Mitochondrial dysfunction as a central event for mechanisms underlying insulin resistance: the roles of long chain fatty acids

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Summary

Insulin resistance is characterized by hyperglycaemia, dyslipidaemia and oxidative stress prior to the development of type 2 diabetes mellitus. To date, a number of mechanisms have been proposed to link these syndromes together, but it remains unclear what the unifying condition that triggered these events in the progression of this metabolic disease. There have been a steady accumulation of data in numerous experimental studies showing the strong correlations between mitochondrial dysfunction, oxidative stress and insulin resistance. In addition, a growing number of studies suggest that the raised plasma free fatty acid level induced insulin resistance with the significant alteration of oxidative metabolism in various target tissues such as skeletal muscle, liver and adipose tissue. In this review, we herein propose the idea of long chain fatty acid-induced mitochondrial dysfunctions as one of the key events in the pathophysiological development of insulin resistance and type 2 diabetes. The accumulation of reactive oxygen species, lipotoxicity, inflammation-induced endoplasmic reticulum stress and alterations of mitochondrial gene subset expressions are the most detrimental that lead to the developments of aberrant intracellular insulin signalling activity in a number of peripheral tissues, thereby leading to insulin resistance and type 2 diabetes. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords long chain fatty acids; insulin resistance; mitochondrial dysfunction; inflammation; lipotoxicity; reactive oxygen species

Abbreviations DNA, deoxyribonucleic acid; LCFA, long chain fatty acid; SCFA, short chain fatty acid; MCFA, medium chain fatty acid; ETC, electron transport chain; FADH₂, flavin adenine dinucleotide; mtDNA, mitochondrial DNA; IRS, insulin receptor substrate; O₂⁻, superoxide radicals; NADH, a reduced form of nicotinamide adenine dinucleotide; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; TCA, tricarboxylic acid; TNF, tumour necrosis factor

Introduction

Type 2 diabetes mellitus is a devastating metabolic disorder that is characterized by insulin resistance and linked to various metabolic syndromes such as hormonal imbalance, hypertension, hyperglycaemia and excess fatty acids in
blood circulation [1]. The biological determinant such as genetic factors is involved in the pathogenesis of type 2 diabetes [2,3]. One of the first-degree relatives who had family history of type 2 diabetes is conferred by threefold increased risk of developing the disease [3–5]. However, during the last few decades, the dramatic increases in incidence and prevalence rates are also significantly observed in developed and developing countries [6], signifying that the complex synergistic roles of both genetic and environmental factors such as malnutrition and sedentary lifestyles are considerably involved in the development of insulin resistance and type 2 diabetes [7].

In spite of extensive researches in deciphering the associated risk factors for insulin resistance, the exact mechanism that contributes to these metabolic perturbations is still largely debated. Excessive calorie intake and poor-quality diet are attributable to one of the major independent risk factors underlying the events of such disorder. Notably, there are growing concerns that surround the roles of certain types of fatty acids in the progression of various metabolic syndromes [8]. Fatty acid is regarded as one of the most important nutrients that are actively involved in the regulation of an energy metabolism. They are classified based on their carbon chain length as short to very long. Short chain fatty acids (SCFAs) are ranging to have aliphatic tails of two to six carbons, while medium chain fatty acids (MCFAs) are the sub-class of fatty acids with aliphatic tails of 7 to 12 carbons. By contrast, long chain fatty acids (LCFAs) are composed of 13 to 21 carbon links, and very long chain fatty acids (VLCFAs) are viewed to have more than 22 carbon lengths [9]. Particularly, different types of fatty acids are metabolized in distinctive ways. It was shown that unsaturated fatty acids are mainly catabolized for energy production, whereas the saturated one is likely to be oxidized with the formation of lipid intermediates [10]. In recent years, there are certain equivocal issues regarding the metabolic roles of LCFAs in the regulation of disease processes. A couple of studies suggested that several types of LCFAs are directed to inhibit the signalling function of thyroid hormones in the liver, leading to the promotion of hyperinsulinemia and fatty liver [11–13]. Accordingly, the chronic exposures of LCFAs such as palmitic acid on L6 skeletal muscle of rat increased the severity of mitochondrial peroxides, mtDNA damages, c-Jun N-terminal kinase-1 (JNK1)-signalling activation and inhibition of intracellular insulin signalling activity, resulting to mitochondrial dysfunctions and insulin resistance [14]. On the other hand, the other type of fatty acids such as SCFA and MCFAs are evidenced to enhance the insulin sensitivity and increase the mitochondrial respirations of the cell [15,16]. Therefore, the oxidation of certain types of fatty acids in various peripheral tissues may affect and behave differently in regulating biological phenomenon of the normal and disease states.

Correspondingly, there is appealing evidence that revealed that mitochondrial dysfunction plays a crucial role in the central pathogenesis of insulin resistance and type 2 diabetes. In fact, the causal pathogenic relationship between mitochondrial dysfunction and type 2 diabetes is largely being discussed [17–19]. Numerous findings [20–26] indicate prominent mitochondrial dysfunction in skeletal muscle, liver and adipose tissues of obese and type 2 diabetic patients with a positive family history of diabetes and in animals with obesity-associated type 2 diabetes. Although clinical investigations on how fatty acids affect the mitochondria function are relatively scarce, compelling experimental studies suggest that the increase of plasma free fatty acid (FFA) induces insulin resistance with the significant alteration of oxidative metabolism in various target tissues such as skeletal muscle, liver, adipose tissue and heart [17,18]. These metabolic impairments mostly occur prior to the development of insulin resistance, which is in the pre-diabetes condition. A number of review articles dealing with the roles of fatty acid-induced mitochondrial dysfunctions in insulin resistance and type 2 diabetes were extensively published [27–32]. The current dogma in the field of mitochondrial dysfunction and insulin resistance is generally focused around the idea that lipid overload induced mitochondrial dysfunction that leads to skeletal muscle insulin resistance. However, the previous research directions are being descriptive to explain the mechanism of insulin resistance in the event of mitochondrial dysfunctions without specifically focusing on what types of fatty acids contribute to these mechanistic processes in other peripheral tissues such as liver, heart and adipose tissue. It is firmly believed that LCFAs play important roles within the disease process by exerting their detrimental effects on mitochondria functions. Thus, the purpose of this article is to provide a comprehensive overview of how LCFAs might contribute to the regulation of insulin resistance by proposing that mitochondrial dysfunction is one of the central hallmarks that causes aberrant insulin signalling activity in a number of peripheral tissues, directing to the progression of insulin resistance and type 2 diabetes.

Mitochondria: it is more than energy factories of the living cells

Mitochondria have its own unique DNA (circular and double stranded) and contain two plasma membranes, an inner and outer membrane. Human mtDNA is maternally inherited and encodes 13 specific gene subunits for mitochondrial biogenesis and metabolism in the electron transport chain (Complexes I, III and IV) together with
ATP synthase, 22 tRNAs and 2 rRNAs. However, certain genes from other specific subunits (Complex II) are encoded from the nuclear DNA [33]. Mitochondria can be recognized as a heart of the cell that modulates various cellular homeostasis such as cell proliferation, calcium homeostasis, detoxification of ammonia (in the liver), programmed cell death (apoptosis), oxidative stress (a hallmark of ageing process) and other related metabolic processes [34,35]. Several important events such as mitochondrial fusions and fissions are indirectly involved in the programmed cell death and autophagy [36]. Without this organelle, cell would be unable to extract the significant amount of energy in order to comply with the metabolic requirements under conditions of high energy demands. Furthermore, it was found that mitochondria are responsible for almost 90% of the whole body metabolism required for the sustainability of cellular process and its physiological components, including the maintenance of the trans-membrane ion gradients, protein synthesis and vesicular transport [37,38].

The ability of cells to perform their function is largely dependent on the amount of energy produced per se during the hydrolysis of the terminal phosphate bond. This is generally known as the free energy of ATP hydrolysis. The amount of ATP generated by a particular tissue depends on the number of mitochondria that can range from hundreds to thousands per cell. The amount of mitochondria varies depending on the age and maturation of the cell as well as type of tissues [39]. The electron transport chain (ETC) system can be regarded as the heart of the mitochondria where the oxidative phosphorylation takes place. The electron transport chain is embedded in the inner mitochondrial membrane that comprises four enzymatic series complexes (Complexes I–IV) together with ATP synthase. These five complexes synergistically work in concert as a biological machine that undergoes conformational changes due to the substrate-induced reduction–oxidation reactions. The electrons that are passed down through ETC are delivered and accepted by oxygen in order to generate an electrochemical gradient that drives the phosphorylation of ATP synthesis.

During the oxidative phosphorylation, the reduction of one molecule oxygen with one electron produces free radical and superoxide molecules. Reactive oxygen species (ROS) such as superoxide (O$_2^–$) radical is converted to hydrogen peroxide (H$_2$O$_2$) by manganese superoxide dismutase and to water by glutathione peroxidase. Several distinct biological processes such as DNA repair, transcription, replication and protein synthesis are indirectly interacting with the mitochondrial matrix. In order to perform these cellular processes, all required enzymes need to be imported from cytosol into the mitochondrial matrix. These complex processes signify that mitochondrial activities require distinct mechanistic interactions from various levels of metabolic activities in the tissue. Thus, any impairment of these processes might inherently affect downstream metabolic activity of the cell, as well as functional and structural behaviour of certain proteins and metabolites in target tissues. In addition, it was evidenced that human mitochondrial genomes (size of approximately 16.5 kb) have a very high mutation rate that is 10–17-folds higher than the nuclear genomes. This could be due to the nature of human mitochondrial structural integrity that lacks histone and mutation-suppressing elements such as intron sequences [40]. As such, it does not come as a surprise why these biological complex systems in the mitochondria are vulnerable to perturbations in the event of metabolic stress and disease process [41]. Because of the fragility of the mitochondrial respiratory chain system, one simple perturbation to the electron transport chain and its downstream complexes might affect the nucleotide pools, tricarboxylic acid (TCA) cycle flux, one-carbon metabolism and ROS signalling [42].

In particular, glucose, fatty acids and amino acid are the important substrates that have to be utilized by the body in order to perform the cellular functions [43]. These important substrates undergo a series of cellular metabolism and break down into several intermediate metabolites. The reducing equivalents such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH$_2$) and other metabolites are directly involved towards energy production. They are generated through TCA cycle and β-oxidation together with the involvement of various levels of oxidative process. Highly energetic tissues such as skeletal muscle, heart and liver are critically dependent on the oxidative metabolism of mitochondrial activities and phosphorylation of adenosine diphosphate (ADP) to ATP [44]. As the excessive energy production takes place, ROS are produced and may cause defects in other macromolecules such as lipid, protein and nucleic acid. Consequently, these conditions cause a series of modification of the mtDNA structures and affect the upper and lower levels of the mitochondrial gene expression and proteins [45]. Excess FFA in blood circulation and together with hyperglycemia may also result in the disproportionate generation of superoxide radical that apparently affects mitochondrial integrity and plays a major role in the pathway leading to many metabolic disorders [46]. One study showed that in parallel with the increase of ROS productions, other stress kinase intracellular signalling pathways via nuclear factor-kB (NF-kB), p38 mitogen-activated protein kinase, JNK or stress-activated protein kinase, and hexosamine are also triggered in the disease process [47,48]. The activation of these regulatory pathways together with the elevation of ROS productions worsens the metabolic activity of the cell, causing the impaired glucose uptake and insulin
resistance. Conspicuously, the underlying mechanism of mitochondrial dysfunction in several peripheral tissues is very complex and intricate to be understood that it requires synergistic analysis of both genetic (nuclear and mitochondrial genomes) and environmental factors to be performed. Given their significance in basic biology and clinical medicine, we should agree with the assumptions to recognize mitochondria as one of the vital organelles in regulating cellular and systemic energy homeostasis of the various metabolic disorders such as insulin resistance and type 2 diabetes.

Cellular metabolic competition of fatty acid and glucose oxidation

Historically, the research works on the metabolic competition between fatty acids and glucose oxidation for mitochondrial respiration were started in the early twentieth century [49]. Several years later, the perfused heart muscle and diaphragm of rats were isolated to elucidate these mechanisms of inhibition of glucose oxidation by fatty acids [50,51]. After that, the first mechanistic explanation for the reciprocal relationship between fatty acids availability and glucose utilization was pronounced by Randle and his colleagues. This finding is famously known as the Randle cycle [52]. In their study, they believed that the elevation of fatty acid level supply to the diaphragm and isolated heart was strongly associated with an increase of fatty acid oxidation rates towards producing acetyl-CoA and citrate. The availability of these metabolic intermediates in heart muscle essentially leads to the impairment of glucose oxidation, glycolytic flux and its utilization. The strong competition of substrate oxidation between fatty acid and glucose was significantly observed in rat heart muscle. According to their hypothesis, the increase of FFA oxidation directly augments the mitochondrial ratio of acetyl-CoA to coenzyme A and NADH to NAD+. An elevation in these ratios results in the increase of citrate levels in the mitochondria and crucially inactivates the pyruvate dehydrogenase activity via inhibition of glucose oxidation in the cytosol. In turn, glycolysis is inhibited via reduction of phosphofructokinase and hexokinase II activities and later contributed to the accumulation of glucose-6 phosphate and glycogen levels in the cytosol, thus contributing to the decrease of basal glucose uptake [53,54].

However, in the following years, Randle’s cycle theory was challenged and overshadowed by numerous evidence [27–29,54,55]. They clearly argued that the accumulation of excess fatty acid intermediates such LCFA-CoA, diacylglycerol and ceramides in various peripheral tissues is noticed. Insulin resistance induced by FFA is primarily associated with the impaired glucose uptake rather than the changes of glucose metabolism in the cytosol [54,55]. The defects in glucose transport system, phosphorylation and its disposal equally contribute to the reduction of glycogen synthesis in rat with high-fat diet-induced insulin resistance. The other similar reports also documented that the elevated level of LCFAs impairs and inhibits the process of glucose uptake rather than affecting the glucose metabolism [56–58]. Thus, it is noteworthy to know that Randle’s cycle does not completely explain and demonstrate the reciprocal relationship between the glucose and fatty acid oxidation, as both metabolic substrate oxidations were reduced in distinct ways. In fact, other intracellular mechanisms such as accumulation of ROS and lipid intermediate metabolite-induced lipotoxicity, activation of novel protein kinase isoforms and the effects of inflammatory mediators in the cytosol might play the most important causative roles in the development of insulin resistance [31,59]. On the other hand, it is speculated that LCFAs could be one of the ‘metabolic culprits’ that can deteriorate the efficiency of mitochondrial activities in the long run. The emergence of the old and new theories in these competing ideas might warrant for numerous potential investigations and studies to be performed in the future.

The metabolic fates of LCFA oxidation in normal and disease state

Fatty acids undergo different metabolic pathways depending on the length of the carbon chain, position and geometry of the double bonds (cis and trans) [60]. These differences in chemical properties might substantially affect their metabolism in the body. The resultant fatty acid substrates subsequently modify their properties by the actions of the respective enzymes involved in the particular metabolic pathways [61]. Physiologically, SCFAs and MCFAs are able to diffuse directly into the plasma membrane of the liver via portal vein during lipid digestion. LCFAs are wrapped into chylomicron and enter lymphatic capillaries via the subclavian vein before going into the blood circulation [62]. LCFAs are subjected to undergo several distinct processes that require protein transporter such as fatty acid translocase (FAT/CD36), fatty acid transport proteins and plasma membrane fatty acid-binding protein in order to cross the membrane barrier of the cell. It was observed that the CD36 mRNA and its protein were significantly amplified in both rodent and human skeletal muscle during several days of the intervention of a high-fat diet [63,64]. Additionally, the defective uptake and utilization of LCFAs in skeletal muscle, heart and adipocytes were observed in CD36 knock-out mice [65]. This might suggest the pivotal role of this...
protein transporter in the relative rate of LCFA uptake into skeletal muscle of both rodents and humans.

The insulin resistance is also characterized by an imbalance between fatty acid oxidation and its uptake in certain target tissues [66]. During fatty acid oxidation, the transport of LCFA is generally known as the rate-limiting step. LCFA are metabolized by the addition of coenzyme A by acyl-CoA synthetase in the cytoplasm, and the resultant LCFA-CoA is transported into the mitochondria via carnitine palmitoyltransferase. Before cytoplasmic LCFA-CoA undergoes fatty acid β-oxidation in the mitochondrial matrix, it must be transported into the mitochondrial membranes. This process would require the aids of several enzymes to be highly expressed in the outer mitochondrial membranes, as they cannot freely diffuse into the mitochondrial. Carnitine-dependent transport system is known as a central system in regulating and modulating the LCFA transportation in the outer and inner mitochondrial membrane. The system consists of carnitine palmitoyl-transferase I (CPT-I), carnitine-acylcarnitine translocase (CACT) and carnitine palmitoyl-transferase II (CPT-II) [67]. CPT-I is the rate-limiting enzyme that catalyses the addition of carnitine into the LCFA-CoA towards the formation of acylcarnitine in the mitochondrial intermembrane. Acylcarnitine is transported into the mitochondria matrix with the aid of shuttle transporter CACT, whereby CPT-II reverts the action of CPT-I by reconverting the acylcarnitine into long chain fatty acyl-CoA and carnitine. Later, carnitine is transported back into the mitochondrial membranes to be utilized for the subsequent reactions [67]. Lastly, LCFA-CoA enters into the β-oxidation process, producing acetyl-CoA, and enters into the TCA cycle and ETC for the cellular energy generation. The overall mechanistic interaction of carnitine transport system in regard to the LCFA oxidations in normal and insulin resistance subjects is summarized in Figure 1.

The capacity of this carnitine system for the transportation of LCFA into mitochondria heavily depends upon the expression of CPT-I enzymes, which is controlled by the allosteric inhibitor malonyl-CoA and citrate. The formation

Figure 1. Schematic representation of the long chain fatty acid (LCFA) oxidation in mitochondria for both normal and disease states. Activation of fatty acids (fatty acyl-CoA) is the rate-limiting step in β-oxidation in mitochondria. The transfer of carnitine to LCFA-CoA to form long chain acylcarnitine is catalysed by carnitine palmitoyl-transferase I (CPT-I). Once inside the mitochondria, carnitine palmitoyl-transferase II (CPT-II) reforms LCFA-CoA, which undergoes β-oxidation generating acetyl-CoA molecules. However, an accumulation of LCFA in the cytosol required high amounts of the carnitine transport system to be expressed in mitochondrial membranes. Besides, in the events of abnormal accumulation of LCFA, malonyl CoA are synthesized by carboxylation of acetyl-CoA carboxylase irreversibly that inhibits CPT-I activity, thus inhibiting β-oxidation by preventing entry of activated fatty acids into the mitochondria. As a result, these mechanisms lead to the build-up of lipid intermediates that can exert their deleterious effects on mitochondrial membranes. CACT, carnitine-acylcarnitine translocase; CAT, carnitine-acetyl transferase; ACC: acetyl-CoA carboxylase; CoA: coenzyme A
of malonyl-CoA in the cytosol is catalysed by acetyl-CoA carboxylase (ACC) via carboxylation of cytosolic acetyl-CoA. Thus, in order to increase the transportation of LCFAs into mitochondria, the inhibition of the CPT-I enzyme must be resolved first. This can be achieved via direct phosphorylation of ACC and the reduction of malonyl-CoA synthesis with highly expressed of CPT-I enzyme activities [68]. In obese and insulin-resistant individuals, the relative rate of mitochondrial LCFAs uptakes in the number of peripheral tissues such as skeletal muscles and liver is significantly reduced. As compared with the healthy control, obese groups exhibit almost 35% lower CPT-I activity with profound 50% decrease of fatty acid oxidations [69]. In addition, the increased expression of ACC activity at the basal level was reported in insulin-resistant subjects. The high-level expressions of this enzyme later stimulate the synthesis of malonyl-CoA to inhibit CPT-I activity, resulting in the accumulation of lipid intermediates in the cytosol [70].

β-oxidation is one of the important processes in mitochondrial fatty acid catabolism to produce acetyl-CoA and shortened fatty acyl-CoA towards energy production in mitochondria. A concept of the incomplete β-oxidation cycle was introduced to manifest the condition of lipid oversupply that increased fatty acid oxidation without the activation of the TCA cycle and ETC in the process [71,72]. This process promotes the unusual accumulation of lipid intermediates such as acylcarnitine in the mitochondrial membrane and exerts its damaging effects into various metabolic signalling activities of mitochondria metabolism. Several experimental and clinical studies revealed that the acylcarnitine profile of marker incomplete β-oxidation in the blood and urine is significantly amplified in both animal and type 2 diabetic patients compared with control [71,73,74]. Acylcarnitine is known to affect the mitochondrial protein within the plasma membrane by acylation and acetylation [75,76]. Additionally, incomplete β-oxidation may also lead to the accumulation of reactive oxygen species (ROS) that exacerbate the lipid peroxidation rates towards damaging the mitochondrial protein and DNA [77]. The inflammatory response through the activation of JNK and IKKβ protein is activated with the increases of ROS production in the mitochondrial membrane [48]. In spite of that, the exact mechanism by which this aberrant process in β-oxidation leads to insulin resistance remained to be elucidated.

**LCFA-induced mitochondrial dysfunction leads to insulin resistance**

The metabolic associations between mitochondrial dysfunction and insulin resistance were studied as early as in the 1963 [78]. Later in 2000, it was found that elder people exhibited skeletal muscle insulin resistance with the significant decrease (~40% lower) of the mitochondrial oxidative phosphorylation level compared with the young group [79]. Mounting evidence indicates that the circulating FFAs in the blood play a vital role in the development of insulin resistance [8,80,81]. Insulin responsive tissues such as skeletal muscle and adipose tissue metabolize both glucose and FFAs for energy production. In the cell, glucose is broken down into pyruvate, which is converted to acetyl-CoA, by the enzyme pyruvate dehydrogenase in the mitochondria. Fatty acids are first metabolized to several lipid intermediates in the cytosol in order to be transported into mitochondria and catabolized towards the formation of acetyl-CoA via β-oxidation. Later, acetyl-CoA is converted to metabolic intermediates in the TCA cycle with the generation of several reducing equivalents such as NADH and FADH2. The regulation of these metabolic activities in fatty acids and glucose metabolism is tightly coordinated in order to prevent any development of metabolic defects. The ability of various peripheral tissues to switch from fat oxidation during fasting to glucose oxidation after a meal is referred to as metabolic flexibility [82]. Insulin resistance and obesity are characterized by the ‘metabolic inflexibility’ due to the total impairment of substrate preference between glucose and fatty acids in fasting and insulin-stimulated conditions [83].

Insulin resistance is an integral event to the pathophysiology of obesity-related metabolic abnormalities and type 2 diabetes [84]. This metabolic impairment is defined as one of the systemic adaptations of the body to the form of underutilized glucose uptake in specific tissues such as liver, adipose tissue and skeletal muscles [85]. Obese individuals are at an increased risk of developing a range of chronic health problems such as cardiovascular disease, diabetes, hypertension, non-alcoholic fatty liver and certain cancers [86]. The influence of obesity on the risk of type 2 diabetes is determined not only by the degree of obesity but also by the site where the fat accumulates [87]. The excess accumulation of FFAs in the adipose tissue may systematically lead to the increased rate of lipolysis in the body. In the event of increased lipolysis, there is an augmented flow of non-esterified LCFA into skeletal muscle, resulting in the excessive muscular fat storage, associated with the insulin resistance [88]. Prolonged exposure of LCFA into numerous target tissues activates deleterious mechanisms of the glucose–fatty acid vicious cycle that inherently lead to the mitochondrial dysfunction and insulin resistance [66]. The present research study provides additional evidence with respect to the role of LCFA-induced mitochondrial dysfunction via several adaptive mechanisms of metabolic dysfunctions in cellular and systemic profiles of the human body. Therefore, we proposed the idea that LCFA may synergistically induce these intricate mechanisms
towards the notions of ROS-induced oxidative stress, lipotoxicity, significant alterations of mitochondrial gene expression and activation of inflammatory signalling pathways in the development of insulin resistance and type 2 diabetes.

High level of reactive oxygen species

One of the popular postulations underlying mechanisms of diabetic complications is oxidative stress, which arises from the excessive production of free radicals from a variety of sources, especially from the diet. It is estimated that 57.9% of diabetes subjects experience microvascular or macrovascular complications that are significantly related to the oxidative stress [89]. The oxidative stress is the cellular adaptation of certain target tissues in response to the overproduction of ROS. The cross-talk between the oxidative stress and insulin resistance has become one of the central issues in diabetes complications [90,91]. ROS can rapidly react with a wide range of molecules such as lipid, amino acid, carbohydrate and mtDNA. The resultant molecules are produced through severe modifications in the chemical structure to become highly cytotoxic and reactive. Consequently, these molecules may exert their biological effects in intracellular insulin signalling activity, which lead to the irreversible destruction of the cell functions [92,93].

Generally, LCFAs alter the mitochondrial respiration towards producing excessive ROS via two ways: (1) partial blocking of mitochondrial respiratory chain activities and (2) depolarization of mitochondrial inner membrane (the uncoupling effects) [94]. The stimulatory mechanism of these processes is believed to be regulated through the damage of cytochrome c from the external side of the mitochondrial inner membrane. In case of oxidative stress, previous studies showed that the impairment of mitochondrial ETC Complexes I and III is the major source of the free radical superoxide production in diabetes [95]. These superoxide molecules are released into mitochondrial intra-membrane space and matrix. When the level of these reactive superoxide molecules exceeds antioxidant capacity of the cell to neutralize these free radicals, cellular damage may occur. The accumulation of oxidized product of lipids, DNA and related glycolytic products is prominent to be detected in the plasma, urine and various target tissues, suggesting that the systemic and local manifestation of these conditions has exacerbated the diabetes complication with dire consequences [96,97].

The production of ROS in mitochondria is stimulated from a variety of complex biological processes by involving certain activation of enzyme kinases such as NADPH oxidase and nitric oxide synthase in the mitochondria [77,98–100]. The elevation of ROS production by the mitochondria is usually followed by the increased concentration of plasma FFAs that are highly susceptible to alter the oxidative metabolism of numerous insulin responsive tissues [101,102]. Indeed, LCFAs, especially the unsaturated ones, are prone to oxidative damage [103]. Several lines of studies have identified that the chronic elevation of lipid intermediates is implicated with the increase of ROS production [104,105]. The accumulation of ROS may worsen the inner membrane of mitochondria (cardiolipin-enriched in polyunsaturated fatty acids) [106]. These accumulations consequently cause damage to the mitochondrial machinery that consists of the electron transport chain and their downstream effectors. This condition indirectly activates the negative feedback mechanism via lowering the expression of various mitochondrial gene expressions and proteins. Eventually, this cellular behaviour results in the decline of mitochondrial activities to metabolize lipid efficiently, giving rise to an abnormal accumulation of lipid intermediates in the inner membrane of mitochondria and cytosol. This metabolic adaptation is usually observed in pre-diabetes patients, as their skeletal muscles and liver contain a high amount of lipid intermediates and increased rate of lipid peroxidation [20,107,108]. Besides, the oxidative stress also activates several oxidation and reduction processes of cellular protein via sulphydryl groups of cysteine in proteins and later affects several major functions of the peripheral cells in regulating the cellular homeostasis and numerous biological pathways [109]. Thus, it is not surprising to know that the imbalance of these redox reactions inherently exacerbates the cellular function in the intracellular signalling process, hence contributing to the development of the disease.

Works by Anderson and colleagues have identified that the level of mitochondrial H2O2 was significantly augmented in both human and rodents following a high-fat diet meal [110]. They also observed several significant changes through the reduction of the mitochondrial redox buffering capacity after a high-fat diet meal. In addition, the 6-week attenuation of these superoxide molecules with mitochondrial targeted-antioxidant resulted in the improvement of glycaemic control and insulin sensitivity, and ameliorates the detrimental effects by high-fat diet. However, in overweight Zucker rats, the emissions of mitochondrial H2O2 were significantly increased in rat skeletal muscle. The study also found that in vivo metformin (anti-diabetic drug) treatment that was continued for about 4 weeks altered the mitochondrial respiration rate through significant decline of mitochondrial H2O2 emission in skeletal muscle of obese rats [111], signifying that the potential antioxidant-attenuated ROS is directed via the improvement of mitochondrial functions. Furthermore, in vitro studies that utilized LCFAs as substrate oxidation...
in endothelial tissue and skeletal muscle also resulted in the increase of ROS productions in mitochondria [112,113]. Current studies also documented that the treatment of LCFA such as linoleic acid on 3T3-L1 adipocytes contributes to the excessive accumulation of ROS with the significant decrease of glucose uptakes/utilizations as well as activates various levels of inflammatory signalling cascade pathways [114]. As diabetes is strongly associated with other complications such as neuropathy, it was observed that the human neuronal SK-NB-E cells treated with VLCFAs (C22:0, C24:0 or C26:0) for 48 h result in severe modifications of mitochondrial integrity and morphology, resulting in the development of oxidative stress and mitochondrial dysfunctions [115].

Several studies suggest that oxidative stress also affects the mitochondrial activities and its morphology. The oxidative damage induces an accumulation of cell-damaged mitochondria and blocks various distinct processes in mitochondrial activity such as the electron transport chain, β-oxidation and TCA cycle [91]. The impairment of mitochondrial function causes a reduced activity of rotenone-sensitive NADH: O₂ oxidoreductase, citrate synthase and creatine kinase activity [116]. The same study also observed that mitochondria in skeletal muscle of patients with type 2 diabetes were much smaller, and abnormal morphology was clearly observed compared with the control group. The impaired mitochondrial functions directly contribute to insulin resistance with a couple of defects in glucose and lipid metabolisms because of the disturbed oxidative phosphorylation capacity as well as the inefficient provision of ATP for hexokinase activity [117]. An in vitro cell-based assay study indicates that chronic exposure of 3T3-L1 adipocytes to high concentrations of glucose and fatty acids caused a decrease in the mitochondrial membrane potential, morphology changes and down-regulation of peroxisome proliferator-activated receptor gamma (PPARγ) co-activator 1-alpha (PGC-1α) with the significant increase of ROS [24]. In 3T3-L1 pre-adipocyte, several mitochondrial inhibitors impaired mitochondrial respiration, leading to the abnormal triglyceride accumulation. High levels of ROS concentrations in mitochondria by respiratory chain were reported to exert several deleterious effects on the adipocyte proliferation and differentiation [118].

A damaged mitochondria membrane induces accumulation of ROS in the cell and may indirectly affect mitochondrial DNA, RNA and proteins [93]. As a result, the activation of the chronic inflammatory pathways is directed to signal the event of these oxidative damages. Increase of an electrochemical gradient across the membrane exacerbates the cell to undergo oxidative damage by elevating the ROS production. Clearly, these findings suggest a role of a high level of ROS that is highly dependent on the proton gradients over the inner membrane of mitochondria that consists of ATP synthase for ATP production. However, in spite of accumulating findings for the role of LCFA-induced ROS accumulation in the development of such disorder, there are also several lines of studies depicting that insulin resistance can occur without any exacerbations of mitochondrial oxidative properties [20,119–121]. Thus, further studies are strongly needed to be performed in order to enhance our understandings related to the exact roles of LCFA-induced oxidative stress in the development of such impairments. Taken altogether, these studies might substantially add into our current understanding of pathophysiological mechanisms underlying insulin resistance and type 2 diabetes pertaining to the interaction between mitochondrial functions and LCFA availability.

**Altered pattern of mitochondrial gene expression**

Mitochondrial activity is a highly coordinated process that requires a considerable amount of controlled signals in the forms of enzyme and growth factors from both mitochondria and nuclear genomes. These complex signals can act as metabolic sensors that mediate any variation in biological perturbations of cellular and systemic profiles of the human body. The metabolic activities in mitochondria such as β-oxidation, the TCA cycle and electron transport chains are heavily dependent upon the regulation of these cellular machinery genes and proteins. Previous studies reported that a significant amount of deletion and mutation in mtDNA (m.3243A > G) and its related nuclear encoded proteins is critically associated with the development of insulin resistance and type 2 diabetes [122–125]. Several distinct mitochondrial single nucleotide polymorphisms are also linked with such perturbation [126]. Any deficiency or disturbance in these mitochondrial genes and protein networks may contribute to the dysregulation of mitochondrial gene expressions and affects the integrity of the mitochondrial dynamic process. Indeed, the reduction of these key proteins is implicated in the pathogenesis of insulin resistance and endothelial dysfunction in diabetes [127]. In the event of metabolic defects such as type 2 diabetes, insulin resistance and ageing, the integrity of these machinery genes and proteins involved in the mitochondrial biogenesis and metabolism is totally diminished at various levels of a disease process [17,20,128].

Recent review from Joseph and his colleagues highlighted several important issues concerning the dysregulated mitochondrial gene expression networks and its protein various metabolic defects in the progression of insulin resistance and type 2 diabetes [129]. A number of experimental studies also showed that the cellular mechanistic
adaptations of skeletal muscle in obese and type 2 diabetes subjects concomitantly alter the integrity of several vital genes responsible for glucose metabolism, fatty acid oxidation and oxidative phosphorylation [21,22]. Works by Turner groups have compared the effects of fatty acids with different carbon chain lengths (LCFA vs MCFA) in rodents by evaluating their insulin actions and mitochondrial functions [16]. They have found that rodents that were fed with MCFA exhibited an increase of the markers in mitochondrial metabolism up to 50–140% (vs low fat-fed controls, p < 0.01) while groups that were treated with LCFA diet only increased 20–50% (p < 0.05) of markers for mitochondrial metabolism in skeletal muscle compared with low-fat diet-fed controls. In addition, mice fed with MCFA diet did not suffer from severe triglyceride accumulation in skeletal muscle compared with mice groups fed with LCFA that exhibited the lipotoxicity properties with a significant increase of 77% intramuscular triglycerides. The same study also found that the mice treated with MCFA diet led to the improvement of insulin sensitivity in skeletal muscle and adipose tissue. In addition, glucose tolerance in this group treated with MCFA was significantly improved as compared with the other group that was fed with LCFA-rich diets [16], indicating the possible roles of LCFA in the regulation of insulin resistance and lipotoxicity-related events.

The PGC-1 family is a member of the nuclear receptor superfamily that is mainly responsible for the mitochondrial biogenesis and metabolism. This protein is metabolically involved in various biological regulations co-activated with other key transcription factors such as nuclear respiratory factor-1 (NRF-1), estrogen-related receptor-α (ERRα) and PPARβ or PPARγ [130,131]. The following studies also identified that PPARs directly participate in regulating the mitochondrial gene subsets and fatty acid oxidation as well as becoming one of the inducers for the uncoupling mechanism [130,132]. The increase of LCFA concentration in a number of peripheral tissues stimulates these receptors and numerous biological signalling activities through direct inhibitions of intracellular insulin signalling and AMP-protein kinase (AMPK) phosphorylation [133]. PPARα is ubiquitously expressed in liver, heart, kidney, intestine, pancreas, skeletal muscle and brown adipose tissue. It is responsible for the expression of diverse mitochondrial genes that are essentially involved in fatty acids and glucose metabolism. This protein is also recognized as one of the molecular sensor for various types of fatty acids and other lipid derivatives – due to its natural ligands that include polyunsaturated fatty acids. The action of this protein is stimulated through various multiple target genes that are strongly correlated with the increase of CPT-1a expression, the rate-limiting enzyme of β-oxidation in mitochondria. It is also a regulator of CD36, lipoprotein lipase, UCP-3 and potentially pyruvate dehydrogenase kinase-4 (PDK-4) [134].

Long chain fatty acids activate the expression of several genes involved in the lipid and glucose metabolism via stimulation of CPT-1 and PDK-4 [135]. However, the elevated level of plasma FFA may induce detrimental effects on the expression of these enzymes to cause loss-of-functions due to the heavy metabolic demands. Corroborating with these facts, it was demonstrated that the chronic exposure of LCFA in skeletal muscle apparently results in significant alteration of PGC-1 functions [104,105,136,137]. In healthy males, 3 days of high-fat feeding resulted in a significant decline of PGC-1α and PGC-1β expression [138], while similar effects were also observed in 6-h lipid infusion into healthy subjects that resulted in the down-regulation of PGC-1α, PGC-1β and PPARα expression [139]. Another study reported that 3 weeks of high-fat diet in healthy mice led to the down-regulation of mitochondrial genes and enzyme expression such as PGC-1α and several protein components of the mitochondrial ETC [140]. To strengthen this finding, the same study also observed that the treatment of Troglitazone (an insulin sensitizer) in Zucker rat showed a significant increase of PGC-1α and NADH dehydrogenase subunit 1 expression level, indicating that the regulation of mitochondrial genes subsets is considerably implicated in the development of insulin resistance and type 2 diabetes.

Furthermore, a high amount of intramyocellular lipid content is linked to the down-regulation of PGC-1α expression and other gene-encoding protein for mitochondrial respiratory chain complexes I, II, III and IV [138]. In adipose tissues, the reduced expression of PGC-1α in white adipose tissue of rat also leads to the accumulation of lipid intermediates that is concomitantly associated with a significant reduction of gene expression regulating lipid and glucose metabolism in the respected tissue [141]. One study confirmed that the NRF-1 is a downstream target transcription of mitochondrial genes responsible for the mitochondrial dysfunction in insulin resistance. The authors found that NRF-1 expression was significantly reduced as parallel with the lowered level of mitochondrial genes and protein expression [142,143]. Besides, these metabolic changes in gene expression also contribute to the accumulation of intramyocellular lipid content in the mitochondrial matrix that leads to the insulin resistance [17,21]. As PGC-1α is a transcriptional co-activator of NRF-1, PPAR and growth associated-binding protein in the mitochondria, it is tempting to hypothesize that any alteration of PGC-1α gene expression may regulate other mitochondrial genes and proteins. Therefore, any suppression or loss-of-function of these vital genes may result in the impairment of fatty acids and...
Glucose oxidation that leads to the development of mitochondrial dysfunction and insulin resistance.

Nevertheless, all the studies reviewed so far in regard to the dysregulated mitochondrial genes and protein networks in the development of insulin resistance suffer from conflicting results, which indicate that the improvement of insulin sensitivity was not necessary to be accompanied by an increase of mitochondrial gene expression and proteins. Oikari et al. (2008) have successfully shown that 4-week treatment of LCFA in rats increased mRNA expression (1.5–3.5-folds) of liver PPARs and sterol regulatory element-binding transcription factor-1 (SREBP-1) compared with the control groups [144]. However, as compared with control, the protein levels of AMPKα were depleted by 48% with no significant changes observed in glucose and insulin levels of rat fed with LCFA diets. This study indicates that any enhancement of insulin sensitivity is not necessarily accompanied with the up-regulated mitochondrial gene expressions. Similarly, the same study also observed that a diet with MCFAs reduced liver PPARγ and SREBP-1 mRNA levels by 43% and 35%, respectively, while the mRNA level of PPARγ expression in adipose tissue was up-regulated by 31%. An improved glucose tolerance and lower serum insulin level were reported in this group compared with the control, signifying that the decreases of several mitochondrial gene expression and proteins were not necessarily associated to the evolution of insulin resistance. In addition, another study found that the reduced mitochondrial protein contents of transcription factors including PGC-1α and PGC-1β and proteins associated with mitochondrial fusion events in skeletal muscle are not correlated with the progression of insulin resistance and obesity [145], clearly warranting further detailed investigations to elucidate and understand these complex mechanisms underlying the disease.

Mitofusin 2 (Mfn2) is known as one of the key proteins that are directly involved in the biological process of mitochondrial membrane metabolism. The expression of Mfn2 genes and its proteins was reduced in the human skeletal muscle of insulin and diabetic Zucker rat (ob/ob) with the significant decrease of glucose oxidation rate [146]. Another study confirmed that the Mfn2lloxP/lloxP mice exhibit insulin resistance with a profound increase of endoplasmic reticulum stress and enhanced inflammatory signalling pathways [147]. The study was then further verified in rats that were fed with a high-fat diet rich with saturated LCFA that exhibit insulin resistance with lowered expression of Mfn2 in the skeletal muscle [148]. Furthermore, in agreement with the aforementioned studies, Gan and colleagues identified that the overexpression of Mfn2 genes in a group of rats showed ameliorative properties against a high-fat diet-induced insulin resistance [149]. The study also specifically observed that the glucose infusion rate was also improved because of the increase of expression and phosphorylation of insulin receptors that lead to the stimulation of glucose transporter synthesis within the liver. Nevertheless, the specific role of Mfn2 in the regulation of energy metabolism of lipid-induced insulin resistance and type 2 diabetes is not well understood. In spite of several conflicting results in the studies, these observations have fostered our understanding by supporting the ideas that accumulation of FFA especially LCFA may have several detrimental effects via rigorous alteration in gene expression of mitochondrial biogenesis and metabolism that become one of the principal contributors to the obesity-associated diminished insulin sensitivity in various peripheral tissues.

**Accumulation of lipid intermediates induced lipotoxicity**

Obesity is one of the major risk factors involved in the progression of insulin resistance and type 2 diabetes [8,87]. An inverse relationship between insulin sensitivity and adipose tissue mass was critically reviewed [150,151], indicating the roles of adipose tissue functions in regulating lipid and glucose metabolism. In healthy humans, the dynamic amount of fatty acid storage and its release into the blood circulation is tightly coordinated based on metabolic demands. Excess accumulation of LCFA in adipose tissues may lead to the hypertrophic adipocytes. These conditions exert a physiological stress on their cellular functions and later induce them to transform into inhaled adipocytes with the activation of inflammatory response via recruitment of tumour necrosis factors-a (TNF-a) and macrophage-migration-inhibitory-factor and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB). The downstream effects of these mediators lead to various cellular dysregulation including apoptosis, mitochondrial dysfunctions, endoplasmic reticulum (ER) stress and insulin resistance [152–154]. Because of these cellular consequences, the inhaled adipocytes exhibit various metabolic malfunctions in terms of glucose and lipid oxidation, storage and uptake that lead to the higher rate of lipolysis and excess non-esterified LCFA [155]. In these conditions, the accumulation of these LCFA is directed to be catabolized in the other peripheral tissues such as skeletal muscle and liver that have a very limited storage capacity for lipid [156]. It usually occurred when the amount of lipid intermediates is amplified in adipose tissue and exceeds its own capacity to store and metabolize lipid for energy production. Lipotoxicity is defined as the accumulation of excess lipids...
in non-adipose tissues such as skeletal muscle and liver, resulting in an increase of mitochondrial stress, activation of inflammatory pathways, reduced glucose uptakes and insulin resistance [86]. Numerous evidence indicated that the accumulation of non-esterified LCFA is strongly associated with the insulin resistance [157,158]. Indeed, high levels of ectopic lipid accumulation are concomitantly associated with type 2 diabetes and lipotoxicity [159,160].

It was scientifically observed that plasma FFA levels were augmented in obese and diabetic patients [101,161]. In the long run, high amounts of FFA levels together with diminished fat oxidative capacity contribute to the accumulation of LCFA intermediates in several insulin responsive tissues other than adipocytes [156]. On the other hand, the consumptions of other types of fatty acids such as MCFAs were not led to the ectopic accumulation of lipid intermediates in the liver and skeletal muscle of rat and mice [162,163]. Intracellular fatty acid intermediates accumulate in myocytes, mainly in the form of LCFA-CoA, monoacylglycerol, diacylglycerol, phosphatidic acid, triacylglycerol and ceramide [18,32,143,164,165]. The primary defects in the metabolic capacity of mitochondrial fatty acid oxidation may result to the accumulation of these lipid intermediates [166]. Numerous studies were published in regard to the obvious observations of non-esterified LCFA build-up in skeletal muscle and liver that can essentially contribute to the development of insulin resistance [29,164,167].

Ceramide and diacylglycerol are believed to be the most deleterious mediators that can interfere and inhibit various levels of insulin signalling cascade pathways through the decrease of mitochondria functions [31]. The elevated concentration of LCFA within the cell exacerbates the abnormal accumulation of diacylglycerol and ceramide to activate protein kinase C theta that inherently results in the phosphorylation of serine 307 residue of insulin receptor substrate 1 (IRS-1). Intrinsically, this occurrence is directed towards the inhibition of tyrosine phosphorylation and impaired insulin signalling [168]. According to these crucial insights, recent work also supports several issues pertaining to the role of PKC in LCFA intermediate-induced insulin resistance via inhibition of insulin-stimulated phosphatidylinositol-3-kinase activity [169]. Nevertheless, there is no reliable evidence that explained on how the activation of PKC is related to serine phosphorylation of IRS-1 in inhibiting the phosphorylation of tyrosine residues [164], suggesting well-designed experimental studies to be further implemented.

Ceramide is a family of intermediate lipid species that profoundly functions as signalling molecules to signal apoptosis and inhibit cell division [170]. There is an expanding amount of studies that indicate the reciprocal relationship between the ceramide contents and the development of insulin resistance [169,171,172]. Prolonged exposure of ceramide in skeletal muscle, liver and heart tissues may directly suppress the activity of Complexes I and III in the mitochondrial electron transport chain system [173,174]. A study confirmed that ceramide treatment affects the mitochondrial integrity via reduced mitochondrial respiration and Akt/PKB phosphorylation in the skeletal muscle [175]. While in liver, it was showed that the excessive formation of ceramide in mitochondria is directly derived from LCFA intermediates such as sphingosine and palmitate [176]. The elevation of ceramide contents in skeletal muscle of obese subjects was also accompanied by a deterioration of oxidative capacity via reduced fatty acid oxidation and mitochondrial functions [177]. Importantly, an excessive accumulation of ceramide contents was reported in the skeletal muscle of insulin-resistant animals [157], obese insulin-resistant humans [161] and lipid infused humans [178]. High amount of ceramide level in various peripheral tissues is a consequence of sphingomyelin hydrolysis (a plasma membrane constituent), which is catalysed by sphingomyelinase [179]. Alternatively, ceramide can be formed via a series of metabolic reactions between two fatty acids and serine in four enzymatic steps [180]. The first step is rate limiting, catalysed by serine palmitoyltransferase, which specifically entails LCFA as the substrate. Thus, the increase of LCFA uptake by the skeletal muscle or inhibition of stearoyl-CoA desaturase-1 activity may promote the accumulation of ceramide in the skeletal muscle [181,182] and exert their effects on mitochondrial functions. Moreover, other studies have shown that a diet rich in LCFA especially palmitic acids significantly leads to the abnormal accumulation of ceramide contents in the skeletal muscle of mice and rats [183,184]. In human, the consumption of polyunsaturated LCFAs such as conjugated linoleic acid (CLA) as a weight-loss supplement is also controversial [185]. The study observed that 4 g/day of mixed CLA isomers given to the subjects for 12 weeks affects the insulin signalling activity via the elevation of intramuscular ceramide contents. The accumulation of ceramide content causes a significant decrease of insulin sensitivity in skeletal muscle of overweight subjects (p ≤ 0.05) compared with controls [185], implying the roles of LCFAs that significantly contribute to a high amount of ceramide contents in this peripheral tissue.

A number of studies have found that the diacylglycerol level in the skeletal muscle of obese and insulin-resistant subjects is negatively correlated with the improvement of insulin sensitivity and glucose uptake [168,172,186,187]. The accumulation of diacylglycerol content in heart tissues of C57BL/6 mice that were fed with a high-fat diet was also found to affect the insulin signalling pathways via phosphorylation of p70s6 kinase and translocation of PKC-α [188]. The intravenous lipid infusion
into 12 healthy men over 6 h results in the total reduction of the whole body insulin sensitivity because of abnormal accumulation of diacylglycerol in the skeletal muscle [168]. Surprisingly, a diet rich in unsaturated LCFA such as oleic and linoleic acids also contribute to the significant accumulation of diacylglycerol contents [186]. Another report by employing 12 lean male offspring of type 2 diabetic patients and 21 men with overweight or obesity found that the elevations of intramuscular diacylglycerol contents are strongly associated with the development of insulin resistance [172]. These findings are also supported by Todd and colleagues who observed that the rats that were fed with a high-fat diet result in decreased insulin-stimulated glucose uptake together with the augmented diacylglycerol contents in skeletal muscle [189]. Additionally, these detrimental effects of increased diacylglycerol level in the peripheral tissue on insulin signalling transduction pathways were associated with the activation of inflammatory response via activation of novel protein kinase C. Later, this metabolic consequence activates the signalling pathways of NF-κB and insulin-dependent activation of mTOR/p70S6K, causing the phosphorylation of serine residues of IRS-1 as well as inhibition of the PI3K/AKT pathway [190–192]. All in all, high amounts of LCFA intermediates such as LCFA-CoA, diacylglycerol and ceramides in cytosol may synergistically act towards diminished oxidative capacity of various peripheral tissues via reduced mitochondrial respirations.

Activation of inflammatory signalling pathways and endoplasmic reticulum stress

The links between the inflammation and insulin resistance in studying the roles of LCFA-induced insulin resistance are recently emerged in numerous publications [193–195]. Obesity has been well recognized as the low grade of systemic chronic inflammatory conditions that activate the metabolic activity of a number of circulating cytokines in the body. The activation of these inflammatory signals in various peripheral tissues may interfere with the intracellular insulin signalling actions and inherently lead to the disruptions of metabolic homeostasis as a whole. In particular, these signals are directed towards the stimulation and activation of JNK isoforms in macrophage and directly induce posttranslational modification of IRS molecules via direct activation of serine residues in liver and skeletal muscle [196]. It has become increasingly apparent that the activations of these signalling pathways are clearly originated from accumulation of LCFA and lead to the formation of lipid intermediates such as diacylglycerol and ceramides in adipocytes and skeletal muscle and result in the excessive stimulations of pro-inflammatory mediators [152,197,198].

The intracellular inflammatory signalling pathways are activated by LCFA by their binding into Toll-like receptor family, especially TLR-4 [199,200]. TLRs are a family of evolutionary conserved receptors that specifically recognize pathogen-associated molecular patterns by facilitating the presence of microbes. A number of studies indicate that the TLR-4 is one of the key regulators of insulin resistance and accompanied by the reduced NF-κB. The activation of TLR-4 acts as a metabolic sensor that is capable to detect high amounts of plasma non-esterified LCFA and exogenous ligands from various dietary fatty acids that are profoundly present in obese states [201,202]. In particular, the possible mechanism of TLR-4 activation in the mammalian system is suggested through binding to a ligand of lipopolysaccharides (LPS) in the cell wall of gram-negative bacteria. LCFA is one of the main components of the lipid part of LPS, which is Lipid A. The presence of Lipid A could be detected by TLR-4 and sufficiently enough to trigger the TLR-4 activation and its downstream signalling cascade in a macrophage cell line [203]. The release of TLR-4 in skeletal muscle directly activates the c-Jun NH (2)-terminal kinase (JNK) and IκB kinase (IKK) complexes that inherently lead to the down-regulation of the inhibitor of κB (IκB) and NF-κB. As a result, these conditions drive the transcription of a variety of pro-inflammatory genes and proteins [204,205]. Further on, another study also showed that the LCFA and lipid infusion through in vitro and in vivo observed the significant activation of TLR-4/NF-κB in the skeletal muscle [195].

Long chain fatty acids initiate the activation of TLR-4 via direct stimulation of JNK and IκB signalling pathways with the alteration of intracellular insulin signalling and inhibition of phosphorylation in tyrosine residues of IRS-1 [195,206]. Consequently, the serine phosphorylation is significantly activated to signal the inhibition of glucose transporter, GLUT4, to the cell membrane. These observations are in accord with one study to understand these metabolic impairments through lipid infusion into. They have found that the TLR-4 knockout mice did not exhibit insulin resistance in the skeletal muscle, which apparently pointed out to an important role of the activation of TLR-4 in LCFA-induced insulin resistance in vivo [207]. Similarly, mutated mice with the loss-of-function in TLR-4 genes escaped from developing any condition of insulin resistance and accompanied by the reduced NF-κB and JNK activation, although feeding with high-fat diet [208]. In addition, the isolated muscles from these mice were protected from the saturated LCFA-induced insulin resistance by deactivating the TLR family [209–211].
in vitro study also showed that the insulin signalling pathway was not altered by deleterious effects of palmitic acids in L6 myotubes by blocking TLR-4 protein, indicating that this fatty acid requires TLR-4 protein to be significantly expressed in various peripheral tissues [210]. Thus, it can be postulated that to hold the idea of TLR-4 activation by LCFAs might be one of the particular events that lead to the activation of inflammatory signalling pathways.

Furthermore, several studies have indicated that LCFAs also facilitate the secretion of several other cytokines, including TNF-α, interleukin-1β, plasminogen activator inhibitor-1, C-reactive protein and interleukin-6 (IL-6) [212–214]. These reports are in accordance with another study where it was clearly observed that the concentration levels of these inflammatory mediators significantly amplified in obese and insulin-resistant individuals, which could be predictive inflammatory markers for type 2 diabetes [215]. The mitochondrial membrane potential and cellular ATP contents were also significantly reduced in 3T3-L1 adipocytes exposed to IL-6 inflammatory cytokines [216]. TNF-α is found to play their metabolic function via direct and indirect inhibition of certain regulatory pathways that are responsible for the glucose and fatty acid oxidations in the skeletal muscle and adipose tissues [154,217]. Similar to the aforementioned mechanisms, the possible mechanism involved in the activation of these inflammatory mediators is adapted via the activation of PKC isoform and NF-κB, which stimulates the serine kinase cascade event in the myocytes [217]. To corroborate with these defined mechanisms of inflammatory cytokines in the development of mitochondrial dysfunction and insulin resistance, recent investigations suggest that the activation of inflammatory cytokines may indirectly cause physiological stress in the mitochondria and substantially contribute to the mitochondrial dysfunction [218,219]. The release of these inflammatory cytokines leads to the advancement of aberrant mitochondrial function as LCFAs may stimulate the release of these cytokines in leukocytes [193,220].

Moreover, the metabolic association between the activation of ER signalling pathways and the inflammatory response in people with obesity and type 2 diabetes is emerging as one of the critical risk factor components that contribute to the hallmarks of such metabolic disease [221–223]. The close interactions of mitochondria and ER functions are observed in modulating various cellular such as mitochondrial fission, calcium signalling and lipid flux [224]. ER is a membranous network that is intrinsically functioning as one of the important organelles involved in trafficking of a wide range of proteins. Therefore, the enhancement of the metabolic signals critical in maintaining cellular homeostasis through proper ER functions is necessary to modulate the metabolic capacity of tissue during the development of metabolic disorders such as insulin resistance and type 2 diabetes. ER stress is linked to various inflammatory signalling pathways because of the failure of ER functions, particularly its folding capacity, thus triggering the activation of unfolded protein response (UPR) [225]. The activation of UPR may exacerbate the metabolic conditions via the stimulation of inert physiological stress and activate multiple inflammatory signals via inflammatory NF-κB/IKK pathways in various peripheral tissues [226]. The role of LCFA-induced mitochondrial dysfunctions in regard to the activation of the UPR and ER stress is implicated in insulin resistance and type 2 diabetes [224,227]. The activation of the UPR and ER stress is strongly associated with the excessive production of ROS molecules in various peripheral tissues, leading to the development of oxidative stress and diminished mitochondrial functions [98].

Several genetic and dietary animal models that induced insulin resistance were used to study the roles of ER stress in the pathophysiological mechanism underlying these complex diseases [98,228]. To several extents, it was reported that several ER stress markers such as the phosphorylated protein kinase-like endoplasmic reticulum kinase and inositol-requiring enzyme 1α were elevated in these animal models of insulin resistance, indicating that these metabolic pathways of ER stress may play a role for the promotion of inflammatory pathways, insulin resistance and type 2 diabetes. The recent findings demonstrated that obesity and type 2 diabetes are strongly associated with the compromising ER functions via the activation of JNK signalling pathways [226]. The activation of these pathways during ER stress directly regulates the expression of various inflammatory genes in response to these adaptive mechanisms. It was clearly showed that JNK knockout mice were being protected against a high-fat diet-induced insulin resistance. Besides, the induction of pro-inflammatory genes and cytokines such as interleukin-6 and monocyte chemoattractant protein-1 in these mice also were blunted [229,230]. Current analysis highlighted several findings pertaining to the metabolic fate of LCFAs such as palmitic acid on skeletal muscle that apparently contributes towards the amplification of the ER stress marker level [231]. Another report showed that the 16-week treatment of high-fat diet-induced insulin resistance in C57BL/6J male mice significantly increased the mtDNA damage, oxidative stress and mitochondrial dysfunction in various peripheral tissues [232]. In addition, the study also found that high-fat diet in mice also contributed to the activation of ER stress, apoptosis and inhibition of intracellular insulin signalling pathways in both liver and skeletal muscle, implying that these synergistic effects are accordingly activated to signal the metabolic
perturbations as a whole. Therefore, it should be concluded that the study has gone some ways towards enhancing our knowledge in mechanistic understanding of the roles of LCFA-induced inflammation, ER stress, mitochondrial dysfunction and insulin resistance that are intimately allied to each other in the disease process.

Inhibition of intracellular insulin signalling pathways

As the nature of insulin resistance and type 2 diabetes is multifactorial through involvement of the whole global body metabolism, the studies of hormone interaction and their regulatory effects in metabolic pathways are less than straightforward [233–235]. Insulin resistance may be induced by the defects in hormonal actions in various peripheral tissues such as skeletal muscle, liver and adipose tissue. Insulin plays a key role in regulating the glucose and fatty acid concentration in the blood circulation. The functions of insulin as a postprandial hormone are well known to exhibit several beneficial effects in cellular and systemic functions of the human body in terms of cellular metabolism, division, growth, differentiation, survival and proliferations [236,237]. The stimulatory mechanisms between insulin and fatty acid oxidative capacities in various peripheral tissues are well defined. To date, the plurality of studies reported that the insulin up-regulates mitochondrial protein synthesis, enzyme activities such as citrate synthase and cytochrome c oxidase (COX). The increase of oxidative phosphorylation with enhancement of mitochondrial gene expression (NADH dehydrogenase I and cytochrome oxidase subunit) and ATP content of normal skeletal muscle in miniature swine and human were also significantly observed in the event of insulin infusion [238–240].

However, in the progression of insulin resistance and type 2 diabetes, the stimulatory effects of insulin are blunted compared with healthy controls [241]. The study has found no significant effects in mitochondrial protein synthesis and COX activity of the disease subjects. In another study, the insulin-stimulated ATP synthesis was found to be diminished in individual and offspring of type 2 diabetes patients [242,243]. Similarly, acute treatments of insulin for 7 h in non-obese patients with type 2 diabetes result in low levels of ATP synthesis [238]. Thus, it is best to hypothesize that the action of insulin is blunted and inhibited in various peripheral tissues, leading to the deterioration of mitochondrial functions. This aberrant effect might be due to the loss-of-function of mitochondrial membrane integrity that inherently leads to the severe inhibition of many distinct processes in mitochondrial activity such as the electron transport chain, \(\beta\)-oxidation and TCA cycle. Based on previous studies, they found that myocytes treated with LCFA exhibit insulin resistance via inhibition of intracellular insulin signalling pathways [58,244–246]. In line with this, elevated concentrations of LCFA intermediates, especially in skeletal muscle and liver, which are major sites of peripheral glucose uptake and disposal, were associated with insulin-resistant states such as obesity and type 2 diabetes [247–250]. Figure 2 depicts the molecular mechanism of LCFA-induced mitochondrial dysfunctions, involving a number of metabolic stress pathways in skeletal muscle.

Roden and his colleagues have identified the mechanisms of fatty acid-induced insulin resistance by proposing the prolonged exposures of high levels of LCFA that lead to the insulin resistance via inhibition of insulin-mediated glucose uptake in human skeletal muscle [58]. In the event of aberrant mitochondrial functions, intracellular lipid metabolites such as acyl-CoAs, diacylglycerol and ceramides tend to be accumulated in the cytosol. This accumulation trend is due to the efficiency rate of fatty acid uptake into the cell that surpasses the oxidation capacity of the cell to metabolize all the substrates in the mitochondria. Subsequently, these accumulated lipid intermediates indirectly activate novel protein kinase C and its downstream effectors [JNK and/or inhibitor of the NF-\(\kappa\)B kinase (IkB)] through the involvement of a serine/threonine cascade signalling to stimulate the enhancement of serine phosphorylation of IRS-1 [83,85]. This in turn leads to the inhibition of IRS-1 tyrosine phosphorylation and inherently down-regulate PI3K and PKB/Akt2 isoform activities via inhibition on the translocation activity of GLUT4 to the plasma membrane to permeate glucose uptake [169,251,252]. The activation of protein phosphatase 2A by ceramides is also known to inhibit the intracellular signalling pathways via inhibition of PKB/Akt2 isoform activities in C2C12 skeletal muscle cell [253]. Accordingly, the glucose uptakes into the cell are inhibited and inherently resulted in high blood glucose, leading to the hyperglycemia and insulin resistance. The production of ATP mainly relies on both substrates (glucose and fatty acids); thus, any dysregulation in their metabolic activities inherently lead to the impairment of mitochondrial functions as a whole. Therefore, this cellular consequence may markedly affect the integrity of cells in terms of growth, proliferation, metabolism and survival, promoting the programmed cell death (apoptosis). The roles of the LCFA in regulating the insulin resistance are heavily dependent on the activation of these signalling pathways. Noteworthy, these mechanisms strengthen the ideas of LCFA-induced mitochondrial dysfunctions and led to the total inhibition of insulin signalling in various peripheral tissues, implicating the progression of insulin resistance and type 2 diabetes.
Mitochondrial dysfunctions as a unifying theme for LCFA-induced insulin resistance

A large and growing body of literature has reported the decreased lipid oxidation rates and reduced oxidative enzymes in the mitochondria of obese, insulin resistance and type 2 diabetes patients [32,254,255]. Numerous studies found that the mitochondrial morphology in the skeletal muscle of insulin resistance and type 2 diabetes patients was significantly altered by the smaller mitochondrial appearance and decreased electron transport chain activity in Complexes I and III compared with the control [116]. The mitochondrial dysfunction in skeletal muscle and adipose tissues of type 2 diabetes subjects is observed concomitantly with the reduced rate of glucose uptake and ATP synthesis, while lipid deposition was greatly increased [256]. Moreover, another study evidenced that the strong positive correlation between mitochondrial dysfunction and insulin resistance as measured by magnetic resonance spectroscopy through in vivo and ex vivo mitochondrial respiration [257].

The excessive accumulation of ROS is also implicated in regulating metabolic signalling in inflammatory activated pathways. For instance, the LCFA such as palmitic acid were demonstrated to exert its detrimental effects on mitochondria functions in rat L6 skeletal muscle cell lines via significant reductions of mitochondrial respirations, mtDNA damage, activation of inflammatory signalling pathways, induced phosphorylation of serine residues of IRS-1 and total inhibition of GLUT4 translocations [258]. Moreover, the significant changes or alterations of mitochondrial gene expression and proteins also equally contribute to the developments of mitochondria dysfunction because of the elevated lipid intermediate-induced lipotoxicity in numerous insulin responsive tissues such as skeletal muscle, liver, heart and adipose tissues.

As mentioned earlier, we have proposed several mechanisms related to the potential implications of LCFA-induced mitochondrial dysfunctions that lead to the progression of insulin resistance in various peripheral tissues (Figure 3). In the past decade, numerous studies showed the strong correlation between mitochondrial dysfunction and type 2 diabetes. The defects in mitochondrial capacity to metabolize intermediate metabolites...
have become one of the major drawbacks in impaired cell metabolism. Given the metabolic roles of LCFA-induced insulin resistance, it should be suggested that mitochondria dysfunctions might play a pivotal role in the central pathogenesis of several metabolic defects such as obesity and insulin resistance. In fact, there is a large volume of recent published reviews and studies describing the role of mitochondria in the pathogenesis of insulin resistance and type 2 diabetes [18,27,31,81,259].

Concluding remarks and future directions

To date, a number of mechanisms are postulated to elucidate the overall pictures of insulin resistance. Nevertheless, it remains unclear on what is the exact mechanism of actions that triggered the alteration and inhibition of insulin signalling the cascade events prior to the development of insulin resistance. As summarized earlier, there is accumulating evidence from previous and present studies on the roles of LCFA in the regulation of insulin resistance and type 2 diabetes. Here, we further postulate that the impairment of mitochondria functions by LCFA could be one of the causative factors in the regulation of insulin resistance. High consumption of food containing LCFA may lead to the excessive accumulation of ROS, lipid-induced toxicity, alteration of mitochondrial genes/protein integrity and inflammation-ER stress in various peripheral tissues, resulting in the disturbance of mitochondria functions. Further studies are needed in elucidating and deciphering the exact mechanism of these impairments. The studies of other subclasses of fatty acids such as SCFA and MCFAs in modulating the energy metabolism and mitochondrial functions should be continued to explicate their ameliorative effects on the mechanism underlying the disease. In addition, multidisciplinary studies such as the metabolic interaction between nutritional components in diets and other physiological factors such as hormones, gut microbiota and environmental factors are important areas to be further investigated for better understanding in deciphering the primary mechanisms that are essentially involved in the pathogenesis of insulin resistance and type 2 diabetes.

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Conflicts of interest

The authors declare no conflicts of interest.
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