STUDY OF PROTEIN, LIPID AND METABOLITE PROFILES IN TYPE 2 DIABETES MELLITUS (T2DM) RATS MODEL UPON HIGH CALORIES FOOD INTAKE AND METFORMIN TREATMENT

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LIST OF ABBREVIATIONS

2D	Two Dimension
2-DE	Two Dimension Gel Electrophoresis
2-OG	2-Oxoglutarate
ABCG5	ATP-binding Cassette Sub-family G Member 5
ABCG8	ATP-binding Cassette Sub-family G Member 8
ACAT	Acyl-CoA Cholesterol Acyl Transferase
acetyl-CoA	Acetyl Coenzyme A
ACN	Acetonitrile
AMPK	Adenosine Monophosphate-activated Kinase
ANOVA	One-way Analysis of Variance
ApoE	Apolipoproteins E
AUROC	Area under the Receiver Operating Characteristic
BMRB	Biological Magnetic Resonance Bank
CDK	Chronic Kidney Disease
CHAPS	3-[3-cholamidopropyl]dimethylammonio]1-propane sulfonate
D2O	Deuterium Oxide
DGAT	Diacylglycerol Transferase
DKD	Diabetic Kidney Disease
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
DNA	Deoxyribonucleic acid
DTT	DL-dithiothreitol
ENOA	Alpha-enolase
eNOS	Endothelial Nitric Oxide-dependent Synthesis
ESI	Electronspray Ionisation
ESRD	End-stage Renal Disease
FA	Formic acid
FBPase	Fructose-1,6-bisphosphatase 1
FFA	Free Fatty Acid
GC-MS	Gas-chromatography Mass Spectrometer
GDM	Gestational Diabetes Mellitus
GFR	Glomerular Filtration Rate
HBB	Haemoglobin subunit
HDL	High-density Lipoproteins
HFD	High Fat Diet
HMDB	Human Metabolome Database
HSP	Heat Shock Protein
IEF	Isoelectric Focusing
IL-6	Interleukin-6
IP	Intraperitoneal Injection

IPG	Immobilized pH Gradient
K2EDTA	Dipotassium Ethylenediaminetetraacetic acid
KCL	Potassium Chloride
kDA	kilo Dalton
LDL	Low-density Lipoproteins
LPL	Lipoprotein Lipase
m/z	Mass to Charge Ratio
MA	Methylamine
MALDI	Matrix-assisted Laser Desorption/ionisation
MGAT	Monoacylglycerol Transferase
MS	Mass Spectrometry
MTTP	Microsomal Triglyceride Transfer Protein
MUFA	Monounstaturated Fatty Acid
MUP	Major Urinary Protein
NA	Nicotinamide
NaCl	Sodium Chloride
NF-κB	Nuclear Factor-κB
NMR	Nuclear Magnetic Resonance
NPC1L1	Niemann-Pick C1 like 1 Protein
OPLS-DA	Orthogonal Projections to Latent Structures Discriminant Analysis
PA	Palmitic Acid
PAG	Pheylacetylglycine
PBS	Phosphate-buffered Saline
PC	Principle Component
PCA	Principal Components Analysis
PHB	Prohibitin
PI	Isoelectric Point
PI	Pre-induced
PO	Palm Oil
ppm	Parts per Millions
PT	Pre-treatment
PUFA	Polyunsaturated Fatty acid
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RP-LC	Reversed-phase High Performance Liquid Chromatography
rpm	Revolutions per Minute
SCAP	SREBP cleavage-activating protein
SDS-PAGE	Sodium Dodeyl Sulphate Polyacrylamide Gel Electrophoresis
SFA	Saturated Fatty Acid
SREBP	Sterol Regulatory Element-binding Protein
STAT	Signal Transducer and Activator of Transcription
STZ	Streptozotocin
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus

TAG	Triglycerides
TCA	Trichloroacetic Acid
TCA cycle	Tricarboxylic Acid Cycle
TLB	Thiourea Lysis Buffer
TLC	Thin Layer Chromatography
TMAO	Trimethylamine-N-oxide
TMB	3,3',5,5'-Tetramethylbenzidine
TMS	Trimethylsilyl
TSP	Sodium 3- (trimethylsilyl)-propionate-2,2,3,3-d4
VIP	Variable Influence on Projection
VLDL	Very Low-density Lipoproteins
WAT	White Adipose Tissue
WHO	World Health Organization

LIST OF APPENDICES

KAJIAN BAGI PROFIL PROTEIN, LIPID DAN METABOLIT DALAM MODEL TIKUS DIABETIS MELITUS JENIS 2 (T2DM) SETELAH MENGAMBIL MAKANAN BERKALORI TINGGI DAN RAWATAN METFORMIN

ABSTRAK

Diabetis jenis kedua (T2DM) ialah penyakit metabolik yang dicirikan oleh peningkatan paras glukosa darah disebabkan oleh kekurangan insulin dan kerintangan insulin. T2DM disebabkan terutamanya oleh gaya hidup yang tidak sihat. Pengurusan gaya hidup seperti diet yang sihat dan aktiviti fizikal adalah penting untuk pengurusan diabetis. Makanan yang dimasak dengan minyak atau berlemak tinggi menambah rasa dan tekstur pada makanan; jesteru, digemari oleh orang ramai. Dalam kajian ini, pembolehubah metebolik, profil protein (buah pinggang), lipid (darah) dan metabolik (urin) dalam tikus T2DM dikaji setelah diberi diet berlemak tinggi yang diperkayakan dengan minyak kelapa sawit dan rawatan metformin. Tikus dinduksi diabetis dengan penggunaan bersama nikotinamida dan streptozotosin. Tikus dalam kumpulan kawalan dan kumpulan diabetik diberi diet biasa dan diet berlemak tinggi, samada dengan atau tanpa rawatan metformin selama enam minggu berturut-turut. Tikus diabetik menunjuk paras trigliserida darah yang lebih rendah, berat badan yang lebih rendah, kumulasi tisu adiposa yang lebih rendah, dan penyerapan fitosterol yang lebih rendah apabila dibanding dengan tikus sihat. Tikus diabetik juga menunjuk paras laktat dan bahan perantaran kitar TCA lebih rendah. Selain daripada itu, diabetis juga menyebabkan peningkatan ekspresi protein kejutan haba (HSP) 60kDa dan penurunan ekspresi “major urinary protein” dan regucalcin dalam buah pinggang. Diet berlemak tinggi meningkatkan paras trigliserida darah, meningkatkan berat badan, meningkatkan kumulasi tisu adiposa dan meningkatkan penyerapan fitosterol diet

tetapi tidak ada kesan terhadap paras glukosa darah tikus diabetik. Tikus diabetik yang diberi diet berlemak tinggi juga menunjukkan peningkatan paras laktat, bahan perantara kitar TCA, allantoin dan jasad ketone dalam urin. Enolase, HSP 90kDa dan prohibitin juga diekspresi dengan lebih tinggi dalam tisu buah pinggang. Rawatan metformin menurunkan paras glukosa darah, menurunkan kumulasi tisu adiposa, meningkatkan paras trigliserida dan meningkatkan penyerapan fitosterol diet pada tikus diabetik. Rawatan metformin melemahkan kesan diabetis dan diet berlemak tinngi terhadap berat badan tikus diabetik. Peningkatan paras laktat dan bahan perantaran kitar TCA dalam urin didapati dalam tikus diabetik yang dirawat dengan metformin. HSP 90kDa, subunit hemoglobin, dan haptoglobin juga diekpresi dengan lebih tinggi dalam buah pinggang bagi tikus yang dirawat dengan metformin. Mikrobioma usus juga diubah oleh diet berlemak tinggi, diabetis dan rawatan metformin. Secara amnya, hasil kombinasi kajian tiga omik mencadangkan diet berlemak tinggi yang diperkayakan dengan minyak kelapa sawit dan rawatan metformin memperbaiki metabolisme tenaga dan lipid yang telah diganggu oleh diabetis. Walaupun begitu, peningkatan ekpresi protein seperti HSP 90kDa dan prohibitin dalam buah pinggang serta peningkatan paras allantoin dalam urin menunjukkan berkemungkinan diet berlemak tinggi yang diperkayakan dengan minyak kelapa sawit meningkatkan tekanan oksidatif dan keradangan, ini adalah berbahaya bagi buah pinggang tikus T2DM.

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by high blood glucose due to insulin resistance or insulin deficiency. Type 2 diabetes is primarily caused by an unhealthy lifestyle. Lifestyle management such as healthy diet and physical activity is essential for diabetes management. Food prepared in oil or high fat content add taste and textures to the palate; therefore, it is more favourable by most people. In this study, metabolic parameters, proteins (kidney), lipids (blood), and metabolites (urine) profile of T2DM rats were studied upon palm oil enriched-high fat diet and metformin treatment. Rats were induced to T2DM states by a combination of nicotinamide and streptozotocin. The rats in control and diabetic groups were fed with normal and high fat diets, with or without metformin treatment for six consecutive weeks. Diabetic rats showed to have a lower blood triglyceride level, lower body weight, lower adipose tissue accumulation, and lower phytosterol absorption compared to the control rats. Diabetic rats also showed lower urinary lactate and TCA cycle's intermediates. Besides, diabetes also caused the upregulation of heat shock protein (HSP) 60kDa and downregulation of major urinary protein and regucalcin in the kidney. High fat diet increased the blood triglyceride level, increased body weight, adipose tissue accumulation and dietary phytosterol adsorption but have no effect on the fasting blood glucose of diabetic rats. High fat diet fed diabetic rats also showed increased urinary lactate, TCA cycle's intermediates, allantoin, and ketone bodies. Enolase, HSP 90kDa, and prohibitin were also upregulated in their kidney tissue.

Metformin treatment lowered the fasting blood glucose, decrease adipose tissue accumulation, increased blood triglycerides level, and increased dietary phytosterol absorption of the diabetic rats. Metformin treatment also flattened the effect of diabetes and high fat diet on the body weight of diabetic rats. Increase urinary lactate and TCA cycle's intermediates were observed in the metformin-treated rats. HSP 90kDa, haemoglobin subunit and haptoglobin were also upregulated in the kidney of metformin-treated diabetic rats. The gut microbiome also was altered by high fat diet, diabetes and metformin treatment. Generally, the results of the combination of three omics study suggested that palm oil enriched-high fat diet and metformin treatment improved the energy and lipid metabolism that were disturbed by diabetes. Nevertheless, the proteins that were upregulated, such as HSP 90kDa and prohibitin in the kidney and the elevated urinary allantoin indicate that palm oil-enriched high fat diet might increase oxidative stress and inflammation, which are harmful to the kidney of the T2DM rats.

CHAPTER 1

INTRODUCTION

Type 2 diabetic mellitus (T2DM) is one of the common metabolic diseases that affect the world. It is characterised by high glucose levels in the blood due to insulin resistance or lack of insulin. An unhealthy diet pattern and obesity have been associated with an increased prevalence of diabetes. Besides the use of antihyperglycemic drugs like metformin, lifestyle management such as diet and physical activity is vital to control the blood glucose level for people living with diabetes. Diabetic patients are required to abide to a controlled diet. Nevertheless, many find this diet restriction difficult. Food cooked in oil, such as frying, adds taste and texture to the palate (Koriyama et al. 2002) as compared to food prepared by healthy cooking methods such as steaming and boiling. Therefore, high fat diet (HFD) serves as the choice of food for many.

Palm oil can be easily obtained, and it is the most commonly use cooking oil in Malaysia. Therefore, in this study, in order to study the effect of high fat diet on diabetes, palm oil was chosen to be the vegetable oil incorporated in the rat's diet, mimicking the diet pattern of our daily meals.

High fat diet is among the risk factors of metabolic syndrome such as diabetes and obesity (Moreno-Fernández et al. 2018). Thus, body metabolic parameters such as fasting blood glucose, body weight, serum cholesterol level, serum triglyceride level and adipose tissue weight were investigated in this study. It is reported that vegetable oil such as palm oil contains plant sterol, which able to help to control lipids level in the body by lowering cholesterol absorption (Plösch et al. 2006). In this study, plant sterol concentration was measured in the blood of diabetic rats after feeding with a palm oil-enrich high fat diet. Diabetes and obesity are the leading cause of chronic

kidney disease (CKD) (Maric-Bilkan 2013). 40% of people with diabetes patients developed diabetic kidney diseases (DKD) or diabetic nephropathy (American Diabetes Association 2014). Therefore, in this study, the changes of the protein profile of kidney tissue were mapped in order to evaluate the effect of high fat diet on the kidney in type 2 diabetic rats. Urine has a high medical diagnostic and biomarker value as it does not involve body homeostasis and it can be easily and non-invasively collected. The urinary metabolic-based analysis was done on food intervention and diseases to discover valuable knowledge or even biomarker for diseases (Bouatra et al. 2013, Tebani and Bekri 2019). Palm oil-enriched high fat diet upon T2DM rat models on the changes of the urinary metabolite in diabetic rats was investigated in this study.

By investigating the protein, metabolites, and lipid profiles of the T2DM rats upon feeding with high fat diet and metformin treatment, this study aims to provide greater insights into biological changes at the molecular levels upon consumption of high fat diet, which is hoped to strengthen diabetes management of patients especially when lifestyle intervention fails.

RESEARCH OBJECTIVES

To investigate protein, metabolites, and lipid profiles upon high fat diet in type 2 diabetes mellitus (T2DM) rats.

Specific research objectives:

i. To investigate the changes of metabolic parameters caused by palm oil-enriched high fat diet and metformin treatment in type 2 diabetes mellitus (T2DM) rats

ii. To evaluate the serum plant sterols concentrations upon feeding of palm oil-enriched high fat diet and metformin treatment in type 2 diabetes mellitus (T2DM) rats.

iii. To identify the changes of kidney tissue protein profile caused by palm oil-enriched high fat diet, and metformin treatment in type 2 diabetes mellitus (T2DM) rats.

iv. To investigate the urinary metabolites profiles upon feeding of palm oil-enriched high fat diet and metformin treatment in type 2 diabetes mellitus (T2DM) rats.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes mellitus

Diabetes mellitus (DM), a chronic metabolic disease, is the most common non-communicable disease of the present era. It is characterised by increased glucose levels in blood plasma (Khawandanah 2019). This disorder will happen when the pancreas does not produce enough insulin, or the body is unable to use the insulin it produces effectively. The threshold levels to be diagnosed as diabetes according to WHO are plasma glucose values > 7.0 mmol/L (126 mg/dl), 2-h post-load plasma glucose > 11.1 mmol/L (200 mg/dl), HbA1c > 6.5% (48 mmol/mol); or a random blood glucose > 11.1 mmol/L (200mg/dl) (World Health Organization 2011). The sign and symptoms of diabetes include polyuria, polydipsia, loss of weight, and polyphagia (American Diabetes Association 2013).

2.1.1 Types of diabetes mellitus

There are mainly 2 types of diabetes mellitus, type 1 diabetes (T1DM) and type 2 diabetes (T2DM). T1DM is also known as insulin-dependent, juvenile, or childhood-onset. T1DM is caused by the autoimmune attack on the pancreatic β cell that produces insulin, causing the body to produce very little or no insulin (Raskin and Mohan 2010). T1DM affects 4-5% of all the people living with diabetes.

T2DM, also known as non-insulin dependent or adult-onset (American Diabetes Association 2013). T2DM is the result of insulin resistance, where the body failed to respond to insulin effectively, causing high blood glucose levels. T2DM is the most common type of diabetes affecting most of the people living with diabetes (around 90%) (Olokoba et al. 2012, American Diabetes Association 2013). T2DM mostly occurs in

older adults. However, due to the rising prevalence of obesity and unhealthy lifestyles, the people living with T2DM are getting younger. T2DM and T1DM share relatively similar symptoms although T2DM symptoms are often less marked and dramatic, sometimes even symptomless. Therefore many people who have T2DM are undiagnosed (American Diabetes Association 2015a).

Besides these two types of DM, another type of DM is gestational diabetes mellitus (GDM). GDM happens when hormones produced by the placenta cause insulin resistance. Women with GDM will experience a higher risk of complications throughout their pregnancy and during their delivery (Plows et al. 2018). On top of all these, the risk of them and their children getting T2DM is also increased in days to come (Vohr and Boney 2008).

Other types of diabetes are, hybrid forms of diabetes, other specific types (e.g., monogenic diabetes, disease of exocrine pancreas, endocrine disorders, drug-or chemical-induced diabetes, infection-related diabetes, uncommon specific forms of immune-mediated diabetes, and other genetic syndromes) and unclassified diabetes. All these are described in the recent WHO report on the classification of diabetes mellitus. (World Health Organization et al. 2019).

2.1.2 Prevalence of T2DM in the world and Malaysia

According to IDF Diabetes Atlas Ninth Edition 2019, 1 in 11 adults were living with diabetes, which is around 463 million adults. The number is predicted to continue to rise to 578 million by 2030 and further to 700 million by 2045 (51% increase compared to 2019) (Cho et al. 2018, International Diabetes Federation 2019).

Diabetes prevalence in Malaysia of ages 20 to 79 is 16.7% of the population (age 20-79), according to the World Bank data (International Diabetes Federation

2019). 3.6 million Malaysian are living with diabetes, which is not only the highest rate of incidence in Asia but also one of the highest in the world. With the current forecast, the prevalence of DM in Malaysia will reach 31.3% by the year 2025, predicting there will be 7 million Malaysian aged 18 years and above will be living with diabetes (Ministry of Health Malaysia 2016).

2.1.3 Pathogenesis of T2DM

In general, the glucose level is maintained by a dynamic interaction between glucose absorption, glucose production and glucose utilisation. All these are controlled by hormones in our body, primarily insulin and other hormones like glucagon and many others (Clapham 2020). After a meal, the food is degraded into glucose. When the body has high glucose levels in the blood after a meal, the glucose-sensitive beta cell in the pancreas will secrete insulin. Insulin acts as the hormone that regulates the metabolism of carbohydrates, fats, and proteins, it will go into action by giving commands to the insulin-sensitive tissue such as liver, adipose tissue, and muscle (Wilcox 2005) to induce absorption of glucose into these organs. The remaining glucose in the blood will be absorbed by the non-insulin mediated organs like the brain and kidney (Baron et al. 1985). The glucose will either be converted into energy for cell consumption (increase cell metabolism) or be stored as glycogen or fats in the liver and adipose tissue. Besides, insulin also will halt the production of glucose in the liver.

T2DM is mainly caused by insulin insensitivity and insulin deficiency. When the feedback loops between insulin action in insulin-sensitive tissue (insulin resistance) and insulin secretion (beta-cell dysfunction) are disrupted, the communication between insulin-sensitive tissue and insulin secretion organ is affected. As the results, the body cannot regulate glucose correctly and resulted in an abnormal level of blood glucose

(Stumvoll et al. 2005). Insulin resistance causes insulin not able to suppress the hepatic glucose output and increase the glucose uptake and utilisation (raised blood glucose). When the beta cells are resistant to insulin, the beta-cell increases insulin secretion to compensate for the lack of hormonal action. Eventually, the beta cell is exhausted by providing the "high demand" of insulin and caused the cell to die, resulting in insulin deficiency where the body produces less and less insulin (Reaven 1995, Tesauro and Mazzotta 2020).

2.1.4 Risk factor of T2DM

The increasing prevalence of diabetes worldwide is due to an interplay of many factors. The wide range of risk factors includes socioeconomic, demographic, environmental, and genetics.

Ageing is one of the risk factors for T2DM. Studies had found that adults aged 45 and above were the age group most diagnosed with T2DM (Cho et al. 2018, Asiimwe et al. 2020). Mitochondrial function declines and changes in skeletal muscle protein/mass (sarcopenia) attributed to T2DM at an older age (Pereira et al. 2008, Yakaryılmaz and Öztürk 2017, Durruty 2019).

Obesity and overweight are amongst the major risk factors for T2DM. High body mass index (BMI >30 kg/m^2), and excess adiposity is associated with many metabolic diseases, including T2DM (Sinha et al. 2002). Besides, independent of BMI, abdominal obesity characterised by waist circumference and waist-hip ratio can also predict T2DM risk (Carey et al. 1997). Obesity results in an abnormal level of non-esterified fatty acids, proteins (A beta isoform of protein kinase C, TNF alpha, interleukin-6), hormones (leptin, adiponectin, and resistin) and many other factors causing insulin resistance (Kahn et al. 2006, Feng et al. 2014, AlSaraj 2015, Durruty

2019). However, a large portion of people living with T2DM in Europe and Asian countries are non-obese. Despite a normal BMI, they generally have a higher degree of abdominal and total fat masses (Vaag and Lund 2007).

Besides, unhealthy lifestyles including insufficient physical activity and poor dietary patterns, which are closely related to obesity, are also the risk factor of T2DM. Major clinical experiments had shown that intensive lifestyle intervention could lower the T2DM incidence by 58% compared to those without (Knowler et al. 2002). A dietary pattern that had a lower intake of fruits, vegetables, whole grains, nuts, legumes, and long-chain fats and higher intakes of red meats, refined grains, sodium, trans fat, and sugar-sweetened beverages increased T2DM risk (Fung et al. 2004, Schulze et al. 2005, Ley et al. 2016, Cradock et al. 2017). Physical inactivity has been reported to have a strong association with T2DM by many studies (Weinstein et al. 2001). Sedentary behaviour characterised by activities of low energy expenditure such as watching TV had been reported to increase the risk of T2DM (3.4% increase per hour spent on watching television) (Rockette-Wagner et al. 2015).

In addition, an unhealthy lifestyle like smoking also increases the risk of T2DM (Willi et al. 2007). Not only the smokers but the second-hand smoke also reported increasing T2DM risk (Hayashino et al. 2008). Smoking induced insulin resistance and affect insulin secretion (Reaven and Tsao 2003). Besides, short and long sleep duration also increased T2DM risk (Shan et al. 2015).

Recent studies had also shown that genetics plays a strong role in the predisposition of T2DM. For the children whose one of the parents had DM the relative risk was 3.6, and the risk was almost doubled to 6 if both parents had the disease (Meigs et al. 2000). Most of the gene involved affects insulin secretion (CDKAL1, CDKN2A, CDKN2B, MTR1B, TCF7L2, KCNJ11) whereas a smaller number of genes affect

insulin action (FTO, IRS1, PPARG) (McCarthy 2010). However, gene predeposition of T2DM is polygenic and complicated (Fuchsberger et al. 2016).

Other risk factors of T2DM include people living with fatty liver, variation, diversity and composition of gut microbiota (people living with T2DM have a lower level of butyrate-producing bacteria), air pollution and use of some medication such as statin, thiazides and beta-blockers (Qin et al. 2012, Rao et al. 2015, Anyanwagu et al. 2016).

2.1.5 Complication of T2DM

Diabetes and its complication caused the death of approximately 4.2 million adults in 2019. It is also estimated with 11.3% global death of adults aged 20-79 years (International Diabetes Federation 2019). Complications of T2DM are divided into macrovascular complications (heart, brain, and blood vessel) and microvascular complications (eye, kidney, and nerve diseases). An observational study had shown that 50% of people with T2DM have microvascular complications, while 27% of them have macrovascular complications (Litwak et al. 2013).

T2DM increases the risk of cardiovascular diseases (CVD), such as heart attack and stroke. CVD attributed to half of the mortality of people living with T2DM, where coronary artery disease (CAD) was most lethal, followed by stroke (Einarson et al. 2018). People living with T2DM had twice the risk of developing CVD than those who are not (Emerging Risk Factors Collaboration 2010).

Microvascular complication includes retinopathy, nephropathy, and neuropathy. People living with T2DM will experience some degree of retinopathy, which eventually causes blindness. The prevalence of diabetic retinopathy ranges from 16% to 35% in Asian countries (Goh 2008, Wong et al. 2008, Jee et al. 2013). Diabetic nephropathy is

also a prevalent complication in T2DM. T2DM is a significant contributor to chronic kidney disease, and 10% of T2DM related death is due to renal failures (Susan et al. 2010). In 2014, 61% of new patients requiring dialysis in Malaysia had diabetes mellitus (Abdul et al. 2017). The clinical sign of diabetic nephropathy is albuminuria, reduced glomerular filtration rate, or both (Afkarian et al. 2016). As for the effect on neuropathy, peripherical neuropathy, affecting the distal nerves of the limb is the most common (International Diabetes Federation 2019). As the condition gets severe, limb amputation is unavoidable. People with diabetes who had their lower limb amputated are 10 to 20 times without diabetes (Moxey et al. 2011). In recent years, it was reported that T2DM is linked with several cancer types and also higher cancer mortality (Jalving et al. 2010, Hua et al. 2016).

2.1.6 Management of T2DM

Besides the usage of blood glucose-lowering drugs like insulin, metformin, and sulfonylurea, lifestyle modification had been reported to be helpful in the care and management of T2DM (Schellenberg et al. 2013). Lifestyle modification includes healthy eating and increases physical activity. People living with diabetes who are obese are encouraged to lose weight (0.5-1 kg per week), and within 6 months, they should lose 5 to 10% of their initial weight (Tuomilehto et al. 2001, Knowler et al. 2002). The way to achieve this goal is by reducing energy intake and increasing energy output.

The amount of food and calories intake will determine body composition. Healthy eating defines as “the ability of the individual to choose a variety of foods from all food groups with suitable portions and healthier food preparations” (Malaysian Diabetes Educators Society 2016). Ideally, one with diabetes should consult a dietitian to have tailored diet management to achieve their blood glucose, lipids, and blood

pressure goals (American Diabetes Association 2015b). Nevertheless, there are a few general recommendations for all the food groups to abide by for one with diabetes. There is no ideal percentage of energy from carbohydrates, protein, and fat for diabetes. A balanced diet containing 45-60% energy from carbohydrates, 15-20% energy from protein, and 25-35% energy from fats are encouraged (Malaysian Dietitians' Association 2013). The type (low in glycaemic index) and the amount of carbohydrates also are very important. Brown rice (whole grain) is able to lower the risk of T2DM by 16% when compared with white rice (refined grains) and substituting nuts, low-fat dairy, or whole grains is able to lower risk of T2DM by 16% to 35% compared with the same amount of red meat mainly processed red meat such as bacon, sausages, and hotdog (Sun et al. 2010, Pan et al. 2011).

People with diabetes should limit total fat (25-35% energy intake), saturated fats (< 7 % energy intakes), minimal trans-fat (<1% energy intake) and dietary cholesterol (< 200mg/day) in their diet to control body weight and lipid profile (Van Horn et al. 2008, Franz et al. 2010). Fatty fish high in omega 3 and 6 is recommended to be consumed at least two servings per week. Low fat food preparation like boiling, steaming, grilling, or baking is preferred over deep-fried food (Malaysian Diabetes Educators Society 2016). Reduced sodium intake is recommended, less than 2000 mg sodium per day or 5 g of salt a day.

Increased physical activity can improve the overall health and wellbeing by improving glycemic control and also lowering the mortality of people with diabetes (Hamasaki 2016). Types, frequency, duration, and intensity of physical activity should depend on an individual's condition. The ideal suggestion is that individuals should exercise 5 days a week (150 minutes) and no more than two consecutive days without physical activity (Boulé et al. 2003). T2DM incidence was lowered by approximately

50% by walking for at least 30 min per day (Hamasaki 2016). Both aerobic and resistance exercises are beneficial for people with diabetes.

Lifestyle intervention is an effective method to reduce the risks of diabetes. Nevertheless, many patients find it difficult to abide. When the lifestyle intervention failed or the inability of a diabetic patient to manage blood glucose, medical or pharmacological treatment becomes an important option. The oral drugs that are prescribed to the people with diabetes include metformin, insulin secretagogues including sulfonylureas (glyburide, glipizide, glimepiride) and meglitinides or glinides, alpha-glucosidase inhibitors (acarbose, miglitol and voglibose), thiazolidinediones (rosiglitazone, pioglitazone), dipeptidyl peptidase-4, DPP4 inhibitors (incretin agents such as sitagliptin, vildagliptin, saxagliptin, linagliptin and alogliptin), sodium glucose co-transporter -2, SGLT2 inhibitors (dapagliflozin, canagliflozin and empagliflozin). Besides, injectable agents such as RA-GLP1 (exenatide, lixisenatide, liraglutide,,exenatide LAR, albiglutide and dulaglutide) and insulin are also used in treatment of T2DM (Marín-Peñalver et al. 2016).

2.2 Metformin

Metformin (dimethylbiguanide) is one of the most common drugs, first-line treatment according to recommendations and guidelines worldwide, to treat T2DM. Metformin belongs to the biguanide class developed from galegine. It was first synthesised in 1922 but has only been used as a treatment of diabetes after Jean Sterne named the compound Glucophage when he published on metformin's properties in the 1950s. Figure 2.1 showed the chemical structure of metformin. Metformin is an antihyperglycemic agent which acts by decreasing hepatic glucose production, increasing peripheral glucose uptake, and halting gastrointestinal glucose absorption

(Bailey 1993). Metformin is effective in reducing fasting plasma insulin levels and improves insulin sensitivity but not insulin production, like sulfonylurea, it does not cause overt hypoglycaemia. Compared to sulfonylurea and insulin treatments, no elevation in body weight or adipose tissue accumulation is the “positive” side effect of metformin. Instead, metformin treatment has shown some weight loss. The influencing characteristic of metformin on AMP-activated protein kinase, a major player in lipid metabolism, leads to a different effect on adipose tissue and muscle mechanism. Moreover, metformin treatment was also reported to lower blood triglycerides, total and LDL cholesterol (Wulffelé et al. 2004). Figure 2.2 showed the mode of action of metformin on the various organ in the body.

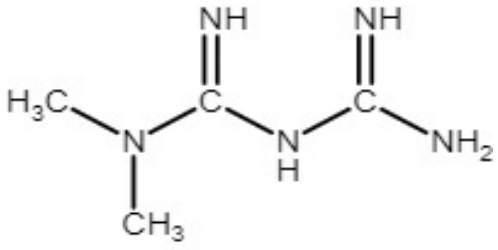

Figure 2.1 Chemical structure of metformin (N,N-dimethylimidodicarbonimidic diamide)

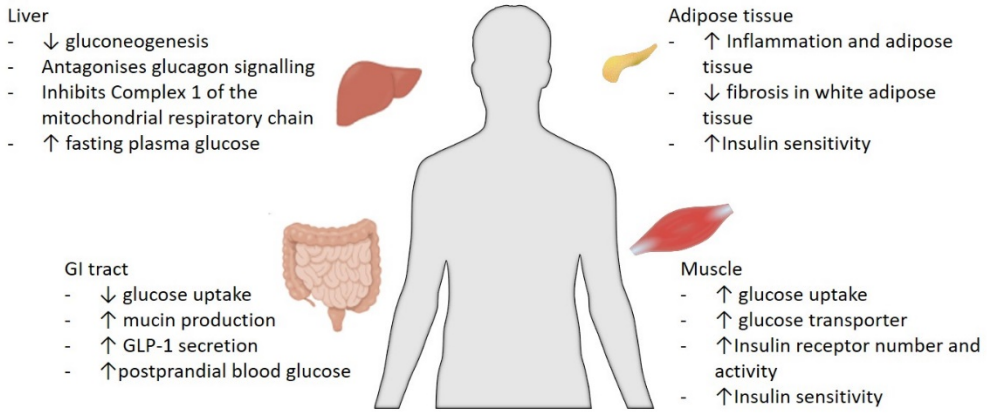

Figure 2.2 Summary of the metformin actions. [adapted from Thomas & Gregg (2017)].

2.3 Lipids and metabolic diseases

Lipid is defined as a group of compounds that are insoluble in water but soluble in the organic solvent (Cammack et al. 2006). Lipids participate in many vital roles in the biological system such as maintaining structural membrane, energy storage, and signalling molecules in cellular response. Perturbation in their expression will lead to metabolic pathways dysfunction and diseases (Finkelstein et al. 2014). The most common lipids that are involved in clinal diagnostic of metabolic diseases such as obesity and diabetes are triglycerides and cholesterol. Obese people and people living with diabetes have an increased prevalence of lipid disorders. Obesity lipid disorder consists of high triglycerides and free fatty acid, decreased high-density lipoprotein (HDL) and normal or increased low-density lipoproteins (LDL) (Klop et al. 2013). On the other hand, the most common lipid abnormalities by diabetes are high triglyceride levels and low HDL cholesterol values. The total and LDL cholesterol are usually not significantly different in those with diabetes and without diabetes (Solano and Goldberg 2006). Changes of lipid profile in diabetes mellitus are due to the defects of insulin action and high blood glucose levels (Merkel et al. 2002). Lipoprotein lipase (LPL),

which is regulated by insulin, promotes the lipolysis of chylomicrons triglycerides and the release of fatty acid. Insulin resistance reduced LPL activity (Merkel et al. 2002). Thus, increase blood triglycerides and decreasing HDL cholesterol in diabetes. The reduced level of HDL in diabetes is also the result of the action of cholesteryl-ester-transfer protein, increased activity of hepatic lipase, increase triglycerides hydrolysis, and the forming of smaller HDL (Hopkins and Barter 1986, Chahil and Ginsberg 2006). The smaller HDL is more readily and rapidly removed by the liver than the large size HDL.

2.3.1 Triglycerides

Triglycerides or triglycerols are under the class lipid of glycerolipids, in which the esterified fatty acid is attached to the hydroxyl groups of the glycerol backbone. Triglycerides naturally occur in animals and plant oils (Butterworth 2005). Triglycerides can be formed by different combinations of FAs with glycerol. Triglycerides found in plants and animals are usually formed by FAs with even-numbered long hydrocarbon chains (Hu et al. 2009). The different composition of FAs in triglycerides is related to various biological synthesis pathways and is essential for cellular bioactivity. Triglycerides not only play a crucial role in energy storage in the body but also play a role in the metabolism process and diseases (Han et al. 2000).

Hypertriglyceridemia was reported to impair β-cell function and cause insulin resistance causing diabetes to deteriorate. Triglycerides deposition in islets and accumulation of metabolites from fatty acid metabolism damaged β-cell function, resulting in a higher blood glucose level due to decreased insulin secretion (Unger 1995, Robertson et al. 2004, Zhang, Wang, et al. 2017). The metabolites of triglycerides such as free fatty acid and diacylglycerol activate several pathways such as serine/threonine

kinases pathways, suppress insulin receptors and eventually lead to insulin resistance (Grundy 1999, Schinner et al. 2005).

2.3.2 Cholesterol and plant sterol

Cholesterol is a simple lipid, belongs to the sterols groups, consists of a tetracyclic ring with a double bond and a free hydroxyl group. Sterols are essential components of membrane lipids and they have important roles in cell regulation related to cell signalling and fluidity modulation. Cholesterol is found in the free state and also esterified state (cholesterol esters). Free cholesterol mainly helps in maintaining membrane fluidity while the body stores cholesterol in esterified form (Zhang and Liu 2015). Cholesterol in the body can be produced endogenously or exogenously where roughly 20-25% of cholesterol comes from the diet (Norum et al. 1983). Cholesterol is found in animals, whereas plant sterol which is also known as phytosterols (structurally similar to cholesterol which only differed by side chains) such as stigmasterol, sitosterol, ergosterol, etc. is found in the plant (Law 2000). Cholesterol levels regulation in blood was attained by the homeostasis between cholesterol absorption and cholesterol synthesis. It was reported that diabetes lowered cholesterol absorption and increase cholesterol synthesis while weight reduction increases cholesterol absorption (Gylling and Miettinen 1997, Simonen et al. 2000). Dietary phytosterols are found in vegetable oils, seeds, nuts, grain products, vegetables, legumes and fruits (Trautwein, Vermeer, et al. 2018).

The plant sterols are reported to be beneficial to the body as they lower cholesterol absorption in the gut and also LDL-cholesterol with intakes of 1.5 to 3 g/day (Best et al. 1954, Trautwein et al. 2018). Besides, the blood concentration of phytosterols was also reported to be correlated with the absorption of dietary cholesterol

(Tilvis and Miettinen 1986). Plant sterols have been reported to be effective in improving blood lipid profile and thereby decreasing cardiovascular disease and other diabetes risks (Calpe-Berdiel et al. 2009, Jones and AbuMweis 2009, Misawa et al. 2012). On top of the cardioprotective effect, plant sterols have been shown to alleviate cancers (breast, prostate, lung, liver, stomach and ovary) or reduce the risk of cancer by 20% (Ramprasath and Awad 2015, Jiang et al. 2019) . Plant sterols can inhibit cell proliferation, metastasis, and apoptosis (Choi et al. 2003, Awad et al. 2005, Baskar et al. 2012). Besides, plant sterols exert antitumor effects by improving the immune system's ability in fighting cancer, affecting hormone-dependent endocrine tumour growth, and regulating sterol biosynthesis (Bouic et al. 1996, Bouic 2001, Awad et al. 2004). Plant sterols can protect against non-alcoholic steatohepatitis resulting from high fat and high cholesterol diet by preventing hepatic inflammation (Plat et al. 2014).

2.4 Palm oil

Fat is one of the essential macronutrients needed by the human body to sustain life by providing calories or energy to the body. Historically, people are encouraged to reduce their dietary fats for better health. One of the shreds of evidence supporting this advice is the association of saturated fat composition and the risk of cardiometabolic diseases (Forouhi et al. 2018).

Nevertheless, in recent decades, more and more studies had shown that the healthiest diets are those rich in fats from vegetable oil, nuts, and seafood (Mozaffarian 2016). Several dietary guidelines suggest a high fat diet for people living with cardiovascular disease, obesity-related diseases, and cancer (Aranceta and Pérez-Rodrigo 2012, Berger 2014). High fat diet that exceeds 35% daily limit of calories,

especially those that are high in healthy fat can reduce the risk of diabetes (Mozaffarian 2016).

Palm oil (PO), which is derived from the fruit of the Palm tree, *Elaeis guineensis*, is the most consumed vegetable oil in the world (Mba et al. 2015). Palm oil's yield per hectare of land is higher compared to other vegetable oil, and PO had overtaken soybean oil as the highest edible vegetable oil in the world (Scanes 2018). Malaysia and Indonesia are the primary producers of PO (86% of global production) (Mancini et al. 2015). Therefore, in Malaysia, PO based cooking oil is easily obtained by the public, and the price is lower compared to other vegetable oil. PO is very popular in the household cooking and food industry for its texture, fragrance, and neutral taste compared to other vegetable oils. Besides, PO has a high smoke point at 230°C, which makes it suitable for most cooking methods. Also, the high content of saturated fatty acid (SFA) causes PO to have a higher resistance to oxidative changes (Edem 2002).

PO has a balanced ratio of unsaturated and saturated fatty acids. It contains 40% oleic acid (monounsaturated fatty acid), 10% linoleic acid (polyunsaturated fatty acid), 45% palmitic acid (PA) and 5% stearic acid (SFA), it is the most consumed saturated fat in Asia, Africa, and Europe countries (Sambanthamurthi et al. 2000, Edem 2002). PA is the most abundant SFA naturally present in vegetable oil, animal fats, and human milk fat (Jensen 1999). Unlike animal fats, PO is cholesterol-free. PO's fatty acids are mainly structured as triglycerides (TAGs). TAGs in PO are mostly made up of PA and oleic acid, PA mainly located at Sn-1 and Sn-3 positions, while oleic acid is mainly placed at the Sn-2 position. Figure 2.3 shows the typical structure of TAGs in PO (Sambanthamurthi et al. 2000, May and Nesaretnam 2014).

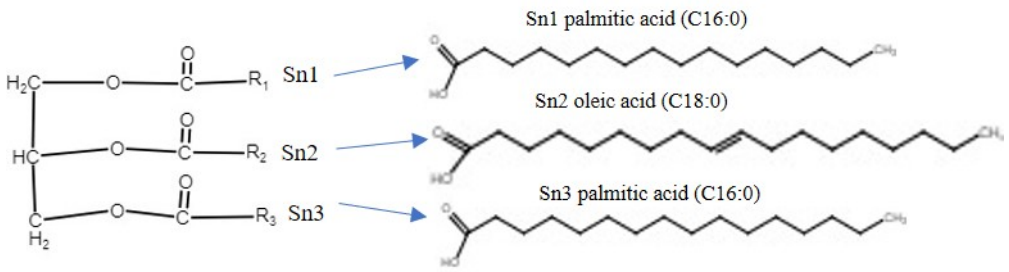

Figure 2.3 Structure of palm oil and its triglycerides.

2.4.1 Palm oil and diabetes mellitus

Excess energy or calories due to the high intake of fats are stored in adipocytes as TAGs, which lead to lipid accumulation, mainly white adipose tissue (WAT) accumulation. The excess TAGs stored in adipocytes caused hypertrophy and hyperplasia of adipocytes (Dixon 2010). Enlarged adipocytes demonstrate decreased insulin responsiveness, decreased glucose uptake, and increased secretion of proinflammatory adipokines. Elevated SFA promotes inflammatory mechanism not only through activating Toll-like receptor 4 (TLR4) but also TLR – independent pathway through reactive oxygen species (ROS) (Bradley et al. 2008, Ting et al. 2008, Franchi et al. 2009, Wang et al. 2013). Laugerette and his coworker (2012) have reported that the PO-enrich diet, high in PA, promotes the secretion of inflammatory markers in plasma (IL-6) and white adipose tissue (IL-1B, TLR4, and CD4). All this alteration on proinflammatory cytokines, such as up TNF-α, leptin, and adiponectin, play a prominent role in the development of insulin resistance, the primary precursor of T2DM. Besides, a PO-enrich diet containing high TAGs results in elevated blood serum TGAs. Scientists had found out that a high level of serum TGA is linked with insulin resistance, supporting some findings of PO enriched diets impairs glucose tolerance in mice (Storlien et al. 2000, Kochikuzhyil et al. 2010). However, some studies conducted

on humans showed conflicting results—no significant effects on plasma glucose levels in T2DM patients after four weeks of PO treatment. High dietary fats had been strongly associated with obesity, especially excess fats in the abdominal region, which have a detrimental effect on insulin resistance. The association between PO and T2DM remains controversial as there are limited studies linking vegetable oil to T2DM. This had inspired Zulkiply and coworkers (2019) to do a review of the effects of palm oil consumption on glucose metabolism biomarkers. They concluded that there is no extra benefit to replace palm oil with other unsaturated fatty acid-rich oil, as the evidence of the effect of palm oil consumption on the glucose metabolism marker is not significant.

2.5 Kidney

Kidneys are the two-bean shape organ located in the retroperitoneal space. The kidney is essential for the body to remove toxin as the toxin and waster products are filtered in the kidney and removed from the body by urine. The kidney also plays critical roles in the homeostasis of the body including fluids osmolality, acid-base balance, electrolyte and salts concentration.

2.5.1 Kidney and diabetes mellitus

The kidney is one of the vital and vulnerable organs that is impacted by diabetes. Diabetes is the most common cause of chronic kidney disease (CKD) and also end-stage renal diseases. CKD is defined as kidney damage or reduced kidney glomerular filtration rate (GFR) of < 60 ml/min/1.73 m^2 for at least three months. Diabetes together with CKD, also increases the risk of cardiovascular diseases. High blood glucose level causes an increase in the workload of the glomerular filtration barrier as more glucose

is filtered through it. Pathological glomerular changes in diabetic kidney diseases (DKD) includes thickening of glomerular basement membrane, podocyte injury, mesangial matrix expansion, and loss of endothelium fenestrations which cause by the production of certain molecules such as growth factors, toxic products, TGFβ, advanced glycosylated end products, protein kinase C, and aldose reductase due to high blood glucose level. These alterations will directly cause changes in microvascular permeability and damage the glomerular filtration barrier. Eventually, these dysfunctions will result in microalbuminuria which is the clinical hallmark of DKD. For example, mesangial matrix expansion increases systemic hypertension advancement and urinary albumin excretion and reduced glomerular filtration (Shahbazian and Rezaii 2013, Reidy et al. 2014, Fu et al. 2019). Besides, DKD is also closely related with systemic and local renal inflammation with the involvement of inflammatory cells such as macrophages, mast cells and T-lymphocytes, oxidative stress, and fibrosis which was thought to be induced by hemodynamic changes and metabolic disorders (Fu et al. 2019). However, the pathogenesis of DKD is complex and multifactorial. More studies need to be done to understand DKD further. With the advancement of microarray and mass spectrometry, changes of several proteins such as α1-Antitrypsin, transferrin, haptoglobin, and Vitamin D-binding proteins are found to be associated with diabetic kidney disease or kidney defects (Bhensdadia et al. 2013, Gluhovschi et al. 2016, Fawzy and Abu AlSel 2018, Kim et al. 2018, Żyłka et al. 2018).

2.5.2 Kidney, high fat diet and obesity

High fat diet induces obesity. The presence of obesity especially excess visceral fat, dyslipidaemia, hypertension and diabetes increase the risk of kidney disease and its progression (Eriksson et al. 2013). High fat diet and lipids can also accelerate the

decline in kidney function by altering the renal morphology and the deposition of fats in the kidney (Altunkaynak et al. 2008, Deji et al. 2009). The deposition of lipids in the kidney may cause damage to the mesangial cell, endothelial cells, and glomerular podocytes. Lipid accumulation induced inflammatory reaction and matrix production in the kidney may also cause glomerulosclerosis (Mesquita et al. 2010). Besides, high fat diet also caused dyslipidaemia (Feng et al. 2019). It is reported that the treatment of lipid disorders reduced kidney diseases. Statin, a cholesterol-lowering drug, also reported being renoprotective. Statin is not only able to reduce lipids level but also able to reduce interstitial inflammation, improve haemodynamic of kidney and lower glomerular proteinuria (Mesquita et al. 2010, Mikolasevic et al. 2017).

2.6 Urine metabolites, diet and diseases

Urine is a transparent, sterile and yellowish-coloured fluid produced by mammals. Urine is the primary route to eliminate the water-soluble waste products from the body by the kidney. The kidney will extract the soluble wastes from the bloodstream together with the excess water, resulting in the presence of sugars, urea, inorganic salts, organic acids, creatinine, ammonia and various toxin and products of metabolism breakdown in the urine (Bouatra et al. 2013). Although urine is considered as a waste product, it has been known as a rich biofluid for medical diagnostic, and many clinical assays are performed on urine (Khamis et al. 2017). The condition and colour of urine may help physicians to diagnose certain diseases. For example, brownish colour urine may indicate jaundice, colourless urine may indicate diabetes, and foamy urine may indicate proteinuria (Bouatra et al. 2013). Although other biofluids such as flood and saliva could also be used, urine has its advantages as it can be collected easily, passively, non-invasively and longitudinally (Wald 2017). Besides, urine lacks homeostasis

mechanism (Gao 2013). There are approximately 4500 metabolites detected in urine, associated with close to 600 human conditions such as obesity, diabetes, cancer, inflammation, etc. (Bouatra et al. 2013, Khamis et al. 2017, Miller et al. 2019). Besides diseases, intrinsic factors (age, gender, and hormonal status) and extrinsic factors (lifestyle, diet, and exercise) will affect by the changes in urinary metabolites (Wu and Gao 2015). Dietary intake can affect kidney filtration and tubular reabsorption by interfering with the acid-base balance, water levels and electrolytes and various metabolism in the body. It is reported that dietary differences can be detected in urine with more sensitivity compared to blood or saliva (Walsh et al. 2006). Many studies had done on the discovery of biomarkers related to dietary exposure or food intervention via urinary metabolomics-based analysis (Beckmann et al. 2013, May et al. 2013, Pujos-Guillot et al. 2013, Gibbons et al. 2015). Besides the direct effect of dietary exposure itself, food intervention like high fat diet also leads to metabolic diseases such as obesity and diabetes. It is well reported that high fat-induced obesity and diabetes altered the metabolites in the urine as the energy metabolism is perturbed (Stella et al. 2006, Shearer et al. 2008, Stec et al. 2015, Wei et al. 2015, Wang, Bergeron, et al. 2018, Pan et al. 2019, Maulidiani et al. 2020).

2.7 Omics study and methods for omics study

Omics study refers to the field that ends with -omics in biological sciences. Omics study includes genomics, transcriptomics, proteomics, metabolomics, or lipidomic. Genomics studies the structure, function, evolution, and mapping of genes and genomes; transcriptomic studies transcriptome, messenger RNA that involve gene transcription and translation. Proteomics is the science that study proteome (all the protein in the organism) while metabolomics studies metabolome (all the metabolites

in the organism). Lipidomic is the study of lipidome produced by the living organism (Vailati-Riboni et al. 2017). Each omics are closely related to each other as the flows of information in the living organism is from DNA (genomics) to RNA (transcriptomics) to protein (proteomics) and finally to metabolites (metabolome).

The transcription and translation of DNA and RNA yield a broad range of structural and functional proteins, which play significant roles in metabolites modulation, which are derived from endogenous metabolism or exogenous sources (Dubin and Rhee 2020). Proteins and metabolites may be influenced by age, sex, diet, environmental factors, and microbiome (Rebholz et al. 2019, Shao et al. 2019). Besides, all fields (DNA, RNA, protein, and metabolites) are interrelated and can influence one another. Changes in proteome and metabolome may result in a feedback loop and ultimately affect the transcription and translation of DNA and RNA. This flow is shown in Figure 2.4. The advancement of science and high throughput technologies, especially in the omics study, had helped in biological and medical research. Commonly each type of omics data shows a list of differences related to the disease, which gives insight into the different metabolic processes or pathways between the diseases and control group. However, analysing one type of data is limited to correlations (Hasin et al. 2017). The integration of multiple omics enables the researcher to have a more comprehensive and holistic picture of biological problems and diseases.