These environmental transformations include work pattern changes from heavy labour to sedentary, increased computerisation and mechanisation, and improved public transportation accompanied by easy access to fast foods and other energy-dense and

nutrient-imbalanced foods (Standl et al., 2019). Dietary intake has been suggested as a major lifestyle risk factors for T2DM for a long time, and evidence from prospective studies evaluating diet in relation to the incidence of diabetes has vastly accumulated since the 1990s (Ley, 2012).

2.2.1(e)(i) Dietary carbohydrate and fibres

Several prospective observational studies investigated the relation between total carbohydrate intake (expressed as the proportion of carbohydrate intake of total energy intake or as energy-adjusted intake quantity in grams per day) and risk of T2DM (Hauner et al., 2012). For example, no significant relation of higher carbohydrate intake at the expense of protein with risk of diabetes was observed in the UK National Health Study (NHS), but such an isocaloric exchange was associated with reduced risk in the EPIC-Potsdam Study (Polemiti et al., 2021). In a recent meta analysis, a greater reduction of HbA1c (approx. 62.7%) and FBG (approx. 82.7%) among T2DM was reported among moderate carbohydrate diets compared to recommended diets (Jing et al., 2023). The team also concluded that moderate carbohydrate diet and low glycaemic index diet (high-fibre diet) were effective for glycaemic control in T2DM patients.

In Malaysia, a recent study reported that T2DM patients mean carbohydrate intake was within the range of recommended nutrient intakes (RNI) for Malaysia at 51.9% (Md Isa et al., 2023). However, the mean carbohydrate intake was lower than the reported previous studies (56.9% and 60.0% of total energy intake) (Tan et al., 2011; Ming et al., 2002). This has raised some concern as fibre intakes, which were known to be inversely related to T2DM, were also relatively declining as it was grouped with carbohydrates (Mao et al., 2021). The meta analysis reported that the

lack of fibre intake, which was below the recommended amount, increases the risk of T2DM progression by 43%. Although the Malaysian MOH has been implementing medical nutrition therapy (MNT) to provide optimal nutrition recommendation for T2DM patients, the compliance was low. The approach only showed a 16.4% of compliance among Malaysian T2DM patients despite its effectiveness in glycaemic control (Abdullah et al., 2018).

2.2.1(e)(ii) Dietary fat

While higher total fat intake has been hypothesised to contribute to diabetes directly by inducing insulin resistance, and indirectly by promoting weight gain, metabolic studies in humans do not support the idea that high fat diets have a detrimental effect on insulin sensitivity (Neuenschwander et al., 2020). In most observational prospective studies, total fat intake was not associated with diabetes risk. Strong evidence also comes from the large scale, randomised Women's Health Initiative (WHI) (Huang et al., 2017). The incidence of treated diabetes was not different among women who consumed a low fat diet (24% energy from fat) compared to women who consumed a standard diet (35% energy from fat). The specific type of fat may be more important than the total intake. Prospective studies suggest that diets that favour plant fats over animal fats are advantageous (Kurotani et al., 2013).

Whether changing the relative proportions of different fatty acid subgroups is related to diabetes risk remains a matter of debate. A higher intake of PUFAs was related to lower diabetes risk in the Nurses' Health Study (NHS), the Iowa Women Study and the Health Professional Follow-up Study (Jiao et al., 2019). Also, exchanging saturated fatty acids with PUFAs was related to lower risk in some studies (Peláez-Jaramillo et al., 2020). Consequently, the relationship between long-chain Ω-

3 PUFA and diabetes risk has been inconsistent: a meta analysis including 46 randomised controlled trials with 4991 T2DM participants reported Ω-3 PUFAs intervention can improve the cardiovascular risk factors in T2DM patients, particularly for total cholesterol, triglyceride, HDL-C and HbA1c, but no significant difference was observed for insulin resistance (HOMA-IR), LDL-C, adiponectin and leptin (Xiao et al., 2022).

In addition to the opposing health effects of saturated versus unsaturated fat, specific PUFAs may differ in their health effects. Some studies have indicated that Ω-6 PUFAs, but not Ω-3 PUFAs, may improve insulin sensitivity (Egalini et al., 2023). Since 2008, there was discovery to suggest that Ω-3 PUFA supplementation in people with T2DM had no significant effect on glycaemic control, whereas vegetable PUFAs, which mainly made from Ω-6 PUFAs, were found to reduce fasting insulin and HOMA-IR in a more recent meta-analysis (Hu et al., 2022). However, more recently, studies involving various sources of PUFAs conducted among the T2DM patients yield mixed results. Several studies incorporating vegetable oils such as safflower-, flaxseed-, canola-oil and a few others reported changes in glycaemic regulation either between or within groups among T2DM subjects (Telle-Hansen et al., 2019). The changes of glycaemic regulation reported include fasting glucose levels, HOMA-IR and fasting insulin. A pooled analysis from a prospective cohort study demonstrated that higher levels of PUFAs in blood were associated with a 43% reduced relative risk for T2DM (Wu et al., 2017). An earlier study also showed that switching from a saturated fatty acid (SFA) rich diet to PUFA-rich diet up to 5 weeks improved insulin sensitivity in people with T2DM, non-obese and obese subjects (Summers et al., 2002).

2.2.1 (f) Physical inactivity

The Global Status Report on Physical Activity reported that 27.5% of adults currently do not meet WHO's recommended levels of physical activity, and this has not only affected individuals over their life span and their families, but health services and society as a whole (WHO, 2022). In Malaysia, the overall prevalence of physically inactive adults was 25.1% (NHMS, 2019). The level of physical inactivity gradually increased in adults from the age group of 55-59 years to 75 years and above. The highest prevalence of physical inactivity was observed among Malays (85.3%), followed by Chinese (84.4%), and Indians (81.0%). Sedentary behaviours, including screen time, are risk factors for T2DM. In a prospective cohort study, higher early childhood television viewing time was associated with increased risk of T2DM at adulthood (Schmid et al., 2021). Based on worldwide estimate, physical inactivity, defined as insufficient physical activity to meet present global recommendations (WHO Guidelines on Physical Activity and Sedentary Behaviour, 2020), is responsible for 7% of the burden of T2DM (Lee et al., 2012).

2.2.1 (g) Adiposity

The prevalence of obesity worldwide is estimated at 1 billion, with 650 million adults are identified as obese and overweight (WHO, 2022). WHO estimates that by 2025, approximately 167 million additional people will become less healthy because of overweight and obesity. In Malaysia, the national prevalence of overweight was 30.4%, while obesity was 19.7%. Additionally, the national prevalence of abdominal obesity among adults in Malaysia was 52.6% (NHMS, 2019). Although the prevalence of overweight is still lower than the global prevalence of 39%, an alarming trend of obesity epidemic still requires immediate action through public health policies to

reduce the relative risk associated with overweight. Excessive body fat is the single largest risk factor for T2DM (Sattar & Gill, 2014). Meta analysis from various geographic regions, namely South East Asia, South Asia, North-, and South America, Europe, the Far- and Middle-East, as well as Africa suggested that the T2DM relative risk is associated with a higher BMI. A larger waist circumference, independent of overall adiposity, was strongly and linearly associated with the risk of T2DM (Jayedi et al., 2022).

2.2.1 (h) Smoking

In a meta-analysis of prospective epidemiologic studies with over 45,000 incident cases over the span of 50 years, active smokers were at an increased risk for developing T2DM compared to non-smokers by 30-40% (CDC, 2014). In Malaysia, the data from 3 national surveys, the GATS 2011, NHMS 2015 and NHMS 2019 showed that approximately 4.7 million (23.1%), 5.0 million (22.8%), and 4.9 million (21.3%) adults ≥ 15 years in Malaysia were current smokers (Yusoff et al., 2022). The underlying mechanism to explain the roles of cigarette consumption in T2DM risk is not entirely clear. In experimental studies, smoking was linked with impaired glucose tolerance and insulin resistance (Artese et al., 2019). In a double-blind, cross-over, placebo-controlled, randomised experimental study, nicotine infusion aggravated insulin resistance among participants with T2DM, but not among non-diabetics (Vu et al., 2014). Therefore, cigarette smoke may not initiate, but instead promotion of the disease. In addition, it has been speculated that nicotine or other agents in cigarette smoke might induce pancreatic injuries and affect insulin secretion by inducing oxidative stress in the pancreas and, subsequently, leading to loss of beta cell functions (Tong et al., 2020).

2.2.2 Metabolic factors associated with risk of T2DM

A few novel biomarkers and intermediate conditions were called as metabolic risk factors for T2DM. A hyperbolic relation between beta cell function and insulin sensitivity is constant among individuals with normal glucose tolerance. In situations where a deviation from the hyperbole occurs, deteriorating glucose tolerance happens thus occurring the progression of T2DM (Lee et al., 2012) (Figure 2.1).

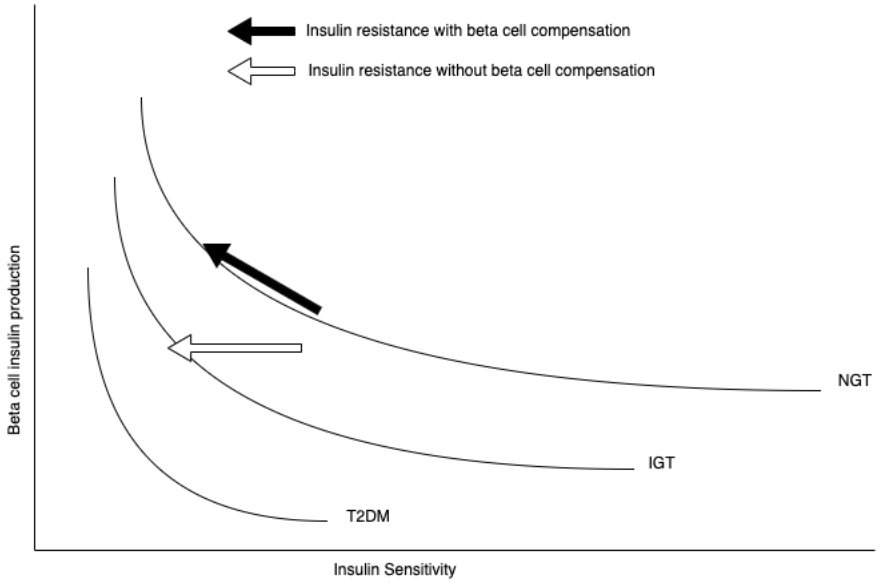

Figure 2.1 Progression from normal glucose tolerance (NGT) to T2DM

2.2.2 (a) Impaired fasting glucose and impaired glucose tolerance

Impaired fasting glucose (IFT) and impaired glucose tolerance (IGT) are "prediabetes" states defined by glycaemic levels higher than normal but below the actual cut-offs for diagnosis of diabetes (Baynest, 2015). In prospective studies, individuals progressed from normoglycaemia to impaired glucose regulation (IFT and/or IGT) before reaching hyperglycaemic levels of T2DM (American Diabetic Association, 2018). Although individuals progressed from normal glucose tolerance to T2DM via IFG and/or IGT, one anomaly does not always precede the other. Both

IFG and IGT in isolation predicts an increased risk, but the combination of IFG and IGT signifies a particularly high risk state. In the Framingham Offspring Study, a standard oral glucose tolerance test (OGTT) was performed, and follow-up assessments revealed that T2DM incident was 1.3% for normoglycaemia, 4.3% in IGT, 9.2% in IFG, and 25.5% in individuals with both IFG and IGT (Ley, 2012). However, there are recent literatures comparing blood glucose levels and insulin sensitivity and resistance during the OGTT and a mixed meal tolerance test (MMTT). Various standardised mixed meals have been pointed out as substitutes for the glucose solution administered in the OGTT, supported by a reported large inter-individual variations in plasma glucose after corresponding meals and found that gut microbiota and food structure are more important in determining differential responses (Skantze et al., 2023) .

2.2.2 (b) Beta cell dysfunction

T2DM is believed to originate from an imbalance between insulin resistance and the capacity of the beta cell to produce insulin during demand. The etiologic factors leading to beta cell dysfunction are still a matter of important research, but genetic variants predisposing to T2DM are often located near genes implicated in beta cell functions (Cerf, 2013). The most commonly recorded indices of insulin secretion is derived from OGTT. In the San Antonio Heart Study (SAHS), a low insulinogenic index was associated with increased risk of developing T2DM. Another report from the SAHS showed that the index estimating the later phase of insulin secretion during OGTT (60-120 minutes) was also associated with risk of developing T2DM, independent of the insulinogenic index (Lorenzo et al., 2012). However, the administration of 75g glucose have been reported to cause unpleasant symptoms, such

as nausea, vomiting, diarrhea, bloating and anxiety (Lages et al., 2022). Multiple studies have also shown that comparing OGTT and MMTT, the MMTT were proven to worked well as a screening method to detect abnormal glucose metabolism (Lages et al., 2022; Chanprasertpinyo et al., 2017). The glucose, insulin, and C-peptide responses within the MMTT tests in a comparison study had good reproducibility and were concluded to able to detect beta cells function in prediabetic and T2DM patients (Shankar et al., 2016).

2.2.2 (c) Insulin sensitivity (insulin resistance)

The Homeostasis model assessment of insulin resistance (HOMA-IR), based simply on fasting insulin and glucose measurements, is commonly used and accepted as a marker of insulin resistance in large scale epidemiologic studies. In the Framingham Offspring Study, individuals in the upper quartile of insulin resistance based on HOMA-IR were twice as likely to develop T2DM during 7-years of follow-up (Ley et al., 2012). In the WHI Observational Study, higher HOMA-IR was associated with increased risk of T2DM across multiple ethnic groups (Michelle-Schmiegelow et al., 2015). Fasting insulin is also used as a surrogate for insulin resistance in large scale epidemiologic studies. Based on NHANES 2019, the prevalence of hyperinsulinemia increased for more than 10% between surveys, resulting in a 35% global increase in the adult population.

2.2.2 (d) Adiponectin

Adiponectin is an adipokine mainly produced by adipocytes, a well-known homeostatic factor for the regulation of glucose levels, lipid metabolism, and insulin sensitivity (Ouerghi et al., 2020). Circulating adiponectin levels are decreased in

excess weight. Weight loss or caloric restriction leads to increasing adiponectin levels, and this increase is associated with increased insulin sensitivity. Smaller sizes adipocytes produce high levels of adiponectin, which is believed to have anti-inflammatory and insulin-sensitising effects, based on *in vitro* and animal studies (Ma et al., 2021). Unlike adipose tissue, the expression of adiponectin in muscle is more likely to depend on gender, with men expressing more adiponectin receptors. This highlights the dimorphism thus appeared to be tissue specific. Intriguingly, circulating adiponectin levels are lower in men than women, which can be explained by the level of receptor expression in muscle (Diep Nguyen, 2020).

Numerous studies have shown the existence of an inverse relationship between circulating adiponectin concentrations and insulin resistance in several pathologies with high cardiovascular risk such as obesity, metabolic syndrome, and T2DM. In mice, injection of adiponectin induces an increase in circulating insulin levels (Khalil et al., 2018). It was found that treatment of pancreatic β cells with adiponectin induces an increase in insulin exocytosis, accompanied by an increase in expression of multiple co-activators of transcription of the insulin gene (Khalil et al., 2018). Adiponectin coherently promotes the consumption of glucose by stimulating the membrane translocation receptor, GLUT4 in muscle cells and adipocytes following phosphorylation of AMPK. Subsequently, adiponectin inhibits the formation of glucose and glycogen. In the liver cells, glycogenesis and gluconeogenesis are slowed down by adiponectin, following the decrease in the expression of two key enzymes in the pathways, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK). In muscle cells, glycogen production is also reduced by adiponectin, following activation of AMPK. Adiponectin induces a drop in blood sugar by its

hypoglycaemic action, helping the body to protect against the onset of T2DM (Diep Nguyen, 2020).

2.2.2 (e) Pro-inflammatory Cytokines

Excess weight accumulation is known to cause adipose tissue infiltration by macrophages that consequently leads to secretion of pro-inflammatory cytokines and impaired secretion of adipokines, a type of proteins secreted by adipose tissue (Diep Nguyen, 2020). Tumour necrosis factor-alpha (TNF-α) is one of the first proteins identified as part of the inflammatory pathways, and is suspected to directly and indirectly contribute to insulin resistance by acting on adipose tissue, liver and skeletal muscles (Tsalamandris et al., 2019). In the adipose tissue, TNF-α is produced primarily by adipose-infiltrating macrophages, and implicated in inflammation, cell cycle regulation, cytotoxicity, and production of other cytokines (Guzik et al., 2017). Interleukin-6 (IL-6) is a pro-inflammatory cytokine that is produced by many cell types, including fibroblasts, endothelial cells, mononuclear phagocytes, neutrophils, hepatocytes, and lymphocytes. Both TNF-α and IL-6 stimulate the synthesis and secretion of C-reactive protein (CRP) by the liver. CRP is a sensitive marker of low grade systemic inflammation (Sproston & Ashworth, 2018).

Each of these three biomarkers; TNFα, IL-6, and CRP, was associated with increased risk of developing T2DM in prospective studies, but CRP seemed to offer stronger and more stable association. When including all three biomarkers, only CRP remained significantly associated with T2DM incidence in the NHS and WHI Observational Study. In the EPIC-Norfolk cohort, the association between elevated CRP and risk for developing T2DM was attenuated to nonsignificance after adjustment for waist-to-hip ratio, serum gamma glutamyltransferase (GGT), and serum adiponectin. In the Multi-Ethnic Study of Atherosclerosis (MESA), IL-6 and CRP