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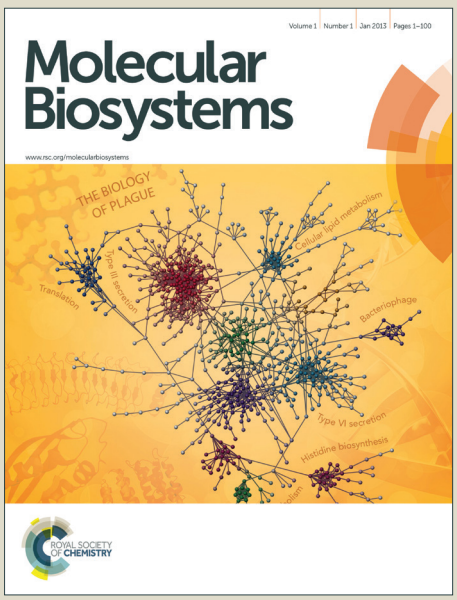

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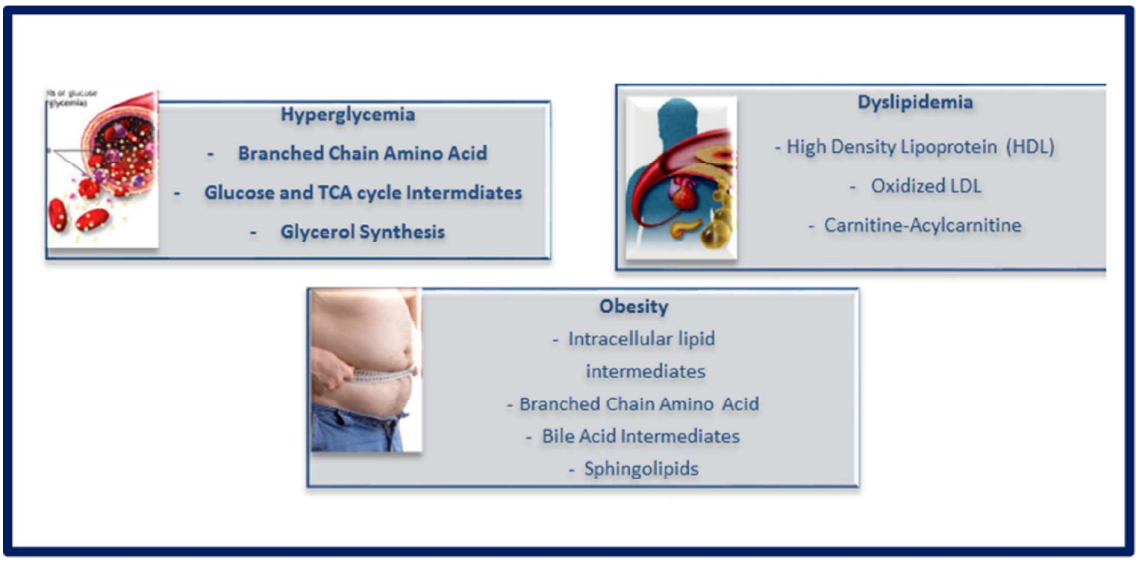
Table of content entry

This paper highlights the metabolomic roles in systems biology towards elucidation of metabolic mechanisms in obesity and type 2 diabetes.



Metabolomics – The Complementary Field in Systems Biology: A Review on Obesity and Type 2 Diabetes

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Abstract

Metabolomic studies on obesity and type 2 diabetes mellitus have led to a number of mechanistic insights in biomarker discovery and comprehending of disease progressions at metabolic levels. This article reviews a series of metabolomic studies carried out in previous and recent years on obesity and type 2 diabetes, which have showed potential metabolic biomarkers for further evaluations of the diseases. Literature including journals and books from Web of Science, Pubmed and related databases reporting on the metabolomics in these particular disorders are reviewed. We herein discuss the potential of reported metabolic biomarkers for novel understanding of disease processes. These biomarkers include fatty acids, TCA cycle intermediates, carbohydrates, amino acids, choline and bile acids. The biological activities and aetiological pathways of metabolites of interest in driving these intricate processes are explained. The data from various publications supported metabolomics as an effective strategy in the identification of novel biomarkers for obesity and type 2 diabetes. Accelerating interest in the perspective of metabolomics to complement other fields in systems biology towards in-depth understanding of the molecular mechanisms underlying the diseases is also well appreciated. As conclusion, metabolomics can be used as one of the alternative approaches in biomarker discovery and novel understanding of pathophysiological mechanisms in obesity and type 2 diabetes. It can be foreseen that there will be an increasing research interest to combine metabolomics with other omics platforms towards establishment of detailed mechanistic evidences associated with the disease processes.

Keywords: **metabolomics; type 2 diabetes; obesity; biomarkers; systems biology, biological fluids**

Article Outlines

Abbreviations

NMR	-	Nuclear Magnetic Resonance
MS	-	Mass Spectrometry
LC-MS/MS	-	Liquid Chromatography Tandem Mass Spectrometry
GC-MS	-	Gas Chromatography-Mass Spectrometry
ROS	-	Reactive Oxygen Species
GWAS	-	Genome-Wide Association Studies
SNP	-	Single Nucleotide Polymorphism
TCA	-	Tricarboxylic Acid Cycle
TOF	-	Time of Flight
ATP	-	Adenosine Triphosphate
BCAA	-	Branched-Chain Amino Acid
PCA	-	Principal Component Analysis
OGTT	-	Oral Glucose Tolerance Test
IFG	-	Impaired Fasting Glucose
IGT	-	Impaired Glucose Tolerance

1. Introduction

In the past decade, metabolomics has made remarkable progress in providing useful systematic insight into the mechanisms underlying numerous metabolic diseases such as type 2 diabetes, obesity and cancer. The findings had mainly led to establishment of biomarkers and risk factors associated with these diseases. This burgeoning area offers opportunities to paint out the dynamic picture of the phenotype of an organism through the comprehensive analysis of endogenous and exogenous metabolites in biological fluids, tissues, and cells. Metabolomics enables simultaneous measurement of a large part of the metabolites present in a biological sample [1]. A number of definitions of metabolomics appear in literature. It is favourable to use the definition from *The Metabolomics Society* that interprets metabolomics as a comprehensive and holistic identification and quantification of intracellular and extracellular metabolites in a biological system. It provides an overview of metabolic picture and global biochemical process in a given sample [2–4]. Metabolome is a final set of small molecular mass (below 1,500 Da) organic compound in a biological system or total complement of metabolites in the cell. These molecules can be either the substrates or products of the complex biochemical networks associated with the cellular and systemic biological pathways. Carbohydrates, fatty acids, and amino acids are the common metabolites involved in various physiological processes for signalling, survival, and structural functions. These key metabolites are particularly present in blood serum, urine, bile, seminal fluid, synovial fluid, amniotic fluid, gut aspirate, cerebrospinal fluid, breath, tissues and saliva [5–9]. As such, the biological regulations of these small molecules have been reported to act through covalent modification (post-translation modification) of the involved transcripts (mRNA) and the protein of the cellular pathways as a whole [4].

Metabolic phenotype is the total reflection (end product) of the biological changes at the systemic level and environmental factors that is synergistically affected by the integrity of genes, enzymes, and related proteins in complex ways. The genome indirectly indicates what may happen, but metabolome defines the end point of the process on what has already happened. The prominent advantage of the application of metabolomics is the ability of analytical tools to detect a variety of metabolites at one time, thereby providing an insight on the functional readout of the cellular biochemical pathways in an organism. Obesity and type 2 diabetes are rapidly becoming common disorders that are precipitated by complex interactions between genetics, hormonal defects, metabolism and environmental predisposition (diet and lifestyles). Therefore, metabolomics is considered as a promising approach to unveil the pathophysiological mechanisms underpinning these disorders. An overview of Web of Science database for the application of metabolomics in obesity and type 2 diabetes research (Figure 1) clearly shows a rapid growth in the number of studies using metabolomics in identifying biomarkers associated with the disease process.

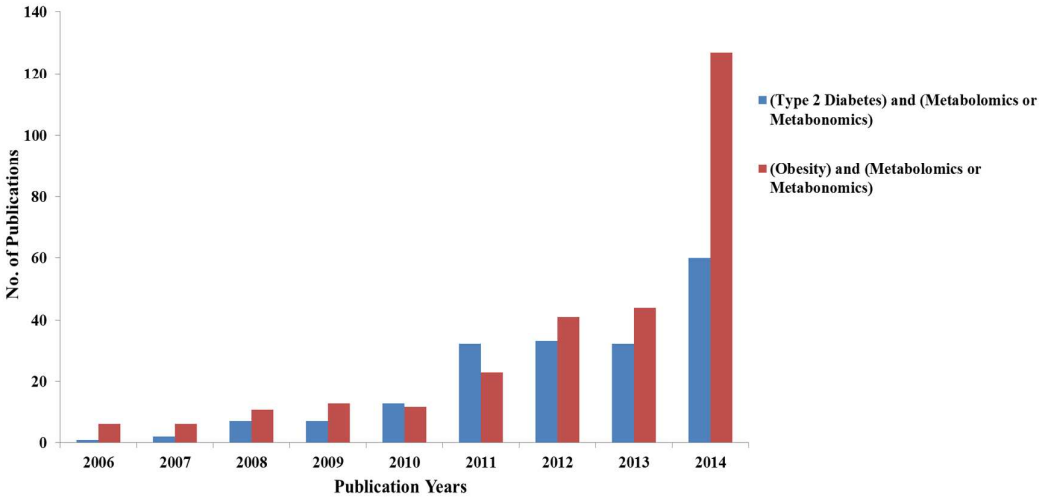


Figure 1. The general trend from Web of Science database for the past nine years shows that the number of metabolomic studies on obesity and type 2 diabetes has undergone unprecedented growth.

In the metabolic studies, human subjects and animal models were extensively used to provide new prospects for potential biomarkers in type 2 diabetes and obesity. The use of body fluids, such as blood and urine, is routinely practised by clinicians to diagnose the risks of developing diabetes symptoms [10,11]. The clinical symptoms such as dyslipidemia, hyperinsulinemia, glucose intolerance, and hypertension can be detected indirectly via the analysis of body fluids in the form of metabolites [12]. The simple clinical tests usually come with the blood sugar and electrolyte balance analysis. These metabolic variations are a result of combined effects of genetic variation and environmental factors [13–15]. Despite decades of intense research, large parts of the metabolic pathophysiological aspect of these disorders still remain elusive. Therefore, there is a lack of non-invasive, sensitive, and specific diagnostic tools based on bodily fluid biomarkers for these disorders. Recent advances in systems biology may add some levels of understanding that can enable us to obtain novel mechanistic insights and uncover potential therapeutic targets. However, a key challenge for scientific communities in these fields nowadays is to integrate the complexities of an astonishing amount of systems biology datasets into useful biological interpretations.

Metabolomes are considered as the most terminal reporters of biochemical pathways that can pinpoint the specific perturbations in a disease process. Metabolic profiling holds many promising features in diagnosing the disease by offering complementary mechanistic evidences in disease progressions. A series of review articles dealing with the metabolomics in type 2 diabetes and other metabolic disorders were published [4,16,17]. Nevertheless, to the best of our knowledge, a specific review article on metabolomic approaches concerning the roles of this field in complementing the progression of other omics fields in obesity and type 2 diabetes has not been published so far. Indeed, it would be interesting to assess the previous and on-going research on both laboratory and clinical application of metabolomics in these disorders in order to draw a broad picture of systems biology views. This will surely enable other researchers, clinicians, scientists and upcoming experts to construct the lag in till date research works and discover the best approach for futuristic advancements in managing and treating these disorders. Therefore, the aim of this paper is to evaluate and review the current

metabolomic applications, which had showed potential biomarkers in delineating associated mechanisms underlying obesity and type 2 diabetes.

2. The Roles of Metabolomics in Systems Biology

There is an increasing appreciation on the potential of combined multiple omics approaches such as genomics, transcriptomics, and proteomics towards comprehension of disease processes. In fact, great insights have been obtained from a large volume of published reviews and reports depicting the role of these fields in unraveling the potential mechanisms and possible risk factors associated with diseases [18–21]. With the current advancement in metabolomic platforms, it provides a new layer of biological information covering comprehensive metabolic perturbation. Small molecular changes in gene expressions, such as non-coding polymorphism and single base transition in certain genes, might result in rigorous amplifications of metabolite level in biological fluids. The relative amounts of metabolites in the human body are more readily amplified compared to those at the gene and protein levels, which may be detectable in biological fluids. It is self-evident that the cellular adaptations in certain peripheral cells and tissues are the result of integration of genetic and environmental factors that regulate the expression of metabolites produced through various complex biochemical regulatory networks. Thus, metabolomics may lead to improved understanding of the disease biological system at the molecular level, especially if it is combined with genomics, transcriptomics, and proteomics. These essential facts lead to the idea that metabolomics could provide insightful information on disease phenotype and disease onset predictions.

Metabolome consists of a diverse set of atomic arrangement of the molecules, which comprise carbohydrates, proteins, fatty acids, organic acids, alcohols, and nucleosides. These small molecules are illustrated through wide variation in their chemical properties such as size, polarity, concentration, molecular weight, and solubility. Currently, human metabolome consists of more than 41,000 small-molecule metabolites (version 3.5) archived in Human Metabolites Database (HMDB: http://www.hmdb.ca/) and some reviews point out that the number is much higher than estimated [22–24]. Metabolomics has just started within the century and advanced significantly. Importantly, metabolomics research nowadays is considered to be the next pillar in the field of omics and many scientists have recently started to divert their focus to this field for studying the roles of small endogenous metabolites from biological fluids, tissue extracts, and cells. This favoritism could be due to the fact that metabolomics is well-suited and reliable in discovering the potential biomarkers in various metabolic disorders such as obesity and type 2 diabetes. Indeed, the on-going accomplishments of this field have unsealed a number of mechanistic insights in biomarker discovery [25–28].

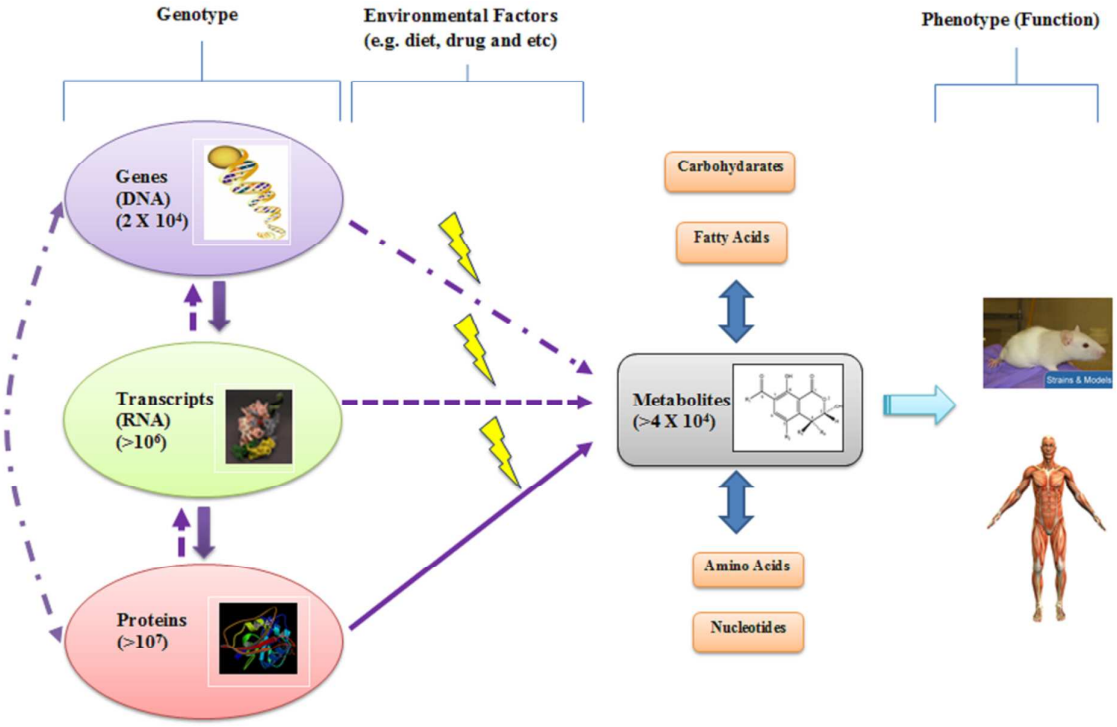


Figure 2. A schematic diagram of systems approach to biology. The correlative functions of each field in systems biology are interacting to each other in a dynamic and complex mechanism. The diagram essentially shows that metabolites are the terminal products downstream of genomes, transcriptomes, and proteomes in the whole organisms.

As shown in Figure 2, metabolites serve as the most proximal potential biomarkers of the disease and are strongly associated with regulatory signals that mediate hormonal changes and its effector [29]. Unlike genes and proteins that are highly regulated by various complex homeostatic functions, metabolites stand for their own expression that results from intermittent differences of gene and protein activities and environmental perturbation, hence making them more usable to be biomarkers [30]. In personalized medicine, the search for biomarkers with highest predictive values has gained considerable attention in studying the pathological aspect of human disease, therefore enabling the identification of novel therapies for the preventive measure. Biomarker is interpreted as a biological parameter that can be measured by a wide array of analytical platforms, which provide relative predictive values for therapeutic strategies in treating disorders. According to the National Institute of Health (NIH) Biomarker Definition Working Group, biomarker can be defined as "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacologic responses to a therapeutic intervention*" [31]. Biomarkers are widely used in the clinical practice and become one of the important applications in diagnostic tools for screening patients and measuring the prognostic values of their survival. Commonly, biomarkers used in the clinical settings are derived from the specific measurement of metabolites, protein, DNA in blood or urine sample. Ideally, the analysis of these samples is non-invasive, widespread, accessible, and low in cost per se.

Metabolites are the substrates, intermediates or products of these biological processes within the metabolic pathways. Many metabolic pathways are observed to be similar in human and rodents [32–34]. Therefore,

it could be easier to transfer data from one species to another in order to extrapolate the changes and perturbation of endogenous metabolites level in an organism. These advantages of metabolomics over genomics and proteomics give some added values for this field to become one of the powerful tools in translational research. This will enable the total assessment of metabolic pattern associated with pathology of the disease in human, animal and other mammalian cell culture model towards novel application to the clinical setting. Metabolic diseases such as obesity and type 2 diabetes represent the systemic alterations of energy metabolism that are tightly regulated with various complex interactions. This condition indirectly creates a highly robust biochemical profile that is accessible in the biological fluid and tissues of an organism, thereby presenting potential markers and risk factors to be identified for prognostic and diagnostic purposes. We believe that if perturbations are directly involved in systemic and local body metabolism, the screened biomarkers could be identified through urine, blood, and biological fluids surrounding the affected tissues.

3. Metabolomics Technologies

Genetic association studies offer a potentially powerful tool for extrapolating causal genes affiliated with disease progression. Yet, only a small number of genes can be explored at one time. The heritability index of general populations estimated for type 2 diabetes was found to be 0.4, but less than 10% of such a heritability in related loci were identified in genome-wide association studies (GWAS) [35–37]. It is estimated that almost 60 genetic loci were analyzed in type 2 diabetes.[38]; still, only 12 loci of these so-called heritability traits were significantly correlated with the development of this metabolic disease. Besides, all possible variants in single nucleotide polymorphisms (SNPs) studies have not yet provided substantial evidence. This is due to the specificity of the current analytical tools in these approaches are merely applicable for those genes with a major allele frequency greater than 1% [39]. Accordingly, SNPs that are less than 0.01 in certain genes still remain mysterious and undiscovered. For that reason, other progressive fields in systems biology, such as proteomics and metabolomics, should take these fascinating opportunities to understand and identify these 'missing links' in elucidating the exact mechanisms underpinning the pathophysiological of such diseases.

Integrated microarray analysis is recognized as one of the most successful approaches in biomarker discovery [40,41]. This analysis offers several advantages in analysing the complex phenotypes of numerous disorders, which may allow identification of risk factors associated with the disease progression. Even so, the application of transcriptomics and proteomics approaches in medical screening of various metabolic disorders are challenging in terms of cost and time. In the case of metabolic disorders, metabolomics analysis for diagnosis and prediction of the complex diseases such as obesity and type 2 diabetes can be a better alternative [42]. In fact, there has been significant attention in the literature to the use of metabolic profile analysis in comprehending the pathogenesis of metabolic diseases at the cellular and systemic level [43,44]. In the history of metabolomics, there have been a volume of methodological advancements that have led to the discovery of novel biomarkers, which provide greater understanding of disease course when metabolomics is combined with other omics fields [45]. It is thus conceivable that the advancement of mass spectrometry (MS) and nuclear magnetic resonance (NMR) has driven forward the vital understanding of various metabolic diseases to its current status as can be seen nowadays through the increase in the number of publications related to metabolomics over the years [46]. This promising field is still in its infancy and yet holds a position as a youngest sibling in the field of systems biology.

Metabolomics analysis is broadly categorized into two generalized experimental strategies, targeted and non-targeted approaches, depending on the goal of the research itself [47]. Targeted analysis is based on data hypothesis-driven research, which requires pre-defined set of metabolites. Typically, the quantification and characterization of the metabolites are focused on selected metabolites and some related etiological pathways. The targeted analysis typically uses internal standards and multiple reaction monitoring (MRM) for the absolute quantification in which the combination of chromatography and tandem MS are necessary. The analytical peaks are usually identified and quantified based on a unique combination of chromatographic retention time, precursor ion m/z ratio, and fragment ion m/z ratio. The data is scanned for specific compounds throughout the reference library and subsequently characterized and quantified. The advantage of this approach is that the identity of the measured metabolites is known. This advantage offers various biological interpretations of data and assists in reducing the confounding effects [48]. A non-targeted analysis is a metabolic profiling that provides a hypothesis-free analysis of metabolites. The prominent advantage of this analysis is the identification of

unknown metabolites that have not previously been quantified and characterized. In spite of that, the identification of new metabolites should be further validated in a targeted approach in order to confirm its identity. It is particularly noteworthy to observe that many findings on metabolomics have started to employ semi-targeted analysis [49,50]. The synergistic combination of various approaches and statistical tools might provide dynamic phenotypic pictures of the physiological systems as a whole.

The expanding field of metabolomics has directed the advancement of highly automated metabolomics profiling. There are a couple of analytical platforms such as NMR and MS coupled to several separation techniques including gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis. These platforms are known to be useful for the investigation of metabolic profiles employing biological fluids such as urine, blood plasma, saliva, and cerebrospinal fluid. These analytical platforms are used to generate a variety of dataset of complex biological samples for biological interpretation. MS-based approaches require a pre-separation of metabolic components using either a LC or a GC following chemical derivatization. The use of ultra and high-performance LC is mounting over the years, and capillary electrophoresis coupled to MS was also reported [51]. Other specialized analytical platforms such as Fourier transform infrared (FTIR) spectroscopy and electrochemical detection were utilized in certain cases as well [52]. The integrations of data from various commonly used analytical approaches are complementary to each other in providing insightful information of different sets of biomarkers classes. The combination of MS-based approaches and NMR result in all-inclusive data for metabolic profiling [53,54]. They also can provide opportunities to identify and discover many unknown metabolites towards further development of this rapidly emerging field. Indeed, there is much promise that an integrated multilayered omics approach is particularly valuable in delivering comprehensive classification of biomarker identity in obesity and type 2 diabetes.

The analytical methods used in metabolomics should be sensitive, specific, quantitative, and highly robust. The developments of these characteristics are formidable and complex as metabolites are classified and characterized from a wide range of compound classes present in the samples. Even though NMR has been the first analytical method used in metabolomics profiling, MS-based approaches are the early methods to be perceived as powerful techniques for the comprehensive metabolic profiling of the metabolome in the samples. Unlike NMR, MS-based approaches exhibit various capabilities ranging from its sensitivity, speed, and ability to interface directly with chromatography. Typically, GC and LC coupled to MS are employed since these methods combine high separation power and reproducible retention times with a versatile, sensitive, and selective mass detection. Numerous review articles on the limitation and technical application of both platforms have been comprehensively illustrated [1,55–58]. Given that MS-based approaches and NMR are the most commonly used methods in metabolomics; this review intends to focus mainly on the application of both approaches in obesity and type 2 diabetes, providing holistic understanding of biological mechanisms and its associated regulatory pathways.

4. Metabolomics in Obesity and Type 2 Diabetes Research

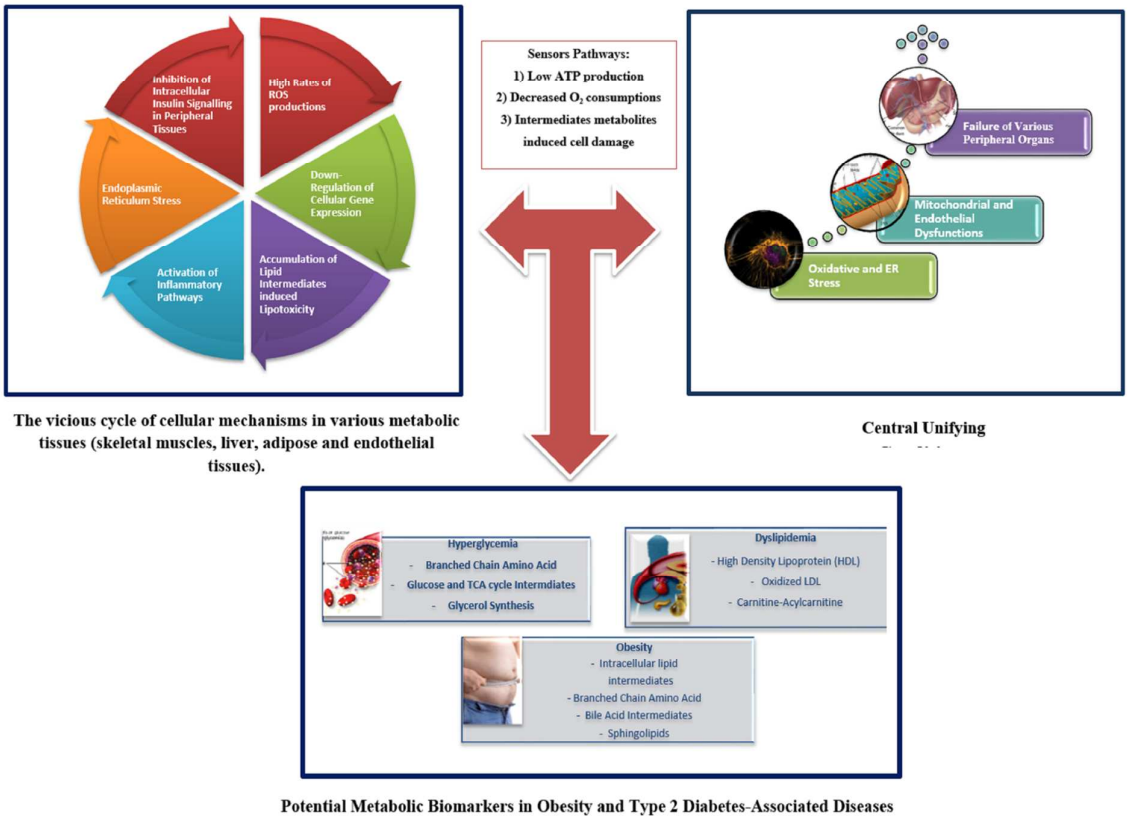
Metabolic disorders are common syndromes that are affecting general population health worldwide. These disorders are considered among the physiological adaptations of body homeostasis on several complications such as malnutrition, sedentary lifestyles, hormonal imbalances, accumulation of metabolic inhibitors and stress, which inherently interrupt with the important cellular and systemic pathways in the human body. The complex interplay between genetic predisposition and environmental factors such as sedentary lifestyle, unhealthy dietary pattern, and other metabolic stress have become pivotal reasons that contribute to the development of the particular disorders [59,60]. The clinical characterizations of these disorders are marked with hyperglycemia, dyslipidemia, impaired glucose tolerance (IGT), elevated blood pressure, and other cardiovascular disease-related complications [61,62]. Importantly, a central event to the entire discipline of metabolic syndromes is insulin resistance [60,63].

Country	Number (million)
China	98.4
India	65.1
United States of America	24.4
Brazil	11.9
Russian Federation	10.9
Mexico	8.7
Indonesia	8.5
Germany	7.6
Egypt	7.5
Japan	7.2

Table 1.0: Top 10 countries for number of people with diabetes (20-79 years) in 2013 [64].

Notably, one of the most prominent metabolic disorders is diabetes. Over the past century, there has been a dramatic multiplication in the prevalence of diabetes. Diabetes mellitus is a looming health issue that has now become a threat to human population. Based on the current statistics provided by The International Diabetes Federation, 382 million people worldwide suffered from this disease in 2013 with 46% of the world population remain undiagnosed. Yet, there is no definitive cure for diabetes due to the scarcity of understanding in pathophysiological features of the disease. Startlingly, these figures are projected to be increased up to 592 million in 2035, particularly in the low and middle-income countries [64]. Table 1 depicts that China, India and United States of America were ranked as top 3 countries with the highest number of diabetic patients. Specifically, global estimates of the prevalence of diabetes have identified Malaysia as one of the top ten countries in the world with highest diabetes cases [65]. Based on the 2011 National Health and Morbidity Survey, Malaysia was the number-one country in Southeast Asia with the highest number of diabetes cases among its population (2.6 million registered adults) and 3.6 million adults were estimated to be affected. Additionally, almost 43% of Malaysians were suffering from abdominal obesity, which is strongly associated with the development of type 2 diabetes and cardiovascular diseases [50]. Diabetes can compromise other related organs such as kidney, eyes, and heart [61]. Due to these broad ranges of diabetic complications, this metabolic disorder becomes one of major causes of both mortality and morbidity [66]. A statistical finding indicates that almost 90% of diabetic patients are suffering from type 2 diabetes that is strongly associated with obesity, aging and sedentary lifestyles [65,67]. Obesity is a whole-body adaptation due to extra calorie intakes and lack of physical activity. Low-grade inflammation, which signals some pro-inflammatory cytokines and immune cells to be

recruited due to excess adipose tissues mass, has been comprehended as one of the fundamental mechanisms in obesity-related diseases [68–70].



The vicious cycle of cellular mechanisms in various metabolic tissues (skeletal muscles, liver, adipose and endothelial tissues).

Central Unifying

Figure 3. The cellular adaptations in various peripheral tissues exert several detrimental effects on the peripheral tissues. These mechanisms of reduced organ-tissues functions indirectly become the fingerprints in the form of metabolites in body fluids.

At the turn of the twenty-first century, the vast progress in genomics, transcriptomics, and proteomics has opened many doors in discovering novel biomarker panels as well as resolving the conflict of the complex mechanism underpinning metabolic diseases such as obesity and type 2 diabetes. The GWAS that utilize large-scale sample populations have revealed that there are more than 56 SNPs. These SNPs are strongly associated with type 2 diabetes susceptibility [14]. Interestingly, the SNPs associated with diabetes are mostly found in non-coding regions of the genome that do not encode protein sequences. The regulations of the genomes inherently affect the gene expression and transcriptions. The SNPs in these metabolic genes are metabolically linked to particular enzymes that expressively lead to the production of specific metabolites of associated genes of interest.

There is evidence that the regulation and modulation of certain gene and protein expressions are known to be affected by the disease-associated alleles on transcript level and splicing, resulting in the formation of distinct protein structures. Certain protein variants can specifically act as transcription factors. For example, transcription factor 7-like 2 (*TCF7L2*) polymorphism rs7903146 is identified to be the strongest genetic marker in type 2 diabetes, especially among the Caucasians [71,72]. A nine-year follow-up investigation shows that SNP in the minor T-allele of rs1260326 in glucokinase (hexokinase 4) regulator (GCKR) might play its role in reducing

the risk of type 2 diabetes susceptibility by lowering triglyceride accumulations and dyslipidemia and improving the fasting insulin and glucose level of the subjects [73]. In addition, the area of epigenetics has also found its place in these disorders. The methylation of certain mRNAs also substantially contributes to the deregulated energy metabolism. The study implied that the demethylation of N-6 methyladenosine in nuclear mRNA was influenced by obesity-associated fat mass and obesity-associated protein (FTO) genes [74]. Undoubtedly, the holistic flow of systems biology in these devastating diseases is tightly regulated to coordinate various biological functions in both normal and disease state.

In principle, the concentration of the metabolites in body fluids such as blood and urine or even in specific organs or tissues can be measured. Cells in the body use blood to communicate in a context dependent manner through the interaction with hormones, cytokines, or various physiological factors. Particularly, obesity and type 2 diabetes are highly marked with certain comorbidities such as hyperglycemia, excess fatty acid, oxidative stress, high blood pressure, and IGT, which are likely to occur prior to the symptomatic development of such disorders. Skeletal muscles, liver, heart and adipose tissues are highly affected in the development of systemic insulin resistance, obesity, and type 2 diabetes [75]. The amplification of plasma-free fatty acid level and hyperglycemia alters the oxidative metabolism of these insulin responsive tissues. The cellular metabolic reprogramming established in these peripheral tissues result in certain level of metabolic adaptation, which involves various destructive mechanisms that induce cell damage and biological perturbations. The excessive accumulation of reactive oxygen species (ROS), lipotoxicity, significant alterations of cellular gene expression, and activation of inflammatory signaling pathways induced endoplasmic reticulum (ER) stress are the central-related mechanisms involved in such a dysregulation [68,76–81]. Subsequently, a plethora of downstream player such as oxidative stress, mitochondrial and endothelial dysfunction may signal the tissues to undergo metabolic stresses, which may cause to the failure of peripheral organ functions [76,82].

As shown in Figure 3, we further propose that these intricate processes involve specific biological mechanisms of interaction among different tissues and biological fluids that secrete and exchange their intracellular content and extracellular fluids. These processes might signal certain molecules in the body to interact with each other. These potential molecules are believed to be measured directly through metabolic analysis to identify candidate markers responsible for perturbations in the cellular and systemic profiles of the human body. A series of previous findings suggest that various regulatory pathways such as fatty acids, bile acids, choline and amino acids are correlated with disease process, thus demonstrating that glucose metabolism is not the only pathway affected in the development of diabetes. Therefore, there is a growing interest to identify and quantify the unknown metabolites and their related pathways that could serve as diagnostic and prognostic markers for metabolic disturbances preceding clinical diagnosis. Table S1 depicts numerous metabolites and metabolic pathways that have been identified in obesity and type 2 diabetes utilizing metabolomics approaches (see electronic supplementary file). Currently, the available metabolic markers in type 2 diabetes need to be proven and validated in a further refined analysis. The identification and use of strong correlation of high-quality biomarker panels in type 2 diabetes remains a cornerstone of scientific communities to provide data for therapeutic treatment and survival among the patients.

Numerous analyses have extensively employed metabolomics as one of the powerful means in spotlighting robust and highly specific biomarkers in the metabolic progressions of various metabolic diseases [83]. In particular, the nutritional and several environmental factors in the early life of human developmental stage

later may also affect the susceptibility to various metabolic diseases in adulthood, including obesity, type 2 diabetes and cardiovascular related events [84]. Gestational diabetes mellitus, intrauterine growth retardation (IUGR) and pre-eclamptic toxaemia are among the metabolic syndromes that can have detrimental effects on the long term health outcomes of the mother, foetus and new-borns. Metabolomics analysis of these syndromes using biofluids from diseased subjects revealed that certain common metabolites such as TCA cycle intermediates, glutamate, glycine, alanine, 2-hydroxyisobutyrate, uric acid, 2-oxoglutarate and myo-inositol were significantly altered [85–90], thereby distinguishing pregnant women at the highest risk of disease with normal groups. The characterization and identification of these early markers-based metabolites in the progression of such syndromes is critically important to strategize how intervention can be performed in person's future health. Essentially, preventive measurement in the early life of developmental stage (perinatal programming) of such diseases is crucial in providing a better healthcare system throughout a lifelong health.

4.1. Fatty Acids

Fatty acids and their derivatives are essential sources of energy, especially in the mammalian system. As the constituents of the cell membranes, their role in modulating biological alterations in the human body is already appreciated [91]. It is known that high concentration of fatty acids and their derivatives in blood can be used as a common indicator for many chronic diseases such as diabetes, hypertension, and coronary heart disease [92,93]. In the case of cardiovascular disorders, almost 100 loci have been determined to predict the biological variations associated with serum lipid concentrations [94]. Lipid metabolism is one of the affected metabolic processes in the progression of obesity and type 2 diabetes. In cells, fatty acids are metabolized in mitochondria through β-oxidation towards the formation of acetyl coenzyme A (CoA) for the energy production in several peripheral tissues such as skeletal muscles and liver, while adipocytes are directed to reserve extra fatty acids in the form of lipid droplets for energy storage. Even so, in the event of dyslipidemia, the excess non-esterified fatty acids are being directed to be over-catabolized in the liver and skeletal muscles, directing to the accumulation of lipid intermediates that induce lipotoxicity in these oxidative tissues [95,96]. Abnormal lipid metabolism was profoundly verified in obese and type 2 diabetic subjects that were exemplified by the deposition of lipid intermediates in these target tissues [97]. Several circulating free fatty acids are believed to exert the deleterious effects in several cell types including cardiomyocyte, pancreatic β-cell, and endothelial cells [96].

The systemic impairments of fatty acids metabolism in insulin resistance and type 2 diabetes subjects have been reported in a series of publications. The accumulation of long chain fatty acids in skeletal muscles and heart induce rigorous alterations in the insulin signaling pathway due to the defective oxidative metabolism [78,81,98,99]. Oxidative stress is thought to play a major role in the pathophysiological features of metabolic disorders, particularly in diabetes and insulin resistance, though full panoply of molecular mechanisms responsible remain ambiguous [100,101]. High metabolic flux in the delivery of free fatty acids into mitochondria may surpass the capacity of mitochondrial fatty acid oxidations, ultimately leading to an abnormal accumulation of ROS and lipid intermediates such as diacylglycerols and ceramides in the cytosol [102]. In turn, these molecules affect the insulin signaling pathways via an upsurge of ROS productions and activation of inflammatory signaling pathways, thus causing stress to the ER [101]. The relative concentrations of free fatty acids were elevated in type 2 diabetes patients with significant changes on the chemical composition of saturated, polyunsaturated, and monounsaturated fatty acids [54,103–105]. In line with this, genetic rodent models of diabetes also experienced the expected changes in plasma-free fatty acid level [106,107].

A study that utilized Framingham Heart Study population examined the lipid profiles of 189 people with type 2 diabetes. The subjects were followed up over 12 years. The authors observed the variations of lipid compositions with the lower number of double bonds and carbon chain lengths in diseased groups, while lipid compositions with higher carbon number and double bonds were strongly associated with minimized risk [108]. This analysis is also in agreement with multi-platform analysis of fasting serum from impaired fasting glucose (IFG) and type 2 diabetes subjects [109]. Low carbon number lipids such as myristic, palmitic, and stearic acid were amplified in diseased subjects compared to control. Additionally, another metabolic investigation demonstrated that subjects with IGT exhibited certain physiological variations in the relative level of fatty acid compositions [110]. Previous analyses have also verified the significant changes in the fatty acid compositions and the relative concentrations of these fatty acids might influence insulin sensitivity and correspondingly contribute to the development of IGT and insulin resistance [111]. Similarly, these observations are in accordance with

metabolic profiling of obese/overweight men. Employing ultra-performance liquid chromatography quadrupole time of flight (UPLC-Q-TOF), a strong correlation of higher level of saturated fatty acid, palmitoleic acid, and stearic acid and lower level of linoleic acid was strongly associated in diseased groups compared to control [112]. Certain fatty acids such as palmitoleate, oleate, palmitate, and stearate were augmented in women suffering from obesity and type 2 diabetes [113]. An UPLC-q-TOF-MS study applying the non-targeted approach substantiated the prominent novel changes in the metabolites concentration of pre-diabetes subjects with an increase in fatty acid level such as C16:1, C18:2, C20:4 and C22:4 [110].

Of note, the relative rates of metabolisms for certain types of fatty acids such as polyunsaturated fatty acids including eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid were also affected in metabolic diseases including diabetes [114]. Metabolic profiling of GC-MS on the investigation of plasma esterified fatty acids (EFA) and non-esterified fatty acids (NEFA) showed that several species of arachidonic acids especially the class of C20 fatty acids might be useful indicators for distinguishing pathological abnormalities among populations in the development of type 2 diabetes and obesity [115]. The study also quantified that the relative concentrations of EFAs were reduced while the relative amounts of NEFAs in plasma were significantly up-regulated. These metabolic adaptations indicate the events of cellular mechanistic injury are thought to occur in order to signal the inflammation process and self-repair mechanism. This clinical observation is particularly beneficial in evaluating the roles of arachidonic acids in the prostaglandin metabolism and inflammatory pathways signaling.

The influence of genomics on metabolic phenotypes is the important key to understand the interaction between genetics and environmental predispositions. These genetically influenced metabotypes (GIMs) are discovered to be in or close to the rate limiting step of enzymatic reactions that correlate with associating protein's function and metabolic match. For instance, one of the important fatty acid enzymes namely fatty acid desaturase, which is directly encoded by a specific genetic variant in the promoter of *FADS1*, was involved in the conversion rate of arachidonic acid in the fasting serum samples of 1809 participants from the population-based KORA (Cooperative Health Research in the Region of Augsburg), followed up in the TwinsUK cohort with 422 participants. The metabolite concentration ratios in proxies with enzymatic reaction rates were used in the study. The statistical associations of these ratios were robust to predict the relationship between genetics and metabolic phenotypes with p-values between 3×10^{-24} and 6.5×10^{-179}. Surprisingly, the identified loci used in the aforementioned study also signified the powerful indication up to 36% of the observed clinical variance in the metabolite concentration [116]. This evidence indicates the strong correlations between the stated metabolic traits and genetic predispositions towards predictions of risk factors associated with the disease progressions.

At the cellular level, the failure of pancreatic β-cells to secrete and release insulin in the event of insulin deficiency and type 2 diabetes is understood as one of the greatest hallmarks in pathophysiological mechanism associated diseases. These mechanistic failures of insulin release are caused by the lipid-induced toxicity in the cells due to the significant changes of sphingolipid metabolism and the abnormal accumulation of the long chain saturated fatty acids-induced ER stress in different peripheral tissues [117]. These metabolic patterns in favoring of sphingolipid metabolism that are quantified in the biological fluids of diseased subjects showed the enhanced inflammatory mediators interactions of macrophage and various lipid-related metabolites. This leads to pathological conditions such as dyslipidemia, cardiovascular disease (CVD), and insulin resistance [118]. Another report demonstrated that obese subjects exhibited higher level of plasma-free fatty acid compared to the healthy

group due to the rapid proliferation in the amount of adipocyte mass [119]. Correspondingly, an advanced flow in the amounts of free fatty acids induces the insulin resistance in the liver and skeletal muscles by minimizing the rate of glycogenolysis and insulin-stimulated glucose uptake [120–122]. Still, the exact process that leads to these metabolic dysfunctions has not been fully elucidated.

High amounts of free fatty acids flux require extra carnitine in the cytosol in order to transport them into the mitochondrial matrix for energy production. When the carnitine level is not enough to support the buildup of free fatty acids flux into mitochondria, these lipid intermediates tend to accumulate in the cytosol, directing to various levels of biological perturbations. Carnitine is markedly involved in dyslipidemia and obesity. As carnitine is strongly associated in the process of burning out fatty acids in the mitochondria, the efficiency of β-oxidation becomes one of the important modulators in this metabolic process. The deficiency of protein transporter such as monocarboxylate transporter 9 (MCT9), encoded by the SLC16A9 gene may result in the fluctuation of carnitine level in the body fluids [14]. The decline of carnitine levels was identified in blood of humans suffering from obesity [112] and in the liver tissue of obese mice [123]. Conspicuously, it is not surprising therefore to see L-carnitine supplementation in pharmaceutical industry is widely used to counteract against obesity and dyslipidaemia [124].

Contrarily, another report specified that the relative concentrations of free carnitine in obese and type 2 diabetic patients were amplified [125]. The key problem with this observation might be due to the overexpression of carnitine palmitoyltransferase-1 (CPT-1) in outer mitochondrial membranes that enhance the process of β-oxidation in response to the elevation of free fatty acids in the blood. Carnitine is an essential intermediate for the fatty acids transportation from cytosol into mitochondria, and its level was elevated in type 2 diabetic patients. A multiplatform analysis (LC-MS/MS, NMR, and microarray) by Gipson and his colleagues identified that the elevation of fatty acids level occurred through the up-regulation of transcript level of fatty acid metabolism-associated carnitine palmitoyltransferase-1 in liver tissues [126]. These synergistic investigations between cellular and systemic physiological functions suggest the clear link between biological fluids (plasma/serum and urine) and cellular metabolites, thus supporting the idea of circulating metabolites in urine, blood, and other biological fluids, are the direct biochemical fingerprints of the cellular events.

Oxidative stress and lipid peroxidation are the most common detrimental occasions that are metabolically linked to the progression of diabetes complications [127,128]. A non-targeted metabolic profiling on 399 non-diabetic subjects with random forest statistical analysis verified that α-hydroxybutyrate was the robust clinical predictors for both insulin resistance and impaired glucose regulations after normalized to age, sex and body mass index (BMI) [129]. The authors postulate that the supporting biochemical interactions of this occurrence involve an increased lipid oxidation and oxidative stress. Subsequently, another report employing a large sample size of non-diabetic individuals from two studies (Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) and Botnia Prospective Study (BPS)) were profiled. Those who went to develop diabetes in 3 years in RISC and 9.5 years in BPS were identified. After normalized to familial diabetes, sex, age, BMI, and fasting glucose level, the study showed that α-hydroxybutyrate was the strongest clinical marker for the prediction of poor glycaemic control in RISC and diabetes in BPS. The treatment of α-hydroxybutyrate on insulin secreting β-cell derived pancreatic cell lines (INS-1e) has substantially diminished the rate of insulin release in a dose-dependent manner while L-GPC treatment on cell lines was discovered to stimulate glucose- and glucose/arginine-induced insulin release in a dose-dependent manner with the presence of low glucose

concentration [130]. Likewise, other investigators applied both LC-MS and GC-MS analysis also stated that the relative concentration of α-hydroxybutyrate in fasting serum of IFG and type 2 diabetes subjects was expressively amplified compared to healthy controls [109]. Therefore, it can be postulated that this marker is potentially implicated with development of diabetes in relative to insulin functions. Interestingly, the same experimental works found that another clinical marker including linoleoyl-glycerophosphocholine (L-GPC) was evidenced to be negatively correlated for both poor glycemic control and diabetes.

In the event of insulin resistance, glucose cannot be fully metabolized by the peripheral tissues such as skeletal muscles and liver, hence contributing to the upsurge of fatty acids flux into these tissues. The diminution of insulin secretion and high rate of free fatty acid flux across the plasma contribute to the ketoacidosis, which was previously confirmed in people with diabetes [131,132]. The escalation of lipolysis rate in adipocytes and induction of ketogenesis (high level of ketone bodies) may exacerbate the insulin resistance in muscle and liver tissues [133]. In return to the low insulin level, glucagon is released into the blood. This phenomenon also stimulates lipolysis in adipose tissues to generate a considerable amount of circulating free fatty acids in the plasma to be transported into the liver. As the levels of insulin in people with diabetes are declined, the rate of lipogenesis is predominantly reduced to synthesize these free fatty acids in liver through β-oxidation. The genes and proteins that are responsible for β-oxidation will then be expressed to modulate the production of energy through fatty acid oxidation. As a result, higher levels of ketone bodies including β-hydroxybutyrate, acetone, and acetoacetate will be synthesized from the liver during the process as seen in several models of diabetic animals [107,134,135]. In parallel, these ketone bodies are lifted in serum [54,136], urine [107], and plasma [113] samples of people suffering from type 2 diabetes compared to control group. These accumulation properties of ketone bodies are also correlated with the disease state as well as age [107].

Before being transported into mitochondrial matrix, fatty acids must be activated in the form of long chain acyl groups before binding to carnitine to form acylcarnitines. Acylcarnitine, a crucial biomarker in the event of insulin resistance is defined as a by-product of fat, glucose, and amino acid oxidation in mitochondria [102]. Utilizing multiplatform analyses, both diet and genetic-induced insulin resistance as well as obesity in animals have a high rate of incomplete fatty acid oxidation, abnormal acylcarnitine profiles and amino acid biosynthesis with the perturbation of mitochondrial metabolites [119,137–139]. Metabolic profiles of obese and diabetic patients revealed significant changes in acylcarnitine (long chain saturated and monounsaturated) concentrations in the blood [125]. The high rate of incomplete β-oxidation could also be evidenced from metabolic profiling of acylcarnitines in blood, urine, and muscle. The idea of mitochondrial dysfunction-induced insulin resistance has become one of the popular hypotheses to represent the overall defects in fatty acid oxidation that leads to the lipotoxicity-induced insulin resistance [140,141]. Metabolomics profiling of mitochondrial metabolic activities in obesity and type 2 diabetes subjects reflect the systemic and cellular function of the whole-body system in a disease process [139,142].

A growing number of evidences reveals the strong association of blunted activity of skeletal muscles insulin resistance in oxidizing free fatty acids in mitochondria [143,144]. The targeted approach of metabolic profiling in type 2 diabetic patients via UPLC-MS platforms signified the incomplete fatty acid oxidation among type 2 diabetes patients with the significant augmented levels of various acylcarnitine intermediates, which mostly consist of the long chain acylcarnitines [145]. Remarkably, a recent study has confirmed that youth who were suffering from obesity also exhibited high amounts of different types of acylcarnitine species ranging from

short, medium and long chains. This finding stresses upon the importance of mitochondrial function to burn out fatty acids. A non-targeted [11] and targeted metabolic analysis [125] quantified that acylcarnitine levels were amplified in both animal and human biological fluids compared to control. Furthermore, works by Newgards and his colleagues via targeted metabolic profiling revealed the amplification of relative concentrations for several acylcarnitine intermediates species such as C3, C5, C6, and C8:1 acylcarnitines [119].

On the contrary, a disparate report found an OGTT-induced reduction in acylcarnitine relative concentrations with the considerable alteration in plasma fatty acids composition [146]. Consistent with this finding, the mechanism reinforcing this condition proposes that the activation of nuclear factor-κB (NF-κB) is the critical regulator that induces various levels of inflammation at the cellular level through accumulation of these acylcarnitine metabolites [147,148]. The other proposed regulation also disclosed that the synergistic effects between the low flux of mitochondrial Krebs cycle metabolic activity and activation of NF-κB-associated inflammation pathways contribute to abnormal and incomplete long chain fatty acid combustion in mitochondria. These low flux of mitochondrial Krebs cycle metabolic activity and activation of NF-κB-associated inflammation pathways are affiliated with the progression of insulin resistance and type 2 diabetes [145]. In addition to these observation, we have recently demonstrated that an inhibition of NF-κB pathways ameliorates mitochondrial dysfunction-induced insulin resistance in adipocytes [149], suggesting that the concerted actions of deteriorated mitochondrial functions and inflammation are among the pivotal features of insulin resistance, the greatest hallmark of type 2 diabetes.

Phospholipids are a class of lipids, mostly in the form of fatty acyl that is mainly involved in distinct biological pathways of membrane signaling activities. These phospholipids constitute the major components of the cell membrane in the form of lipid bilayers. Membrane phospholipids can be affected by various compositions of phospholipid mixtures. Polyphosphoinositides, another form of phospholipids is directly involved in several signaling activities [150]. Physiologically, phospholipid pools in the human body serve as the reservoir for certain polyunsaturated fatty acid such as arachidonic acid concerning the formation of prostaglandins, lipoxins, leukotrienes, and thromboxanes [151]. Even though diabetes is regarded as a metabolic disease with severe functional impairments of glucose utilization, certain findings have indicated that diabetes is the result of dysregulation of lipid metabolism in the form of phospholipids [152,153]. Based on targeted metabolomics approach, it was determined that the level of phospholipids in obese and type 2 diabetes patients was greatly raised up compared to control group [154]. Different types of lipoproteins and phospholipids such as lysophosphotidylcholine (lysoPC), sphingomyelin, and phosphatidylcholine (PC) were associated with the events of type 2 diabetes. Following the availability of GWAS profile in these years, these metabolic traits of lipid metabolite fluctuations in plasma are elucidated in mechanistic investigations that utilized GWAS with NMR-based metabolomics and lipidomics. For example, a study has successfully proved that certain loci (*CETP, SORT1, GCKR, APOA)*) are directly responsible for encoding specific lipoproteins associated with serum total lipids [155]. While obesity and diabetes are strongly correlated with CVD, the finding from this study could also be used an indication to give a strong justification on the systemic flow of systems biology in contributing to the regulation and modulation of lipid level in various biological fluids of diseased subjects.

Ha and colleagues have examined the association of metabolic profiles and vascular stiffness between the patients with newly diagnosed type 2 diabetes [11]. The authors demonstrated that oxidized-LDL, lysoPC (14:0), lysoPC (16:1), high sensitive-C reactive protein (hs-CRP), and interleukin-6 (IL-6) were strongly linked

to the events of diabetic complications and higher arterial stiffness. Among these, they also discovered that the repetitive inflammation, oxidative stress, and cardiovascular complication events synergistically act together in exacerbating such metabolic impairment. Moreover, the metabolic mechanisms and its downstream sequelae underpinning these situations are coupled to fatty acid-induced inflammation. Various classes of polyunsaturated fatty acids in the body such as prostaglandins and other inflammatory mediators are affected in the disease process [156]. Similarly, the relative level of lysoPC was declined in the population-based observational study [157]. In the progression of obesity exploiting high-fat diet mouse, LC (GC)-MS metabolic profiling of liver tissue has confirmed the variation in phospholipids level such as PC and lysoPC to be increased and decreased, respectively [158]. This finding suggests that the relative level of phospholipids in the pathogenesis of type 2 diabetes can be greatly altered in a disease process. The dynamic changes of these metabolites level in lipid metabolic pathway could be one of the important events in the development of the metabolic disorders such as obesity and type 2 diabetes.

4.2. Carbohydrates

Carbohydrate metabolism is the vital metabolic pathway that regulates and modulates cellular energy productions in the human body [182]. Glycolysis is the major regulatory pathway in which glucose is broken down to pyruvate in the cytoplasm. The first step of glycolysis is phosphorylation of glucose to glucose-6-phosphate by hexokinase and glucokinase. Hexokinase plays its role in the metabolic conversion of glucose in the skeletal muscles and adipose tissues, while glucokinase is the enzyme used for the metabolic conversion of glucose in the liver and pancreas [183]. This process is irreversible and rate limiting step to ensure that glucose does not directly diffuse out of the cell [184]. Each glucose-6-phosphate is converted into two pyruvate molecules that, under aerobic conditions, are transported into the mitochondria and converted to acetyl-CoA by the pyruvate dehydrogenase complex. Finally, acetyl-CoA enters the tricarboxylic acid (TCA) cycle in which it is oxidized to carbon dioxide to release energy [185]. In normal physiological conditions, the conversion of glycolytic pathways is highly regulated in regard to the roles of respective genes and enzyme expressions that are responsible for the metabolite productions. On the other hand, in the event of perturbation like obesity, the regulations of these enzymes are defected. In particular, a study that utilized C57BL/6 mice as a model of high-fat diet-induced insulin resistance implied that the expressions of certain glycolytic enzymes such as enolase were down-regulated in peripheral tissues [186]. It is most likely that this finding might give some true indications on how these metabolic changes are critically linked to each other at various levels of systems biology.

In the event of these occurrences, the accumulation of glycolytic intermediates becomes one of the resultant phenomena that reflects the disturbance of the carbohydrate metabolic pathways [187]. Hyperglycemia is considered as an adaptation of several peripheral tissues due to the excessive amount of glucose in the blood circulation, and it may persist for a long time in pre-diabetes conditions [188]. Insulin deficiency may also lead to hyperglycemia, which is the central fact about diabetic complications escalating with time and driven by many intricate biochemical and molecular processes. Insulin deficiency is one of the common risk factors that is strongly implicated with the development of pre-diabetes and insulin resistance with cellular glucose utilization by certain peripheral tissues was altered and impaired [189]. As highlighted in the previous points, one of the established features triggering insulin resistance is oxidative stress, which arises from the high production of free radicals from a variety of sources. It is commonly accepted that hyperglycemia induces oxidative stress through intense productions of superoxide molecules due to the impairment of antioxidant defenses system in the mitochondria. This condition would lead to the situation where numerous biochemical regulatory pathways of carbohydrate metabolism are altered in response to these biological adaptations.

It is well known that mitochondria are the medium of nutrient oxidations through various metabolic pathways. The defects in the mitochondrial respiratory chain system may affect certain metabolites (e.g., pyruvate, alanine, and lactate) level that are often elevated in neurological disorders such as Alzheimer's and Parkinson's disease [190]. Impairment of this system might decelerate the capacity of pyruvate oxidation into acetyl-CoA, preceding to accumulation of pyruvate in the cytosol and overproduction of alanine and lactate [191]. Correspondingly, through metabolomics approach, patients with type 1 and 2 diabetes were confirmed to have 9.4 times higher level of pyruvate than control [136]. In addition, ^{1}H-NMR-metabolic profiling of plasma and urine from high-fat diet mice [163] and rats [166], respectively, also documented the unusual accumulation of pyruvate in the whole system organism. The sudden upsurge in pyruvate level in the plasma of type 2 diabetes subjects may also indicate the metabolic adaptation of high-fat diet system towards transamination of α-ketoglutarate in the

mitochondria by alanine trans-aminotransferase (ALT) [165]. The augmentation of ALT expressions in the plasma level of type 2 diabetes subjects subsequently affect other TCA cycles intermediates and other central carbon metabolisms. Potentially, hyperglycemia might also boost the rate of glucose oxidation to the excessive formation of pyruvate. These situations signify that the metabolic adaptations of the whole-body system organism are directed towards glycolytic pathways in the progression of type 2 diabetes and insulin resistance.

The enzyme responsible for the conversion of pyruvate to acetyl-CoA is pyruvate dehydrogenase kinase complexes that play a vital role in the pathogenesis of type 2 diabetes [192,193]. There are four PDK isozymes (1-4) that have been identified in humans and rodents so far [123, 124]. In particular, the pyruvate dehydrogenase kinase isozyme 1 (PDK-1) mRNA expressions were reduced in the pancreatic islet β-cells after treatment with high level of fatty acids and glucose [194]. Pyruvate dehydrogenase kinase 4 (PDK4) is predominantly expressed in various specialized peripheral tissues such as skeletal muscles, heart, kidneys, pancreatic islet, and liver. Mechanistically, pyruvate dehydrogenase kinase (PDK) phosphorylates the serine residue in a pyruvate dehydrogenase complex (PDC) by sustaining three important carbon compounds namely pyruvate, alanine, and lactate through gluconeogenesis for the maintenance of blood glucose level [195]. In diabetes, PDK4 is up-regulated and eventually dysregulated due to the overload of glucose entrance into cytosol. These situations occur through a series of biological processes, which intrinsically inhibit the entrance of pyruvate into mitochondria. Indirectly, this metabolic response could be accountable for the abnormal accumulation of pyruvate that was characterized in various biological fluids of both animal and human subjects with type 2 diabetes and obesity [136,163,164]. Taken together, these metabolite changes showed the transition state of mechanism underlying the disease, from euglycemia to hyperglycemia, with considerable alteration in the carbohydrates-related metabolic pathways.

The basic concept of mitochondrial dysfunction as a significant factor in lipid (fatty acid)-induced insulin resistance and type 2 diabetes is one of the critical areas in metabolic disorders that is growing in appeal. Failures in the mitochondrial energy-generating system may also lead to the abnormal accumulation of lactate in urine, blood, and cerebrospinal fluids. Under anaerobic conditions, pyruvate, instead of being converted to acetyl-CoA, can be directed to conversion of lactate through the lactate dehydrogenase enzyme. Physiologically, the homeostatic levels of lactate in the blood and urine is maintained by the metabolic coordination of several organs, tissues, and cells such as skeletal muscles, brain, erythrocytes, and adipose tissues [196,197]. Lactate is one of the carbon sources for gluconeogenesis to take place [198]. The concentration of lactate was elevated in blood, urine, and liver tissue of obese and type 2 diabetes patients. In obese mice induced by high-fat diet, the lactate level in the urine [163] and blood serum [164] was higher than mice with normal diet (control). Through metabolic profiling, abnormal accumulation of lactate was exemplified in liver, urine, and blood of Zucker rats that lacks the leptin receptor [159,166]. Besides, Newgard and his colleagues noticed that the level of lactate in obese subjects was generally higher compared to the control group [119]. In line with to such changes, another study reveals that one of the main sources of lactate accumulation in the blood is because of the accumulated subcutaneous fat deposition and larger adipose mass, as obesity and type 2 diabetes events are apparently associated with large adipocyte mass [197]. This metabolic condition might be caused by the impairment of lactate dehydrogenase A (LDHA) expression in the white adipose tissues of the high-fat diet rat [199], leading to the abnormal accumulation of lactate in the biological fluids.

In the same way, high level of lactate also signify the reduction in cellular energy productions as well as dysregulation of CoQ_{10}-antioxidant system, biotin, lipoic acid, and thiamine deficiencies [200]. Moreover, high rate of transamination of pyruvate by alanine aminotransferase may also lead to the abnormal accumulation of lactate and alanine in the biological fluids of IFG and type 2 diabetes subjects [109]. Nonetheless, several investigations that applied ^{1}H-NMR spectroscopy platforms showed contradicting results in which the relative concentrations of lactate were dropped in serum [169] and urine [134] of high-fat diet mice and Zucker rats, respectively. Specifically, it is vital to note that the low levels of lactate were ascertained in the serum of diabetic patients but with considerable small sample sizes [122,123]. To this end, regardless of the inconsistent fluctuation in the relative concentrations of lactate in the blood, these metabolic networks of carbohydrate pathways are altered at many levels. An accumulation of plasma lactate could reveal the perturbation of hepatic glucose production and lipid synthesis [198]. These observations indicate that the metabolic shift of lactate concentration in these biological fluids and tissues might propose the diversion of the glycolysis pathways to anaerobic respiration.

In obesity and type 2 diabetes, the main problems in diagnosing these disorders in the clinical settings are the symptoms, and prognosis care tends to be developed in the late stage of the disease. The most prominent, dominant, and detectable biomarkers used in diagnosing the disease for years are glucose and glycated hemoglobin (HbA1c). Despite their long clinical success, both markers have a lot of shortcomings in various clinical applications as they are not really strong markers in predicting the onset of such disease. This is highly relevant, because of the metabolic alterations of both markers are likely to occur at the late stage of the disease [201]. Therefore, the need to identify related markers to be complemented with glucose and HbA1c are critical for establishing and providing guidelines for the clinical preventive measure and diagnosis. In recent years, several analyses have recommended the potential of having a glucose-related marker, which is 1,5-anhydroglucitol (1,5-AG) to complement with other established metabolic biomarkers. 1,5-AG is a metabolically occurring carbohydrate in the form of the dietary inert polyol that is discovered in many foods. Physiologically, its levels within the blood are usually maintained at the normal state of physiological conditions relative to the balance rate of the kidney filtration at the glomerulus. Under normal physiological conditions, 1,5-AG is not usually secreted through urine as it is prone to reabsorb into the blood through the renal proximal tubule with constant balance of the urine excretions. However, during times of hyperglycemia and insulin resistance (>180 mg/dL), 1,5-AG is not fully reabsorbed in the kidney due to the competitive inhibition by glucose in the renal proximal tubular reabsorption. During glycosuria, a small amount of glucose in the kidneys block the reabsorption of 1,5-AG into the blood, causing to the excessive amount of excretion of this molecule in the urine.

The first attempt to understand the role of 1,5-AG in diabetes started when Akanuma and colleagues observed that the concentration of 1,5-AG was lowered in biological fluids compared to control [202]. Several reports have confirmed that 1,5-AG has a capability to be a sensitive and validated marker of short-term glycemic index relative to cardiovascular and kidney-related disorders [203–205]. In metabolic profiling by exploiting multiplatform investigations, the concentration of 1,5-AG was down-regulated in the serum of type 2 diabetes patients [54,136]. Comparably, another research also found that the concentration of serum 1,5-AG was 10 times higher in control groups compared to people suffering from diabetes [136]. This finding indicates the potential of this metabolite in the blood as one of the vital markers to be used as a complementary to glucose and HbA1c. Hence, it could be assumed that 1,5-AG might be one of the robust predictors in evaluating the

diagnosis of related metabolic disorder such as type 2 diabetes and renal dysfunctions. It remains to be determined whether this marker may be useful to be replicated across numerous population.

Acetate is one of the glycolytic intermediates that is partially involved in certain lipid and carbohydrate metabolic pathways and well absorbed through the intestine [206]. Physiologically, liver is responsible for many metabolic processes including anabolic and catabolic pathways. Under starved conditions, fatty acids derived from adipose tissues are metabolized through β-oxidation in the liver to form acetate and ketone bodies. Free acetate produced by the liver is utilized through extra-hepatic tissues as a source of energy [207]. Under normal physiological conditions, acetate is activated by acetyl-CoA synthetase to produce acetyl-CoA for the various levels of oxidative phosphorylation involving TCA cycle, amino deamination, and cholesterol biosynthesis [208]. As obesity is one of the strongest risk factors in the pathogenesis of type 2 diabetes, the concentration of acetate in obese animals was significantly altered compared to control. ^{1}H-NMR spectroscopy-metabolic profiling of urine and blood of obese animals showed that the relative level of acetate was augmented among other metabolites related to mitochondrial dysfunctions [159,166]. The increment of the acetate level in these biological fluids may indicate the inhibition of acetyl-CoA synthetase enzyme, which is responsible for the conversion of acetate into acetyl-CoA. On the other hand, several lines of evidence discovered that the high amount of acetate is not necessary to reflect the idea of metabolic dysfunction in obesity-related disorders as low acetate level was also detected in high-fat diet mice through metabolic profiling of blood serum and urine [169,172,173]. There are many situations where the level of acetate in the blood is expressively elevated, such as applying acetate during renal dialysis [209], high consumption of dietary fibers [210], and severe ingestion of ethanol [211]. In view of the above discussion, it is hoped that these conflicting results in the exact level of acetate in the biological fluids can be resolved in further refined investigations.

4.3. TCA cycle Intermediates

TCA cycle, which is also known as citric acid cycle, is a series of amphibolic reaction (anabolic and catabolic) in mitochondria. This vicious cycle is responsible for the oxidation of acetyl-CoA into intermediate metabolites for energy productions. TCA cycle is tightly regulated with the electron transport chain and linked with the generation of ATP (38 ATP per citrate molecule). TCA cycle is also known as a central pathway for the aerobic oxidation of most of the fatty acids, carbohydrate, and amino acid intermediates that are metabolized to acetyl-CoA before entering the cycle (Figure 4). On the other hand, several metabolic processes such as gluconeogenesis, lipogenesis, cholesterol and heme biosynthesis, urea cycle, and inter-conversion of amino acids occur directly in the mitochondria and are associated with the regulation of such cycle [212]. Owing to the importance of this cycle in the central metabolic pathway of mitochondria, certain metabolic disturbances through this metabolic pathway has been affected in obese and type 2 diabetes subjects.

Numerous metabolic mechanisms underpinning obesity and type 2 diabetes are postulated in these recent years, and one of them is mitochondrial dysfunction [81]. In fact, there is a large volume of published literature and reports that describes the role of mitochondria in the pathogenesis of insulin resistance and type 2 diabetes [75,81,149,213,214]. In short, mitochondrial dysfunction refers to the disruptions and disturbance of several distinct metabolic processes in mitochondria (β-oxidation, TCA cycle, and oxidative phosphorylation). For instance, the metabolic reprogramming of adipose tissue gene expression in obese interleukin-1 (IL-1) gene knockout (IL-1RI$^{-/-}$) mice also showed that the expressions of genes involved in the lipolysis and TCA cycle were altered [215]. In the event of peripheral insulin resistance, the concentration of lipid accumulation in various peripheral tissues such as skeletal muscles and liver is very persistent to be accumulated, resulting in a mismatch and confusion of various metabolic activities including TCA cycle [216]. Afterwards, the regulation of the chronic inflammatory pathway is deregulated in order to signal the event of oxidative damages [149].

The crosstalk among oxidative stress, mitochondrial dysfunction, and insulin resistance has become one of the central issues in obesity and type 2 diabetes complications [217–219]. Oxidative damage in numerous insulin responsive tissues may induce an accumulation of cell's damaged mitochondria and block various distinct processes in mitochondrial activity, such as electron transport chain, β-oxidation, and TCA cycle [100]. Damaged mitochondria would directly stimulate such abnormal accumulation of ROS within the cell and affect various metabolic genes and proteins [220]. Indeed, high-fat-fed mice and culture of the skeletal muscles cell lines with high concentration of saturated fatty acids noticed the decrease of peroxisome proliferator-activated receptor gamma co-activator 1α (PGC-1α) level (a regulator of mitochondrial biogenesis and metabolism) and an upsurge of acylcarnitine levels in the cells [138]. PGC-1α is one of the important protein regulators of mitochondrial biogenesis and metabolism [221]. As a transcriptional co-activator in various metabolic pathways such as gluconeogenesis and β-oxidation, its role in modulating cellular and systemic oxidative metabolism cannot be reckoned with. One of the main canonical functions for this protein is to detoxify the effects of reactive oxygen species (ROS) accumulations [222,223]. The accumulation of ROS in various peripheral tissues might occur due to the high level of free fatty acids and glucose intermediates in the event of obesity and type 2 diabetes. The protein merges as a metabolic mediator that can up regulate the expression of ROS-detoxifying enzymes in the respective peripheral tissues such as skeletal muscles, liver, and adipose tissues. In regard to the TCA cycle activity and its individual components, elevated PGC1α level in the various peripheral tissues inherently affect the compositions and functional structural of the mitochondrial integrity. The enhancement of PGC1α expression activity lead to elevated activity of TCA cycle flux and its related enzymes and metabolites

[221]. Furthermore, the expression of other electron transport chain (ETC) complexes and ATP synthase activity was down-regulated. The rise of relative concentrations for several TCA cycle metabolites such as fumarate, citrate, malate and α-ketoglutarate also suggests cytochrome c dysfunction in the transfer of electron and energy transmission for oxidation and reduction process. An elevated rate of DNA methylation and reduced mRNA levels of PGC1α expression has also at the same time been exhibited in various peripheral tissues of obese and type 2 diabetic subjects [224–227]. In addition, the expression of other mitochondrial enzyme gene subsets such as the cytochrome c oxidase, complex I, and complex III subunits of mitochondrial ETC was evidenced to be depleted in overweight and obese insulin-resistant subjects [228]. The metabolic changes in this gene expression and enzyme activities disturb the TCA cycle flux, resulting in an abnormal level of TCA cycle intermediates.

Due to the complexity of deteriorated mitochondrial functions, multiple organ systems such as muscle, eye, endocrine, blood, kidney, and central and peripheral nervous systems are affected in the disease processes [229]. These clinical features are likely to be similar to the complications of diabetes and other metabolic disorders. The abnormality of metabolite concentrations in the biological fluids and its enzymes are vital components to look for in diagnosing mitochondrial dysfunction in various chronic diseases such as type 2 diabetes and obesity. An altered TCA cycle flux was determined in the offspring of diabetic volunteers and exercise trainings in patients with type 2 diabetes [230,231]. Re-evaluation study using unbiased approaches of stable isotope tracer methodology (acetate recovery factor (ARF)) corroborated that the rate of ARF via [^{13}C] palmitate oxidation in TCA cycle of type 2 diabetes subjects was reduced during exercise, indicating a diminution in muscular TCA cycle flux in type 2 diabetes subjects [230]. Moreover, further inhibition of TCA cycle enzymes in lean myotubes by malonate (an inhibitor of succinate dehydrogenase) and significant reduction of acetate oxidation rate might force the target tissues to undergo several metabolic changes into diabetic phenotypes [232,233]. In the progression of type 2 diabetes, other experiments that used microarray analysis has confirmed the decreased ATP production from myotubes of type 2 diabetic subjects due to impairments of various oxidative phosphorylation (OXPHOS) genes in the mitochondrial respiratory chain [226,227]. Likewise, several pieces of evidence showed that isolated mitochondria from type 2 diabetes volunteers exhibited a diminished ATP production relative to the lowered rate of TCA cycle flux and oxidative phosphorylation compared to normal [231,234]. In spite of these findings, which parts of the exact impairments are involved in the regulation of this cycle remain obscure.

Citrate is present in all plants and animal tissues. Citrate can be formed through the removal of acetate units of acetyl-CoA, which subsequently combine with oxaloacetate to form citrate by requiring citrate synthase enzyme to be constitutively expressed. It is here worth highlighting that citrate levels within the blood are controlled by glucose and insulin level, cholesterol synthesis, liver clearance, and renal excretion [173]. The fluctuations in citrate level could also suggest the aberrant of protein metabolism and several amino acid structures [235]. The conditions of metabolic acidosis might also induce the least amount of citrate to be excreted through urine while vice versa for alkalosis conditions. Essentially, relative concentrations of citrate in the blood are regulated based on the acid-base status of the human body [236]. In the analysis of the urinary profiles of Zucker rats, the relative concentrations of TCA cycle intermediates such as malate, citrate, fumarate, 2-ketoglutarate, and succinate have significantly lessened [106]. The depletion of TCA cycle intermediates such as citrate and α-ketoglutarate in the serum of diseased subjects showed the up-regulation of ALT enzymes that are responsible for the transfer of an amino acid group from alanine to α-ketoglutarate favoring in the accumulation of pyruvate and acetyl-CoA [165]. Patterson et al. [237] have determined the twofold increase of citrate level in

animal with type 2 diabetes compared to control subjects. The analysis of ^{1}H-NMR spectroscopy using urine samples of type 2 diabetes subjects also revealed the perturbations of the various TCA cycle intermediates with the significant elevation of citrate level compared to control groups [107,168]. Another report employing GC-MS-TOF has discovered that the metabolic patterns of urine and serum *db/db* mice were dysregulated with the elevated TCA cycle metabolites such as citrate, malate, succinate, and aconitate in the early weeks. However, these metabolites were depleted in the following weeks [174]. Surprisingly, the cross sectional investigation has successfully quantified that the metabolic profiles of children who later progressed to diabetes also had lowered citrate and succinate levels at the time of birth [238].

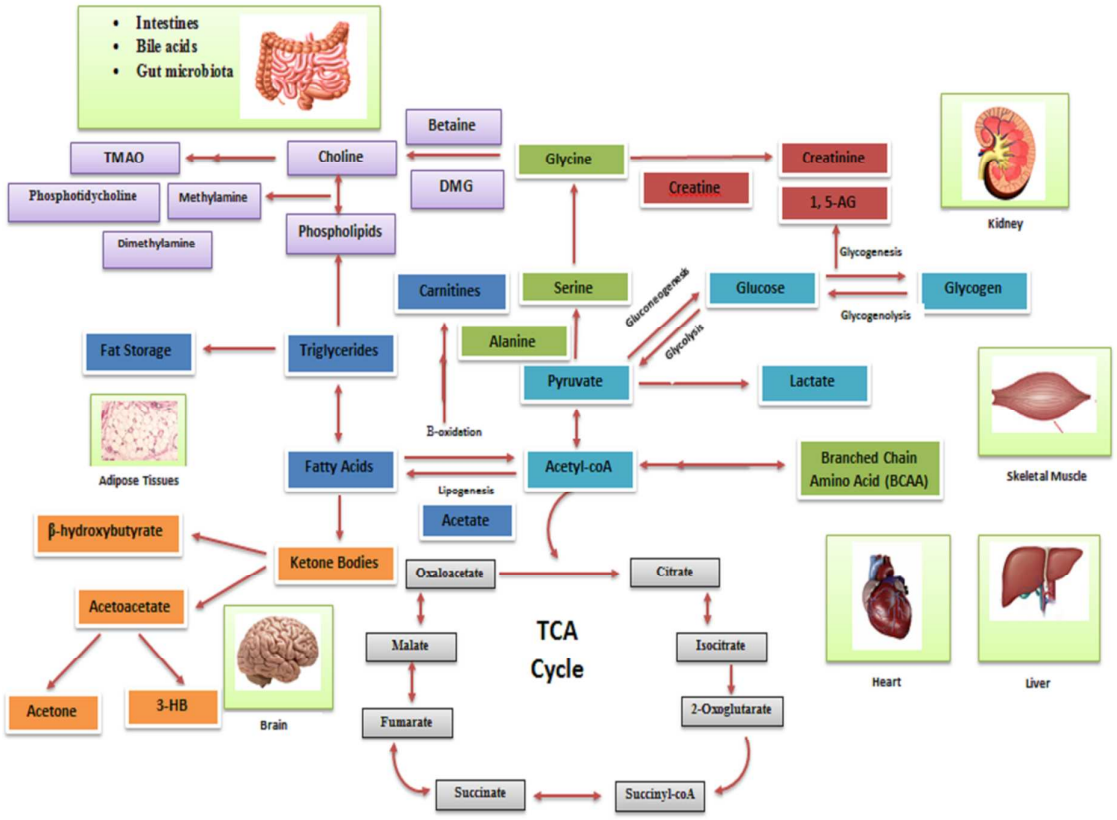


Figure 4. Schematic representation of the metabolic pathways in in the events of obesity and type 2 diabetes. The dynamic changes of these metabolic fingerprints in various biological fluids such as urine and blood is caused by the cellular perturbation of metabolic pathways in these insulin target tissues.

In obese and diabetic animals without insulin resistance, the levels of citrate in blood were amplified when compared to controls [160,173,239]. A study on streptozotocin-induced-diabetic rat revealed that the corresponding relative ratios of TCA cycle intermediates in the rat urine were deregulated at each time point (1, 5, 10, and 15 weeks) of metabolic progression from normal to diabetic rats. The relative concentrations of fumarate and succinate were escalated during 15 weeks of metabolic progressions to become fully diabetic rats while the amounts of citrate and 2-oxoglutarate were maintained at higher concentration at each time point compared to control groups [161]. A high level of fumarate in the urine could indicate fumarase and CoQ_{10} deficiencies while boosted excretion of 2-oxoglutarate level might reveal an association of 2-oxoglutarate

dehydrogenase (OGDC) deficiency and mitochondrial dysfunctions [200]. This paper signified that a reduced TCA cycle flux could be one of the metabolic adaptations that occurs during the metabolic progression of normal rat to exhibit diabetic phenotypes. In a similar methodology, metabolomics analysis of urine samples from high-fat diet mice indicates the significant multiplication of the citrate concentration compared to normal mice [163].

Moreover, ^{1}H-NMR spectra of serum samples [164] and GC-MS of liver samples [160] showed that the amounts of succinate in obese animals (mice and rats) induced by high-fat diet were concomitantly augmented, while metabolic profiling of urine and plasma samples of diabetic rat induced by streptozotocin also demonstrated the elevation of succinate and fumarate [240]. Consistent with these findings, one therapeutic analysis reported that through animal studies, the inhibition of citrate metabolism by fluoroacetate could reduce the body fat mass [241]. It is also conceivable that the increment of succinate level suggests an indication of abnormal accumulation of branched-chain amino acids such as leucine and isoleucine. Additionally, the multiplication of succinate concentrations in the biological fluids and tissues indicate an excess amount of free CoA that is circulating within the blood. The excess amount of free CoA aids the succinate metabolism through reaction with α-ketobutyric acid (see the review in [212]). On the other hand, the findings discussed earlier on the escalation of citrate level in the blood and urine of obese and diabetic subjects might not be in agreement with other papers. The effect of low chronic inflammation with the elevation of metabolic acidosis leads to less excretion of urinary citrate [242]. In obese rats and mice with insulin resistance, low urinary excretions of citrate with other TCA cycle metabolites were discovered [107,160,175,243] while a declined level of urinary citrate also was established in people with severe insulin resistance [244]. In addition, Salek et al. [107] also report the decreases of another TCA cycle intermediates in the urine of diabetic patients, such as fumarate, succinate, and malate. In parallel to this, recent study that utilized type 2 diabetic mice (BKS.Cg-m+/+Lepr^{db}; db/db) as a model of diabetic neuropathy found that several TCA cycle intermediates such as citrate and isocitrate were depleted in sural nerve, sciatic nerve, and dorsal root ganglia (DRG) of the mice [187].

The reduction in isocitrate level also indicates insufficient availability of amino acids in the nervous system due to the systemic dysregulation of ubiquitin-poteosome systems [245]. This findings also stated that the rate of oxidative stress was lifted with the abnormal accumulation of by-product of oxidized lipid and proteins. This abnormal accumulation is evidently linked with dwindled aconitase enzyme activity. In agreement with this, it has been examined that in diabetic rat heart, the activity of mitochondrial aconitase, which is responsible in converting citrate to aconitate, is strongly associated with these manners of reduced TCA cycle flux [246]. Several attempts have been made by Gaster and his colleagues to understand this complex mechanism (down and up-regulation of TCA cycle intermediates) in skeletal muscles of type 2 diabetes subjects. The results indicate that the perturbation of this cycle is far more complex than suggested [247,248]. Deeper insight on these complex interaction at systems biology level would therefore be welcome. It is intriguing to note that these levels of metabolite variations are indicative of the disturbance of metabolic activities in mitochondrial metabolism with dire consequences, necessitating the further detailed studies are imperatively needed.

4.4. Amino Acids

Amino acids are important biological organic compounds that comprise essential and non-essential groups. As the constituents of membrane building blocks, amino acids in the form of protein are the largest components of skeletal muscles. Several distinct proteins are indirectly involved in various biological activities such as hormone biosynthesis and neurotransmission [249,250]. Essential amino acids such as branched-chain amino acids (BCAAs) are required by the human body through diet (meat, dairy, and legumes) for many major metabolic processes. Under normal physiological state, BCAA plays several important roles in regulating numerous physiological functions such as the regulation of food intake relative to the leptin release from adipose tissues [251], biosynthesis of protein in muscle [252], modulating glucose metabolism and oxidation [253], and promoting brain health [254].

The strong association between BCAA metabolism and diabetes has been documented more than 70 years ago [255,256]. In recent years, there are several attempts in applying metabolomics to examine several alterations in various amino acid metabolic pathways. Indeed, amino acid analysis also reveals the defective enzyme activity in amino acid metabolism. A rapidly expanding body of literature confirms that the relative concentrations of wide range of amino acids such as homocitruline, leucine, isoleucine, valine tyrosine, phenylalanine, alanine, and tryptophan are augmented in type 2 diabetes [54,168], IFG [109] and obese subjects [119]. The melatonin-receptor gene, *MTNR1B* was linked to the changes in fasting glucose concentrations [257]. The polymorphism rs10830963 in this gene was parallel with other studies where the same SNP associates with tryptophan:phenylalanine ratios were involved [116]. As phenylalanine is the precursor of melatonin, the deficiency of these genes may indirectly promote to the aberrant of glucose metabolism that is well characterized in the pre-diabetic state. It's worth mentioning that the dysregulation of the phenylalanine hydroxylase gene by hepatocyte nuclear factor 1α (Hnf1α) in phenylalanine metabolism was also coupled to the similar biological phenotypes observed in type 2 diabetes subjects and aminoaciduria related conditions [258].

In the event of systemic metabolic disturbance, it is suggested that the abnormal amplifications of BCAA level might indicate the severe dysfunction in E3 (ubiquitin ligase) enzymes that are commonly shared by various subclasses of the BCAA dehydrogenase, pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase [259]. These complex mechanisms within ubiquitin-proteosome system may inherently lead to the intracellular protein degradation into circulating amino acids in the biological fluids. Emerging findings in human [260] and animals [160] revealed that an intense alteration of amino acid profiles was robustly linked to obesity. In another observational report of 74 obese and 67 healthy subjects, Newgards et al. had identified three robust BCAAs (leucine, isoleucine and valine) that are concomitantly correlated with obesity and glucose intolerance. This finding was verified through targeted MS-based approach with unsupervised approach of principal component analysis (PCA). The concentration of these amino acids was elevated in both obese and overweight subjects with significant disturbance of lipid metabolism [119].

Likewise, the integrity of the plasma level of obese/overweight subjects was greatly perturbed with the significant increase of valine and leucine, i.e., 23% and 14% higher respectively, compared to control groups [112]. In the same way, employing multiplatform analysis, Suhre and his co-workers identified that these high level of three amino acids in the participants in the population-based KORA [54]. Similarly, a recent metabolic study by Stankacova et al. utilizing high throughput NMR platform in the population-based Metabolic Syndrome in Men (METSIM) Study that includes 9,369 non-diabetic or newly diagnosed type 2 diabetic Finnish men also

signified two aromatic amino acids (phenylalanine, tyrosine) and three BCAA (alanine, leucine and isoleucine) in blood plasma as the best clinical predictors for the development of hyperglycemia and type 2 diabetes [261].

Subsequently, to determine whether any of these BCAAs may directly contribute to insulin resistance and obesity, Newgard and his co-workers [119] conducted one refined experiment to decipher the mechanism of these elevations of BCAAs in obese and insulin resistance subjects. They designed the list of experimental works by preparing three groups of rats fed with different diets, which were standard chow (SC), high-fat (HF), and high-fat-BCAA (HF-BCAA), respectively. After normalization, the rats fed with high-fat dense food with the combination of BCAAs (HF-BCAA) were insulin-resistant while the rats fed with HF and SC groups were normal. From this study, the authors proposed that BCAA might play some mechanistic roles within the context of amino acid dietary pattern that includes high-fat consumptions. It is believed that the changes of amino acid profiles in this findings are not the direct cause of obesity and insulin resistance, but it is rather the result of synergistic effects among CoA species (succinyl coA and propionyl coA) that cause stress to the mitochondrial functions. Indeed, the chronic phosphorylation of mTOR (mammalian target of rapamycin), which subsequently leads to the activation of c-Jun N-terminal kinases (JNK), and insulin receptor substrate 1(IRS1)-Ser307, are contributing to the development of insulin resistance in rats fed by HF-BCAA diets.

Besides, the accumulation of multiple acylcarnitine intermediates due to high-fat consumptions may also exert several detrimental effects on these activation processes. A strong justification for the role of amino acids and fatty acids in the development of insulin resistance has been discussed in a recent review that reports numerous changes of various regulatory pathways such as tissue redox balance ($NADH/NAD^+$), methionine oxidation, and cysteine productions [262]. At the cellular level, the down-regulation of BCAA oxidative enzymes in adipose tissues of obese and type 2 diabetes was observed [263,264]. One further report that used branched-chain aminotransferase (BCAT)-knockout mice as a model of genetically induced insulin resistance demonstrated that the BCAA metabolism in mouse adipose tissues was coordinately impaired, hence leading to the abnormal accumulation of BCAA level in the serum [265]. This finding strengthens the hypothesis that defective enzymes in metabolizing BCAA in adipose tissues of diabetes model may inherently affect the equilibrium level of circulating BCAA in the blood. In short, these studies may provide the impetus for the development of metabolomics markers and its associated mechanisms complementing other fields of systems biology in the novel discovery of various amino acid metabolic pathways and signaling molecules in the disease state.

The emerging findings about significant alteration of amino acid profiles in type 2 diabetes and obesity have urged more investigation to be performed in the subsequent years. Wang et al. [266] applied semi-targeted analysis of LC-MS in two independent cohorts and corroborated that five aromatic amino acids and BCAAs such as tyrosine, phenylalanine, leucine, isoleucine and valine were vastly amplified over time with the strong adjustment into age, body mass index, homoeostatic model assessment for insulin resistance (HOMA-IR), homoeostatic model assessment for β-cell function (HOMA-β) and fasting glucose-matched control ($p>0.001$). The authors used two nested case-control reports from the Malmo Diet and Cancer study in Europe and Framingham Offspring Study in the USA. The study involved 2,422 healthy subjects who were followed for 12 years. However, only 201 subjects who later progressed to develop diabetes. The investigators discovered that an alteration of amino acid profile (twofold higher compared to control) in these people who progressed to develop diabetes in time. This study could be acknowledged as one of the stepping stones in establishing and

documenting various markers related to amino acid intermediates associated with insulin resistance, obesity and type 2 diabetes.

Importantly, this diabetes-predictive amino acids score (DM-AA score) for three amino acids (tyrosine, phenylalanine, and isoleucine) also associated with development of cardiovascular disease in a matched case–control study derived from the population-based Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC) [267]. The buildup of these circulating BCAAs concentrations also considered to be useful in differentiating the metabolic profiles of obese, IFG, type 2 diabetes and lean subjects [109,268]. Significantly, Wang et al. [266] also reported that a single biomarker was not fundamentally sufficient in predicting the development of diabetes. The uses of biomarker panels are more reliable and feasible than single biomarker as these panels provide the highest diabetes risk associations across various populations. In spite of all reports above, several lines of investigations on amino acid profile in both human and animal models have been verified to be negatively correlated with the studies discussed earlier. The relative concentrations of BCAAs were down-regulated in urine [107] and blood serum [171] of obese and insulin-resistant patients. Furthermore, the level of BCAAs were also depleted in the blood serum of high-fat fed-induced obese mice [266,269]. Nonetheless, these different findings may have been caused by other interventions such as the variety of dietary components and other parameters used in the investigation that were not reliable to establish cause-effect relationships.

As such, it is interesting to note that obesity and insulin resistance-associated events may involve some severe alterations in cellular amino acid catabolism. The most prominent organ affected by insulin resistance in human is the skeletal muscles as 75-85% of insulin-stimulated glucose disposal system ensues through the amino acid catabolism, which is the major determinant of resting energy expenditure that accounts for 40-50% of the variability in human basal metabolic rate. Therefore, the dysregulation of oxidative metabolism in this insulin responsive tissue was causally added to the pathogenesis of type 2 diabetes and insulin resistance [270]. Insulin is one of the key hormones in maintaining and preserving the mass of skeletal muscles within the body [271]. Insulin regulates various level of amino acid, glucose and lipid metabolism. Physiologically, insulin enhance the cellular uptake of amino acids and protein synthesis in skeletal muscles [272].

In the event of metabolic inflexibility, the cellular damage of skeletal muscle functions occurs via direct mechanism of hyperglycemia induced cell damaged and accumulation of high flux lipid intermediates, ensuing to a low insulin response in peripheral cells and tissues [273]. It is firmly believed that the molecular interaction of metabolic pathways in obesity and type 2 diabetes involves an extensive network of complex intracellular signals. In the condition of metabolic inflexibility such as in diabetes and obesity, the process of muscle wasting is directed through the result of detrimental effects on intracellular signaling pathways that are specifically involved in the regulation of protein degradation (IGF-1/PI3K/Akt pathway) and synthesis (MuRF-1-orMAFbx-dependent pathway), as well as significant alteration in NF-kappaB activation and expression [274,275]. These protein degradation processes give rise to the leakage of these metabolic signatures in the form of amino acids such as BCAAs that are detectable in various biological fluids such as urine and blood. These findings not only lends a support but also offer several potential mechanisms to link diabetes with the changes of amino acid compositions at metabolic level.

Another study contributes additional evidence to existing knowledge that suggests several amino acids such as BCAAs are highly degraded in the skeletal muscles rather than in liver [276]. The products of glycolysis such as pyruvate and acetyl-CoA are the intermediate metabolites than can be linked to other metabolites such

as glucose, amino acids and fatty acids intermediates. These metabolites also can be linked to the BCAA catabolism, leading to the perturbations of various intermediates in the mitochondria [13]. These deleterious consequences that were established in insulin resistance and type 2 diabetes subjects may promote to the accumulation of BCAA by-products in biological fluids of diseased subjects. Metabolic analysis indicated that a higher concentration of these metabolites was exhibited in obese/overweight subjects than control group [112]. In the events of mitochondrial dysfunctions, several metabolic pathways related to amino acid metabolisms are impaired. It has been studied that in peripheral tissues of obese Zucker rats and mice, the actions of two important enzymes such as mitochondrial branched-chain amino acid aminotransferase (BCATm) and branched-chain alpha-keto acid dehydrogenase (BCKD E1α subunit) complexes are expressively blunted. These enzymes are responsible for the catabolic pathways of BCAA [277].

A non-targeted metabolic profiling of 447 fasting plasma metabolites in a large population-based cohort of 2,204 females (115 type 2 diabetic case subjects, 192 individuals with IFG and 1,897 control subjects) from TwinsUK revealed that 42 metabolites from three major fuel sources (carbohydrates, lipids, and proteins) were confirmed to significantly correlate with type 2 diabetes progression after data normalization [278]. The authors believed that branched-chain keto-acid metabolite 3-methyl-2-oxovalerate was the strongest predictive biomarker for IFG after glucose (odds ratio [OR] 1.65 [95% CI 1.39–1.95], $p = 8.46 \times 10^{-9}$). The association of this metabolic pattern was also replicated in an independent population (n = 720, OR 1.68 [1.34–2.11], $P = 6.52 \times 10^{-6}$) and validated in biological fluids of 189 twins. Overall, the study has confirmed the important role for catabolism of branched-chain amino acids in the development of IFG and type 2 diabetes.

Glutamine is a non-essential amino acid that is synthesized within the body. It is proven that oral administration of glutamine and other amino acids may promote overall good health by maintaining muscle mass [279], enhancing growth hormone secretions [280], boosting the immune system [281], and supporting gastrointestinal health [282]. In the development of dietary supplements, glutamine is known as one of the essential amino acids for promoting health [276,283]. Glutamine supplementation might enhance the trafficking rate of glucose transporter 4 (GLUT4), insulin release from pancreatic β-cells as well as release of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [284–286]. A metabolic profiling analysis signified that serum levels of glutamine were depleted in obese people with the potential increase of risk in developing type 2 diabetes [178]. Furthermore, a study of plasma samples from 1015 volunteers of the Framingham Heart Study specified that the strong inverse associations between both fasting glutamine and glutamine-to-glutamate ratio towards insulin resistance were significantly linked after data normalization for age, sex, BMI and baseline fasting glucose. The standardized regression coefficients of both clinical predictors (fasting glutamine and glutamine-to-glutamate ratio) was strongly correlated with relative value of -0.04 to -0.22, change in log-fasting glutamine ($p<0.001$) while -0.05 to -0.20, change in log-glutamine-to-glutamate ratio ($p<0.001$) [179]. Interestingly, the authors also signified that the lifted concentrations of glutamate in the plasma are strongly associated with insulin resistance with the standardized regression coefficients of 0.05 to 0.14 ($p<0.001$). Importantly, the results from this observation were also further confirmed through *in vivo* where mice fed with glutamine result in the improvement of glucose tolerance and decreased blood pressure [179].

Consequently, an effort was successfully devoted in investigating the potential association between gene-transcript-metabolite and gene-metabolite-transcript networks in several models of common laboratory

strains of mice (C57BL/6 and BTBR) [287]. The *ob* gene was introduced into both strains of mice to mimic the model of genetically induced insulin resistance animal. The quantitative trait loci (QTL) metabolic networks identified that the regulations of Glx ratio were directly interlinked to physiological traits of diabetic mice in terms of relative concentrations of certain metabolite intermediates, as well as relative changes in mRNA abundances compared to control. Employing the integrative approach through genomic and metabolic profiling, the authors found that the relative concentrations of glutamate/glutamine (Glx) ratio in the liver was co-coordinately associated with the expression of phosphoenolpyruvate carboxykinase 1 (Pck1), isovaleryl coenzyme A dehydrogenase (Ivd), and argininosuccinate synthetase 1 (Ass1). The ratio of Glx and the level of other amino acids such as alanine and glycine were also interconnected via the relative rate of alanine:glyoxylate aminotransferase (Agxt) mRNAs expression in liver [287].

Analogous to earlier mentioned metabolites, the relative amounts of other nonessential amino acid such as glycine was also significantly altered in various metabolic disorders. Considerable evidence indicates that the relative concentration of glycine was depleted in plasma and serum of diabetic subjects compared to control [113,154,162]. The results from GC-MS analysis confirmed that the serum and liver of high-fat diet rat exhibited with a declined level of glycine concentration compared to control [160]. As per reports, this finding is consistent with the outcomes of other animal studies [166,176]. Several published reports also established an inverse relationship between glycine concentration and obesity or diabetes [119,154]. However, other investigations proved that the level of glycine in the urine [107] and serum [136] was augmented in type 2 diabetes subjects. An observational study utilizing the population-based KORA also proposed the glycine concentration as negative predictor for both IGT and diabetes [13]. The findings were also further replicated and independently confirmed in the population of European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort study [13]. The reduction in the relative concentrations of glycine in plasma of diseased subjects was linked to the event of lipid oxidation and oxidative stress observed in diabetes subjects. An intrigue aspects of the current analysis in this condition are engaged to glutathione utilization by sacrificing glycine as a precursor [130]. Another potential mechanism reconciling the previous and current reports is that the rigorous productions of 5-amino-levulinic acid from glycine due to over-expression of δ-amino-levulinic acid synthase 1 (ALAS1) is required to ensure the metabolic perturbation in disease subjects [13]. Nevertheless, the exact roles of glycine fluctuation in various biological fluids still remain vague. Altogether, these findings reveal that the perturbations of the amino acid profile in these devastating disorders may involve intricate biological processes depending upon the severity and biological variation of population studied. The significant changes of the amino acids level in biological fluids of diseased subjects are correlated with the transient alterations at the cellular level of phenotype functions.

4.5. Choline

Choline is a water-soluble essential nutrient [288], which plays an important role in modulating various physiological functions for transportation of lipid/cholesterol, neurotransmitter synthesis (acetylcholine), methyl group metabolism, and cell membrane signalling [289,290]. Due to these crucial roles in human metabolism, it's deficiency has been associated with numerous metabolic disorders such as diabetes, cancer, liver diseases, neurological disorders, and atherosclerosis [288]. The relative amounts of choline in biological fluids must be maintained at optimal levels for health integrity. Choline intermediates such as PC, lysoPC, and sphingomyelin are known to take part in the synthesis of the membrane phospholipids of the cell membranes [291,292]. Choline is acquired through diet and *de novo* biosynthesis. The adequate intakes of choline-rich food set by the Institute of Medicine, Food and Nutrition Board are 550 mg/day for men aged 19 and older and 425 mg/day for women aged 19 and older [293]. For *the de novo* biosynthesis, the methylation of phosphatidylethanolamine (PE) contributes to PC pools in various tissues. Nevertheless, the amounts of choline supplied by this pathway are not sufficient to meet the human demands in maintaining good health [288]. The combination of these two homeostatic mechanisms in acquiring choline must be adequately maintained at a constant rate.

Several reports are employed to observe the metabolic changes in dietary patterns with choline deficiency [294,295]. Methionine and choline-deficient (MCD) diet introduced to animals results in the accumulation of fatty acid intermediates in the liver and the metabolic regulations supporting these notions are linked to down-regulation of very low-density lipoprotein (VLDL) production and intermittent influx of fatty acids into the liver [296]. Indeed, metabolomics analysis of livers and serum from high-fat diet-induced obese mice found that serum and hepatic PCs were significantly amplified compared to healthy groups [123]. Particularly in the kidneys and liver, choline is converted into betaine and subsequently leads to the transfers of methyl group to homocysteine by betaine-homocysteine methyltransferase, and consequently results in methionine and dimethyl-glycine [295]. Several studies have utilized hepatic and urine analyses of high-fat diet-induced obese mice [123] and Zucker rats [159]. The results in both investigations showed a reduced level of betaine compared to control groups. In addition, in type 2 diabetes subjects, the relative concentrations of betaine in urine were elevated. These perturbations of metabolic activities are believed to be involved in the event of mitochondrial dysfunctions as betaine could not be converted to methionine, giving rise to the accumulation of betaine in various metabolic tissues [291,297]. In other aspects, betaine is also directly involved during the excessive production of carnitine in the disease state [124,295]. These total dysfunctions may inherently affect the efficiency of β-oxidation in disease processes as carnitine is the important metabolite in the regulation of β-oxidation in the mitochondria.

The first serum-based GWAS with LC/MS-targeted metabolomics observed a strong indication on the functions of specific loci such as *PLEKHH1* and *SYNE2* for several associated metabolic pathways involving metabolism of PCae36:5 and PCaa28:1. The authors spotted that the solute carrier family protein such as SLC16A9 (monocarboxylate transporter 9) was responsible for the transportation of acylcarnitine C5 observed in serum plasma of study population [116,298]. Another GWAS that involved 8,816 individuals from three populations with targeted replication association analyses in 18,554 independent subjects revealed that the polymorphisms rs964184 in the apolipoprotein cluster are rigorously associated with the abnormal accumulation of triglyceride level in the blood [299]. In agreement to this, other metabolomic genome-wide association studies (mGWAS) also identified the same SNP to be involved in the PC metabolism [300]. Work by Schäfer and

colleagues suggested that the metabolic remodeling and expansion growth of adipose tissues in genetically obese mice was facilitated by a high demand of PC metabolism [177]. A major recessive gene defect on chromosome 3 (*jobes1*) was linked to the biological variation in the metabolic profile of serum metabolites between obese and lean mice. The LC-MS/MS QTRAP approach observed that the relative concentration of 22 diacyl-phosphatidylcholines (PC aa), two lyso-PC and three carnitines was lowered in obese mice compared to control group. A metabolite–protein network analysis revealed that PC aa C42:1 was functionally associated with the genes *Ccna2* and *Trpc3* via the enzymes choline kinase alpha and phospholipase A2 group 1B (PLA2G1B), respectively. An elevated *Ccna2* expression in adipose tissues of obese mice and unique mutation in the *Ccna2* promoter was causally impacted to the different metabolic profiles observed in obesity-related disorders. This mutual information might find its connection in structuring the link between all levels of systems biology. Conversely, several publications have reported the elevated choline level in diseased group than normal groups in the various biological fluids and tissues such as blood, urine and liver. In the event of high-fat diet-induced obesity, various biochemical pathways are highly affected. For instance, metabolic profile of choline level in the blood [166] and urine [134] of Zucker rats was amplified and the subsequent combination of metabolomics platforms (NMR and LC-MS) using high-fat diet mice as a diabetes model also observed the significant alteration of choline level in the liver [176]. These accumulations of choline are linked to the overproduction of *de novo* biosynthesis of choline that is impaired with the choline pools acquired from diet.

Recently, the roles of intestinal microflora metabolism become one of the interesting issues that are being addressed in various publications pertaining to the issues of metabolic disorders [301–303]. The previous research has identified the potential human intestinal microflora families that are strongly associated with the obesity, namely the phylum Firmicutes and phylum Bacteroidetes [304–308]. In type 2 diabetes, phylum Firmicutes and the class *Clostridia* in the gut of patients were relatively depleted compared to control group [309]. A recent two-stage mGWAS-based study that focused on gut microbiota in type 2 diabetes noticed the decline of global butyrate-producing bacteria due to the dysbiosis conditions as well as augmentation of opportunistic pathogen level in the fecal samples of type 2 diabetes subjects. These metabolic adaptations of microbiomes compositions were also being accompanied by the intensification of gut oxidative stress and sulphate reductions The study used deep shotgun sequencing of the gut microbial DNA from 345 Chinese individuals. [302]. On the other hand, some investigations also indicated that intestinal microbiomes are directly involved in dietary lipid PC metabolism towards the formation of trimethylamine N-oxide (TMAO) metabolites in urine [310–312]. TMAO metabolites are newly discovered metabolites that are strongly associated with added risk for incident major adverse CVD-related problems [313,314]. People with diabetes and insulin resistance often have the following conditions with substantial risk to develop various complications in CVD [313]. The changes of metabolic profiles in several metabolites that are strongly interconnected with the development of CVD-related problems can also be found in people with pre-diabetes state. Wang and his colleagues [311] identified three important metabolites namely choline, TMAO, and betaine are strongly correlated with the development of CVD in an independent large clinical cohort. They also suggested that intestinal microbiota in the *in vivo* studies may play some crucial roles to convert dietary choline to TMAO.

In human, the augmented level of TMAO is seen in urine of type 2 diabetes patients with the significant alteration of other metabolic pathways that are linked with choline metabolism [107,168], while in the knockout mouse of m391, TMAO levels are higher than control groups [175]. Two recent analyses indicate that the

buildup of TMAO level in urine and blood is due to high consumption of foods containing lecithin [310] and carnitine [312]. These foods inherently activate and facilitate several circulating microbes (e.g., species of Acinetobacter) in the human body to convert those metabolites to TMAO. The level and concentration of TMOA in biological fluids could serve as indicators for the status of gut microbiota systems. On the contrary, there is an inconsistency with the findings pertaining to the level of TMAO in the biological fluids. Relatively, a limited number of published papers evidenced that the levels of TMAO were depleted in serum and urinary of Zucker rats [173] and high-fat diet-induced obese mice [169,172]. The discrepancy in these findings could be caused by biological variations of the fatty acid compositions in dietary components. Importantly, the fluctuations in TMAO level in urine and blood may signify the importance of microbiota-derived metabolites in regulating the physiological adaptation of obesity and type 2 diabetes. These metabolites have the potential to be used as putative prognostic biomarkers in the development of diabetes and deregulated microbiota systems.

4.6. Bile Acid Intermediates

Even though obesity and type 2 diabetes are classically associated with the dysregulation in glucose and fatty acid homeostasis, its links to the alteration of bile acid pool size and composition were also documented [315]. Bile acids, also known as bile salts, are the end products of cholesterol utilization. Numerous classes of bile acid compositions are produced in the hydroxylation of various carbon atoms of the molecules. The major classes in men are monohydroxycholanoic acids, dihydroxycholanoic acids, trihydroxycholanoic acids, tetrahydroxycholanoic acids, triketocholanoic acids and hydroxy-keto cholanoic acids. Cholic acid, the subclass of trihydroxycholanoic acids is among the most abundant bile acid in human. Physiologically, bile acids are synthesized in multiple-step processes and stored in the gall bladder, and the synthesis is generated in the liver through cholesterol oxidation [316]. The regulations of bile acid fluxes are modulated through certain subclasses of G protein-coupled bile acid transmembrane receptor, TGR5, and orphan nuclear receptors FXRs [316]. The release of bile acid is engaged through the complex feedback process by lowering blood plasma glucose and triglyceride level and by increasing cholesterol and lipid absorptions [317]. When food is ingested into stomach, bile acid is released into the duodenum and small intestine in order to stimulate the smooth absorption of lipid intermediates. Then, the remaining bile acid is returned to the liver through enterohepatic circulation. In spite of the role of bile acid on various dietary lipid metabolisms and its absorptions, its functions in other metabolic processes such as glucose and protein metabolisms are initiated to be comprehended [318].

There are growing evidences that indicate the incidence of type 2 diabetes is strongly associated with an altered bile acid pool size and composition [319]. In a landmark initiative, for the very first time, Pelkonen et al. [320] demonstrated that the level of bile acid metabolites such as glycocholic acid, glycochenodeoxycholic acid, and taurochenodeoxycholic acid was raised following OGTT. Since then, many clinicians and scientist have started to specifically focus on the instigate roles of bile acids in numerous metabolic disorders related to dysregulation in glucose metabolism. Consistently, another study that used OGTT revealed a comparable result as bile acid pools were amplified in treated subjects compared to control [321,322]. Additionally, one report in 1982 demonstrated that the bile acids in the serum were multiplied following with the response to glucose ingestion [323]. CYP7A1 is considered as one of the vital enzymes for the synthesis of bile acid pools. The connection between glucose metabolism and bile acids synthesis was further confirmed. The highest expression of *Cyp7a1* mRNA was observed in fasting mice following oral glucose and insulin ingestions [324]. Particularly, the excretion rate of fecal bile acid and size of the bile acid pools are significantly intensified during hyperglycemia compared to normal condition (euglycemia) [325]. Corroborate with these findings, it is apparent that the regulation of bile acid pool might play some crucial roles in various metabolic disorders such as obesity and type 2 diabetes [319].

FoxO is known as one of the crucial modulators for hepatic glucose synthesis in favoring the metabolic pathways of gluconeogenesis and glycogenolysis by insulin signaling cascades. However now, it is also linked to the metabolic dysfunctions involving bile acid release in the event of systemic and peripheral insulin resistance. Haeusler and colleagues has identified that the aberrant of FoxO proteins and their posttranslational modifications affect the rate of bile acid synthesis, leading to deterioration of various metabolic pathways such as cholesterol and triglyceride synthesis and their absorptions [326]. These metabolic perturbations may also disturb the action of certain lipoproteins associated with a concomitant increase of cardiovascular-related events. The metabolomics analysis had observed that mice lacking liver FoxO1 (L-FoxO1) experienced an amplification of non-12-hydroxylated bile acids (hydrophilic) while the level of 12-hydroxylated bile acids

(hydrophobic) was expressively reduced in the liver [54]. These metabolic adaptations are strongly associated with the deficiency of their own enzyme, CYP8B1 (also known as sterol 12-alpha-hydroxylase) that is mainly responsible for encoding the 12-hydroxylase in mice. These conditions lead to the abnormal accumulation of non-12-hydroxylated bile acids that subsequently hinder the lowering effects of TG synthesis through orphan nuclear receptors FXR. Concurrently, the upsurge of non-12-hydroxylated bile acids level that are in the hydrophilic forms also results in the enhancement of cholesterol synthesis in knockout mice, possibly due to the decrease of cholesterol absorptions in small intestines. In parallel, the relative level of secondary bile acid such as deoxycholate acid (secondary bile acid metabolite) was augmented in plasma of self-reported diabetic patients and yet one of two primary bile acids, which is cholate (primary bile acid metabolite) is verified to be higher in control groups (normal) than in patients with type 2 diabetes [54]. The metabolomics analysis of plasma level of patients with IGT confirmed the higher level of glycochenodeoxycholic acid compared to subjects with normal glucose tolerance [110]. Furthermore, another study has also identified the difference in the composition of bile acid pools between type 2 diabetes patients and controls [327]. Collectively, these findings provide further insights into novel changes associated with bile acid profiles between control and patients groups as the person with type 2 diabetes is having a greater rate of conversion from primary to secondary bile acids in the gut of patients compared to normal group.

The therapeutic intervention of the bile acid pools in the people with type 2 diabetes was further elucidated with the small scale pilot study of clinical treatment using colesevelam hydrochloride as a bile acid sequestrant. This sequestrant is designed to bind the bile acid left in the intestine and to improve whole-body insulin resistance in diabetic patients. The low total levels of cholesterol and low-density lipoprotein (LDL) cholesterol were confirmed in type 2 diabetes subjects with this therapeutic intervention [328–330]. Nonetheless, the authors report that no significant changes were observed in peripheral insulin resistance in terms of glucose absorptions and metabolism [331]. Moreover, further analysis on the role of FoxO and activation of subclass family of orphan nuclear receptors FXR in the event of bile acid synthesis revealed that FXR ligand (GW4064 or cholic acid) improved the hepatic and plasma triglyceride synthesis rate in a lower state and ameliorated the effects of bile acid imbalance in L-FoxO1 and double mutant L-FoxO1:LDLR−/− mice [326]. These novel explorations might draw some further investigations on therapeutic application of the role of FXR deactivation associated with hepatic lipid abnormalities and bile acid pools in obesity and type 2 diabetes. In total, the above analyses have identified a series of related mechanisms accountingthe roles of various bile acid metabolites that could be potential signatures to differentiate people with disease and normal groups. These metabolites could be exploited and validated in the further longitudinal study to understand the regulation underpinning such diseases.

Taurine is an intermediate metabolite in bile acid metabolism. It mediates the (1) relative rate of calcium homeostasis, (2) conjugation of bile acid and cholesterol, and (3) osmoregulation [332]. Besides, taurine also plays some important roles in other biological processes such as nerve cell activity and detoxification. The relative changes of taurine level in diseased subjects is linked to several metabolic disorders such as diabetes and CVD [333–335]. These functions may signify the importance of taurine as one of the crucial signatures that may modulate and regulate many biochemical pathways. Several reports have applied ^{1}H-NMR spectroscopy analysis coupled with other platforms such as microarray technologies. These studies have revealed the decrease of urinary and hepatic taurine level in GHR mutant obese mice and the expressions of cysteine sulfinic acid

dehydrogenase (*Csad*) were inhibited [175]. In Zucker rats, the urinary excretion of taurine was depleted compared to normal groups [166,181]. These results indicate that a declined synthesis of taurine in body fluids is due to the inhibition of taurine biosynthetic enzymes during the development of obesity and type 2 diabetes. Still, few conflicting results regarding the relative level of taurine in obesity-related events were also reported. Schirra et al. [175], for example, have found that the relative levels of taurine in liver were significantly escalated in mice fed with high-fat diet. In liver, taurine is supplied from diet and *de novo* biosynthesis in the blood. These are few studies that showed the perturbed homeostatic functions in these metabolic disorders may be affected by some unknown factors which warrant further investigation.

5. Concluding Remarks and Future Perspectives

Notably, a number of disease-related metabolic signatures have been identified including fatty acids, TCA cycle intermediates, carbohydrates, amino acids, cholines and bile acids. As a final note, a number of disparate studies have proven the potential of metabolomics in the identification of novel biomarker associated with progression of obesity and type 2 diabetes. To avoid spurious false-positive associations, it is yet to be seen if these potential markers can be reproducibly found in different populations towards establishment of specific biomarker validation for obesity and type 2 diabetes. The biomarker validation process is expected to advance with improved sensitivity and specificity of metabolomics platforms. Several potential metabolic markers are now in the validation stages that require a large sample size and defined prospective investigations. Nevertheless, one of the unsolved problems in metabolomics is to verify whether these metabolic signatures have a causative role in the pathogenesis of the disease. An overarching combination of multiple integrated omics approaches may provide useful insight into causation via application of mGWAS [302]. This can be achieved through quantification and detection of small signatures in biological fluid, comparing metabolic profile of patients and healthy controls over the years. With various integrative approaches become more available, it is a matter of time that we can soon ascertain the early markers with highest prediction values for patient's stratification and survival. Recent reports have discussed several important findings of metabolomic studies with the development of diabetes knowledge in other post-genomic fields such as GWAS, epigenetics, and microarray analysis for mRNA profiling [14,116,336,337]. It is pertinent to mention that the combination of these fields will enable simultaneous analysis of all grounds in systems biology on the whole-body homeostasis of human metabolism, bringing potential opportunities to integrate the efficient diagnosis and treatment closer to reality. In light of the available evidence, it can be foreseen that the concerted action of these fields in systems biology will deepen our understanding on the intricate regulatory pathways as well as its detailed molecular mechanisms associated with the development of obesity and type 2 diabetes.

Conflict of Interest

All authors have no conflicts of interest to disclose in completing this manuscript.

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