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Mitochondrial inhibitor as a new class of insulin sensitizer

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Abstract

Insulin resistance is a major risk factor for type 2 diabetes. AMP-activated protein kinase (AMPK) is a drug target in the improvement of insulin sensitivity. Several insulin-sensitizing medicines are able to activate AMPK through inhibition of mitochondrial functions. These drugs, such as metformin and STZ, inhibit ATP synthesis in mitochondria to raise AMP/ATP ratio in the process of AMPK activation. However, chemicals that activate AMPK directly or by activating its upstream kinases have not been approved for treatment of type 2 diabetes in humans. In an early study, we reported that berberine inhibited oxygen consumption in mitochondria, and increased AMP/ATP ratio in cells. The observation suggests an indirect mechanism for AMPK activation by berberine. Berberine stimulates glycolysis for ATP production that offsets the cell toxicity after mitochondria inhibition. The study suggests that mitochondrial inhibition is an approach for AMPK activation. In this review article, literature is critically reviewed to interpret the role of mitochondria function in the mechanism of insulin resistance, which supports that mitochondria inhibitors represent a new class of AMPK activator. The inhibitors are promising candidates for insulin sensitizers. This review provides a guideline in search for small molecule AMPK activators in the drug discovery for type 2 diabetes.

Keywords

Insulin resistance; Mitochondria; Insulin sensitizer; Mitochondria inhibitor; Type 2 diabetes; Obesity

1. Introduction

Type 2 diabetes (T2D) is characterized by hyperglycemia coupled with hyperinsulinemia. The pathogenesis of T2D is insulin resistance, in which body has a low response to insulin although insulin remains at the normal or even higher levels in the blood. In compensation to insulin resistance, pancreatic β cells produce more insulin leading to insulin elevation. Long-term such compensation increases burden in β cells and often contributes to β cell failure at the end, which is responsible for hyperglycemia and diabetic complications in the late stage. Correction of insulin resistance has been a therapeutic approach in the treatment of type 2 diabetes. However, the medicines are very limited in the treatment of insulin resistance[1]. There is a strong demand for identification of new insulin sensitization drugs[1].

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Mechanism of insulin resistance is a focus in search for new diabetes medicines. There are several hypotheses regarding the cellular and molecular mechanisms of insulin resistance[2,3] (Fig. 1). Those include mitochondrial dysfunction, endoplasmic reticulum (ER) stress, lipotoxicity, AMPK reduction, insulin elevation, oxidative stress, inflammation and adiponectin reduction. All of those mechanisms are proposed according to observations in the study of obesity, which represents energy surplus (fatty acids or glucose) in the body. Therefore, obese subjects have a high risk of insulin resistance. Fatty acids contain high density of energy and elevation of fatty acid in blood is a major risk factor for insulin resistance. In an early review, we proposed that fatty acids in combination with insulin is a major risk factor for insulin resistance in obesity[3]. In this review, we will extend this view point with a focus on mitochondrial function.

Mitochondrion is a subcellular organ where fatty acids and glucose are used in the production of ATP through the oxidative phosphorylation process. The mitochondrial dysfunction is a hypothesis for insulin resistance[4]. The hypothesis suggests that when mitochondria cannot completely burn the fatty acids, the intermediate product of triglyceride such as diaglyceride (DAG) will accumulate in the cells to activate protein kinase C (PKC). In turn, PKC will induce insulin resistance by suppressing insulin signaling pathway through phosphorylation of insulin receptor substrate 1 (IRS-1)[5]. However, in two recent reviews, pharmacological and genetic evidence consistently suggest that mitochondrial dysfunction is not a cause of insulin resistance[6,7]. Instead, mitochondrial dysfunction is a consequence of insulin resistance.

2. Mitochondrial regulation

Mitochondria are the power-generating subcellular organ in cells and are required for ATP production in most cell types except red blood cells. The most important role of mitochondria is to produce ATP through oxidation of carbohydrate, fatty acids and amino acids. The key mitochondria enzymes include those such as pyruvate dehydrogenase, NADH dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome bc1 (Complex III), and cytochrome c oxidase (Complex IV). In glucose catabolism, glucose is first converted into pyruvate through glycolysis in the cytoplasm. Pyruvate is converted into acetyl-CoA in mitochondria by pyruvate dehydrogenase, and then sent into TCA cycle to generate ATP, CO_2 and H_2O. This process is dependent on oxygen, and the activity is inhibited by hypoxia. In the absence of oxygen, pyruvate cannot be used in ATP production in mitochondria, but is converted into lactic acid in the cytoplasm in glycolysis (Fig. 2). In this condition, each molecule of glucose can only yield 2 molecules of ATP in net. In terms of fatty acid catabolism, long chain fatty acids are broken down into acetyl-CoA in mitochondria through β-oxidation, and acetyl-CoA is then used for ATP production in TCA cycle (Fig. 2). In hypoxia, TCA cycle is inhibited and thus, fatty acids cannot be used in ATP production. Fatty acid catabolism is completely dependent on oxygen supply.

Mitochondrial function is regulated by several hormones and substrates. In the hormones, insulin regulates mitochondrial function through direct and indirect mechanisms. The direct mechanism includes insulin-induced gene expression and mitochondrial protein modification by phosphorylation. Indirectly, insulin increases substrate (glucose and fatty acid) supply to mitochondria through stimulation of the transporter activities. Glucose transporter 4 (GLUT4) and fatty acid transport protein (FATP) are activated by insulin leading to enhanced uptake of the substrates by cells. The gene expression, protein modification and substrate supply all promote mitochondrial function in ATP production. In obesity, glucose, fatty acids and insulin are all elevated in the blood. These factors promote mitochondrial function and contribute to the pathogenesis of insulin resistance. The

mitochondria activation provides a mechanism for insulin resistance in the presence of insulin and fatty acids[3].

Mitochondria function is determined by mitochondria number, size and quality. Those are controlled by mitochondrial biogenesis and mitochondria autophagy. The biogenesis is determined by gene expression and PGC-1α is a primary activator of the gene expression. Mitochondrial biogenesis is regulated by multiple factors. Insulin stimulates mitochondrial functions at multiple steps including oxidative phosphorylation, gene transcription and protein expression[8]. Mitochondrial recycle is regulated by autophagy, which removes damaged mitochondria from cells and uses them in energy production through recycling membrane lipids. In addition, mitochondrial function is also regulated by calcium homeostasis, reactive oxygen species (ROS), apoptosis and thermogenesis. These factors are influenced by substrates, nitric oxide, hypoxia, and hormones.

3. Mitochondrial dysfunction and insulin resistance

Mitochondrial dysfunction was first proposed as a mechanism of insulin resistance by Dr. Shulman's group[4]. This hypothesis is supported by evidence in human and in animal. ADP is a substrate in the synthesis of ATP, and is able to stimulate mitochondria to produce ATP. This ADP activity is reduced in type 2 diabetes patients with obesity[9]. In animal studies, mitochondrial dysfunction is associated with insulin resistance[10]. All parameters of mitochondria functions are decreased in the adipose tissues of diabetic mice. Those include mitochondria number, mitochondria DNA content, and respiratory enzymes, oxidative phosphorylation (OXPHOS) and fatty acid β-oxidation. Mitochondrial defects were induced in murine C2C12 myotube cells using the respiratory chain inhibitors. In this cellular model, insulin actions such as insulin-stimulated glucose uptake and AKT activation in the insulin signaling pathway are decreased[11]. Inhibition of mitochondrial function by knocking down mitochondrial transcription factor A (mtTFA) suppressed insulin-stimulated glucose uptake. mtTFA is required for the replication and transcription of mitochondria DNA. These findings suggest that mitochondrial dysfunction from pharmacological treatment or genetic manipulation has close relationship with insulin resistance in adipocytes. In diabetic subjects, mitochondria number and mass are reduced in skeletal muscle [12,13]. Several factors contribute to mitochondrial dysfunction.

3.1. Genetic factors

Mitochondrial biogenesis determines mitochondria mass and number. The biogenesis is regulated by mitochondrial DNA and genomic DNA. Mitochondrial DNA mutation presents in approximately 2% of patients with type 2 diabetes mellitus and in elderly individuals. In addition, a polymorphism of the mitochondrial coding region of the ND1 gene (a subunit of reduced NADH dehydrogenase) is associated with resting metabolic rate (RMR) in a large group of non-diabetic Pima Indians, a population with high risk of diabetes[14]. Mutation in mitochondrial DNA that encodes tRNA impairs insulin secretion in pancreatic β-cells[15]. Polymorphisms in the promoter DNA of UCP2 gene are associated with decreased incidence of obesity, reduced insulin secretion, and prevalence of diabetes[16,17]. In addition, nuclear DNA may determine mitochondrial function through encoding mitochondrial proteins. These association studies indicate that mitochondrial or nuclear DNA may influence mitochondrial function, and mitochondria may influence glucose metabolism through regulation of insulin sensitivity or insulin production. Nair et al.[18] suggests that race/ethnicity determines the role of mitochondria in insulin resistance. In certain ethnic groups, there is dissociation between mitochondrial dysfunction and insulin resistance, and mitochondrial dysfunction cannot account for insulin resistance.

3.2. Oxidative stress

Mitochondrial function is dependent on oxygen during production of ATP. Derivatives of oxygen such as O_2^- are generated during the process of ATP production. These intermediate products of oxygen are reactive oxygen species (ROS, a group of free radicals), and highly toxic for oxidation of lipids, proteins and DNA. Mitochondria usually prevent the toxicity by removal of those products through the antioxidant system, which convert the free radicals into H_2O and CO_2. If these intermediate products are not eliminated immediately in mitochondria, they will induce oxidative stress and damage protein, lipid and DNA functions through chemical reactions. In aging, oxidative stress is a factor for the reduced mitochondrial biogenesis. Oxidative stress is considered as risk factor for insulin resistance for inhibition of mitochondrial function. In high-fat diet models, H_2O_2 production is enhanced in mitochondria of human and rodent skeletal muscles, and redox-buffering capacity is reduced in the mitochondria[19]. When rodents were treated with a mitochondria-specific antioxidant or were genetically engineered to overexpress mitochondrial catalase, H_2O_2 emission was attenuated, and insulin sensitivity was preserved even in the face of a high-fat diet[19].

Mitochondrial dysfunction increases the risk for oxidative stress, which in turn, activates various serine kinases (such as JNK, IKK, PKC, et al.). These serine kinases contribute to insulin resistance by phosphorylation of insulin receptor substrate (IRS) proteins. In hepatocytes, palmitate accelerated fatty acid β-oxidation and ROS generation, which inhibits insulin signal transduction through activation of JNK[20]. In addition, fatty acid metabolites such as DAG and long-chain fatty acyl-CoA (LCFA-CoA) may contribute to insulin resistance[21,22]. The intracellular accumulation of DAG activates PKCs, which increase serine phosphorylation of IRS proteins in the inhibition of insulin signaling. Enhanced oxidation of long-chain fatty acid in mitochondria is sufficient to reverse insulin resistance in liver and correct glucose intolerance in *db/db* obese mice[23]. In this study, expression of malonyl-CoA-insensitive carnitine palmitoyltransferase 1 (CPT1mt) in liver increases hepatic mFAO capacity and improves glucose tolerance in *ob*/*ob* mice. In the study, hepatic steatosis was not affected in CPT1mt mice, indicating dissociation between hepatic steatosis and insulin resistance. A recent research suggests that genetic deletion of the AMPK β1 subunit in mouse macrophages reduced fatty acid oxidation, mitochondrial content, and increased risk for insulin resistance. It suggests that increased fatty acid oxidation in macrophages by AMPK activation may represent a new therapeutic approach to the treatment of insulin resistance[24].

Sedentary lifestyle and high-fat intake have deleterious impact on muscle mitochondrial oxidative capacity, and endurance exercise partly normalizes mitochondrial function and prevents age-associated insulin resistance[25]. Lim et al.[26] reported that insulin resistance is associated with impairment of mitochondrial function. They demonstrated that chronic exposure to atrazine (ATZ) could block the electron transfer at Q site of electron transport chain and led to mitochondrial dysfunction, including morphological disruption, decreased activities of complexes I and III, and decreased oxygen consumption rate in the liver and skeletal muscle. Consequently, these resulted in insulin resistance in skeletal muscle by reducing AKT phosphorylation at Thr308 and Ser473 upon insulin stimulation. These findings indicate that ATZ may result in type 2 diabetes induced by impaired mitochondrial function.

Although a lot of articles suggest that mitochondrial dysfunction contributes to the pathogenesis of insulin resistance, more and more evidence suggests that inhibition of mitochondria actually improves insulin sensitivity. Mitochondrial dysfunction hypothesis is mainly based on the association studies, not cause/effect relationship studies. Recent studies using pharmacological and transgenic approaches have made a huge progress in the analysis

of the cause/effect relationship for mitochondrial dysfunction and insulin resistance. The results consistently suggest that mitochondrial dysfunction is a consequence, but not cause of insulin resistance. Instead, mitochondrial over activation contributes to insulin resistance in obese conditions[7]. Following two points is supported by strong evidence: (I) mitochondrial dysfunction is a protection mechanism against insulin resistance; (II) mitochondrial inhibition is able to improve insulin sensitivity.

4. Mitochondrial inhibition as a strategy for insulin sensitization

Study of respiratory chain function in patients of type 2 diabetes suggests that mitochondrial dysfunction is not an intrinsic defect, but rather a consequence of the impaired insulin response[27]. In this study, the mitochondrial gene transcripts encoding proteins in the electric transport chain were expressed at higher levels in type 2 diabetic than in nondiabetic subjects. Increased insulin at postprandial stage caused an increase in mitochondrial ATP production rate in nondiabetic but not in type 2 diabetic subjects. Increased insulin reduced PGC-1, COX1, and citrate synthase expression in type 2 diabetic patients, but not in nondiabetic subjects[27]. It was reported that a reduction in oxidative phosphorylation in the liver or muscle of mice did not trigger the onset of diabetes but instead has the opposite effect, protecting mice against both diabetes and obesity[28]. The study suggests that the changes in respiratory chain function in insulin-resistant humans may be compensatory response in type 2 diabetes.

Mitochondrial dysfunction is not a cause of insulin resistance [6,7], but it may be a component of a “vicious cycle” exacerbating insulin resistance. Severe hyperglycemia can reversibly inhibit mitochondrial respiration in skeletal muscle cells[29]. Fatty acid also damages mitochondria functions[30,31]. Insulin resistance will reduce mitochondrial biogenesis[12]. In type 2 diabetes, hyperglycemia, hyperlipidimia and hyperinsulinemia together inhibits mitochondrial functions. In the mechanism, ROS may play a role, which inhibits mitochondrial proteins through oxidative stress and activation of stress-related signaling pathways.

In a time-course study, the relationship of insulin resistance and mitochondrial dysfunction was investigated in mice on a high-fat/high-sucrose diet[32]. Mice exhibited insulin resistance and evidence of oxidative stress in muscle at 1 month on the diet. Mitochondria impairment such as disrupted mitochondrial biogenesis, loss of mitochondrial structural integrity and respiratory functioning were found after prolonged exposure (>1 month) to the diet[32]. These results show that the mitochondrial dysfunction occurs after insulin resistance.

A growing body of evidence has revealed the importance of mitochondrial over activation in the pathogenesis of insulin resistance[7]. Transient inhibition of mitochondrial function is an approach in the improvement of insulin sensitivity. Many insulin sensitizing medicines and small molecules can transiently inhibit mitochondrial functions. Following pharmacological and genetic evidence supports that mitochondrial over activation contributes to insulin resistance and mitochondrial inhibition improves insulin sensitivity.

4.1. Pharmacological evidence

Pharmacological and genetic approaches have been used to test the relationship of mitochondrial dysfunction and insulin sensitivity. All of the clinically-approved medicines for insulin sensitization are able to inhibit mitochondrial function. Berberine, an herbal insulin sensitizer that is widely used in China, represents a class of herbal drug in the treatment of type 2 diabetes. Berberine down-regulates the expression of genes involved in lipogenesis and up-regulates those involved in energy expenditure in adipose tissue and muscle[33]. Besides, berberine treatment results in activation of AMPK, which stimulates

oxidation of glucose and fatty acids in mitochondria. Berberine activates AMPK through inducing AMP/ATP ratio by suppressing mitochondrial function[34].

The mitochondrial inhibition is transient and reversible in response to berberine. The mitochondrial function will be enhanced by AMPK after disappearance of the berberine-induced inhibition[34]. This study suggests that combination of inhibition and stimulation of mitochondrial function may contribute to improvement of insulin sensitivity. ATP depletion in response to berberine is associated with inhibition of gluconeogenesis and lipogenesis in the liver[35]. The study suggests that inhibition of mitochondrial function in liver may contribute to the therapeutic activities of berberine. In another study, insulin sensitizing medicines including berberine, thiazolidinediones (TZDs), and metformin all inhibited mitochondrial function[36]. The respiratory chain complex I is likely the target of those drugs in the inhibition of mitochondrial function[37–40], while resveratrol and quercetin inhibit the ATP synthase [41].

Metformin is an effective anti-diabetic drug, which decreases hyperglycemia through insulin-like and insulin-sensitizing effects in liver and skeletal muscle cells. Metformin does not directly stimulate AMPK. It activates AMPK by inducing AMP/ATP ratio after inhibition of complex I of the electron transport chain in mitochondria. Metformin also enhances glucose utilization by uncoupling oxidative phosphorylation in isolated mitochondria[42].

Thiazolidinediones (TZDs) are activators of PPAR and function as insulin sensitizers. Troglitazone is the first TZD-derived insulin sensitizer. Troglitazone significantly increased phosphorylation of AMPK and ACC at 5 mM in 15 min treatment. There was a transient increase in the AMP/ATP ratio. In adipose tissue, TZD promotes mitochondrial biogenesis [43,44]. This activity may be related to AMPK activation. Release of adiponectin by adipocytes and inhibition of mitochondria may lead to AMPK activation. In addition, TZD may enhance mitochondrial biogenesis through induction of gene expression, such as PGC-1, which has strong activity in the mitochondrial biogenesis *in vivo* as shown in transgenic mice.

Resveratrol, a polyphenolic compound extracted from grape skins, has been demonstrated to increase the activity of SIRT1[45] as well as AMPK[46], which in turn activates PGC-1[47]. Resveratrol markedly attenuated weight gain in association with increment of mitochondrial biogenesis in muscle and adipose tissue in the high-fat-fed mice[48]. In parallel, the resveratrol-treated mice show enhanced endurance capacity, higher oxygen consumption as well as improved glucose tolerance[48]. Resveratrol inhibits the mitochondrial ATP synthase by binding to the subunit[49], and leads to the activation of AMPK. This mechanism may contribute to insulin-sensitizing effect of resveratrol. The dose of resveratrol used in mice is not feasible in humans, although the effects of lower dose resveratrol was addressed in a human study[50]. Resveratrol analogs, such as combretastatin A-4 (CA-4), can function similarly as resveratrol and activate AMPK[46].

4.2. Genetic evidence

In genetic study, inhibition of mitochondrial function by gene modification leads to protection of insulin sensitivity[51]. Several reports suggest mitochondrial hyperactivity and overload as major cause of insulin resistance. This point has been demonstrated in Asian Indian immigrants in the United States[18] and in animal models of diabetes and obesity[28,52,53]. Previous genetic studies have shown that transient overexpression of the “master regulator” of mitochondrial biogenesis, PGC-1, increases mitochondrial content and insulin sensitivity in skeletal muscle cells[54,55]. However, persistent overexpression of PGC-1 in skeletal muscle induced systemic insulin resistance by reducing GLUT4

expression[56]. In contrast, skeletal muscle-restricted PGC-1 inactivation reduced mitochondrial content and protected mice from insulin resistance[57,58]. These mice show reduction in the mitochondrial biogenesis, low activity in TCA cycle and a shift from oxidative to glycolytic metabolism[57,58].

When PGC-1 is reduced by knockdown, the skeletal muscle mitochondrial function was reduced in parallel with an increase in glucose uptake and glucose tolerance[59]. Similarly to the PGC-1 over expression mice, PPAR over-expressing mice have an increase in mitochondrial function and a decrease in insulin-induced glucose uptake in skeletal muscle [60]. In these mice, insulin resistance is associated with diminished AMPK activity.

Mitochondrial flavoprotein apoptosis-inducing factor (AIF) is required to maintain the integrity of the mitochondrial respiratory apparatus, and this gene inactivation results in a progressive disruption of mitochondrial respiratory function with a long-term disruption in organ integrity[61,62]. Liver- and muscle-specific AIF ablation induces OXPHOS deficiency in mice, elevates glucose tolerance, reduces fat mass and increases insulin sensitivity[28]. Interestingly, the absence of PPAR in skeletal muscle results in down-regulation of mitochondrial biogenesis and function[63]. Moreover, these mice are resistant to high-fat-induced weight gain and have higher glucose tolerance even in the absence of exercise[64].

A recent work on cardiolipin (CL) remodeling by ALCAT1 (a lyso-CL acyltransferase) suggests an alternative molecular mechanism by which mitochondrial hyperactivity causes insulin resistance[51]. ALCAT1 overexpression leads to CL deficiency and enrichment of docosahexaenoic acid (DHA) content, which is known to increase mitochondrial membrane potential, oxidative stress, and lipid peroxidation[65,66]. The ATP production rate was stimulated by ALCAT1 overexpression and insulin sensitivity was reduced in the condition. In opposite, insulin sensitivity was improved in muscle of knockout ($ALCAT1^{-/-}$) mice. $ALCAT1^{-/-}$ mice are protected from diet-induced obesity and insulin resistance. In genetic obese *db*/*db* mice, ATP production rate is significantly higher in liver mitochondria relative to the control mice. Besides, ATP production rates were significantly lower in isolated mitochondria from liver and heart of $ALCAT1^{-/-}$ mice. These data support the notion that mitochondrial inhibition is able to improve insulin sensitivity. Muscle-specific deletion of the mitochondrial transcription factor Tfam does not induce insulin resistance in mice, suggesting that mitochondrial dysfunction in skeletal muscle is not a primary etiological event in type 2 diabetes[59].

5. AMPK as a target for insulin sensitization

AMPK is a major cellular energy sensor and a master regulator of metabolic homeostasis. It is a heterotrimeric enzyme containing catalytic subunit and two regulatory subunits. AMPK is activated by two distinct signals: a Ca^{2+}-dependent pathway mediated by CaMKK and an AMP-dependent pathway mediated by LKB1[67]. AMPK serves as a unique metabolic control node as it senses cellular energy status through modulation of its activities *via* phosphorylation and allosteric activation by AMP. A number of physiological processes have been shown to stimulate AMPK, including conditions that lead to alterations of the intracellular AMP/ ATP ratio, which are hypoxia, glucose deprivation; and calcium concentration, as well as the action of various hormones, cytokines, and adipokines. The AMPK activation leads to the inhibition of energy-consuming biosynthetic pathways (such as fatty acid synthesis in liver and adipocytes, cholesterol synthesis in liver, protein synthesis in liver and muscle and insulin secretion from β-cells) and the activation of ATP-producing catabolic pathways (such as fatty acid uptake and oxidation in multiple tissue, glycolysis in heart and mitochondrial biogenesis in muscle). AMPK can also modulate transcription of specific genes involved in energy metabolism, thereby exerting long-term

metabolic control. Activation of AMPK in the liver and muscle is expected to elicit a spectrum of beneficial metabolic effects with the potential to ameliorate the defects associated with insulin resistance. Because of its favorable effects on energy metabolism pathways, it is reasonable to consider AMPK as a potential therapeutic target in the prevention and the treatment of type 2 diabetes and insulin resistance.

There are a number of hormones and pharmacological agents reported to activate AMPK *in vivo* upon treatment of cells and tissues, such as metformin, TZDs, berberine, resveratrol, leptin, IL-6 and adiponectin. Changes in mitochondrial coupling and cellular energy state could account for the cellular AMPK activation. Metformin activates AMPK through reducing ATP levels after uncoupling oxidative phosphorylation in mitochondria in skeletal muscle cells; TZDs can activate AMPK by a mechanism inducing adiponectin expression in adipocytes[68]; adiponectin induces activation of AMPK in skeletal muscle and liver, increasing phosphorylation of ACC and fatty acid oxidation, enhancing glucose uptake and lactate production, and reducing glucose levels *in vivo*[69], IL-6 can rapidly and robustly increased AMPK activity in myotubes, enhancing fatty acid oxidation as well as basal and insulin-stimulated glucose uptake[70].

AMPK activation in the liver and skeletal muscle generates beneficial metabolic effects in the diabetic patients. However, the widespread AMPK activities make it hard to regulate the kinase activity in a tissue-specific manner. We need to keep in mind that AMPK activation could be beneficial for diabetic patients[71,72].

6. Mitochondrial PDH and insulin resistance

Pyruvate dehydrogenase (PDH) is an enzyme with multiunit complex that catalyzes the conversion of pyruvate to acetyl-CoA in the glucose catabolism pathway. PDH is localized within the inner mitochondrial membrane, converting pyruvate to acetyl-CoA that is then oxidized in the trycarboxylic acid (TCA) cycle for ATP production. PDH activity is also required for fatty acid synthesis from glucose as acetyl-CoA is the substrate in fatty acid synthesis. When PDH activity is inhibited, glucose cannot be oxidized or converted into fatty acids. PDH activity is tightly regulated by reversible serine phosphorylation that is catalyzed by PDH kinase (PDK). Phosphorylation of the E1 catalytic subunit by the kinase inactivates the enzyme activity. The phosphorylation is catalyzed by four specific PDH kinases: PDK1, PDK2, PDK3 and PDK4. The activity of these kinases is enhanced by ATP, NADH and acetyl-CoA and inhibited by ADP, NAD^+ and CoASH[73,74]. The activity of PDH is also regulated by Mg^{2+}, Ca^{2+} and insulin.

PDH activity is reduced in type 2 diabetes condition, and the reduction is likely a result of insulin resistance. Insulin induces PDH activity through de-phosphorylation to enhance glucose utilization, which includes glycolysis, glucose oxidation, and fatty acid synthesis. PDH is required for glucose conversion into acetyl-CoA. Inhibition of PDH leads to reduction in ATP generation and fatty acid synthesis from glucose in most tissues. This point has been approved in transgenic mice with PDH inactivation by α subunit knockout. PDH inactivation in muscle, leads to heart hypertrophy in mice and on chow diet, the KO mice die from heart failure within a month after birth[75]. The inactivation in liver completely blocks fatty acid synthesis from glucose in liver[76]. However, glucose oxidation was not inhibited locally since liver expresses pyruvate carboxylase, which sends pyruvate into TCA cycle through oxaloacetate. In the liver-specific KO mice, insulin sensitivity is enhanced and body weight is reduced on chow diet[76]. A low PDH activity leads to a decrease in glucose utilization and an increase in fatty acid utilization in ATP production[77]. PDH activity is reduced in diabetes or obesity conditions in various tissues in animals or patients[78,79]. The reduced PDH activity is likely a result of insulin resistance as insulin induces PDH function.

However, an increase in PDH activity is beneficial in the control of blood glucose in obese condition. PDH activity is enhanced in PDK4 knockout mice, and is responsible for hypoglycemia during starvation of the mice[80]. The hypoglycemia is a result of decreased gluconeogenesis from lack of gluconeogenic substrate. PDH converts all pyruvates that come from amino acids or glycerol into acetyl-CoA, which is used in ATP production. However, this PDH activity protects mice from hyperglycemia and insulin intolerance in mice on HFD[81]. The KO mice have less weight gain on HFD, and this may contribute to the improved glucose tolerance. These studies suggest that an increase in PDH activity is beneficial in the control of blood glucose in obese condition. Current literature on genetic studies suggests that inhibition and activation of PDH activity both have beneficial effects in the control of insulin sensitivity in mice. More research is required to determine if PDH is a therapeutic target in the improvement of insulin sensitivity.

7. Mitochondrial inhibitors

Many mitochondrial inhibitors have been reported in literatures [82]. Some of them are known to increase insulin sensitivity. These include berberine, metformin, TZDs, resveratrol, quercetin, curcumin and estrogen. Other inhibitors remain to be investigated for their activity in insulin sensitization. We list examples in Table 1.

8. Conclusions

Mitochondrial dysfunction is not likely a risk factor for insulin resistance in type 2 diabetes. Instead, over activation of mitochondria is a potential risk for insulin resistance. A growing body of evidence has revealed the importance of mitochondrial over activation in the pathogenesis of insulin resistance. In obesity, substrate over-supply plus insulin over-production is a mechanism for the mitochondria over-activation. The mitochondria alteration leads to inhibition of AMPK. Insulin resistance occurs after AMPK activity is reduced (Fig. 3). These evidence-based views have advanced our understanding of mechanism of insulin resistance. Inhibition of mitochondrial function is an approach in the improvement of insulin sensitivity. Many insulin sensitizing medicines and small molecules inhibit mitochondrial functions. These activities of insulin sensitizing agents enforce our proposal that mitochondrial inhibitors may represent a new class of insulin sensitizer.

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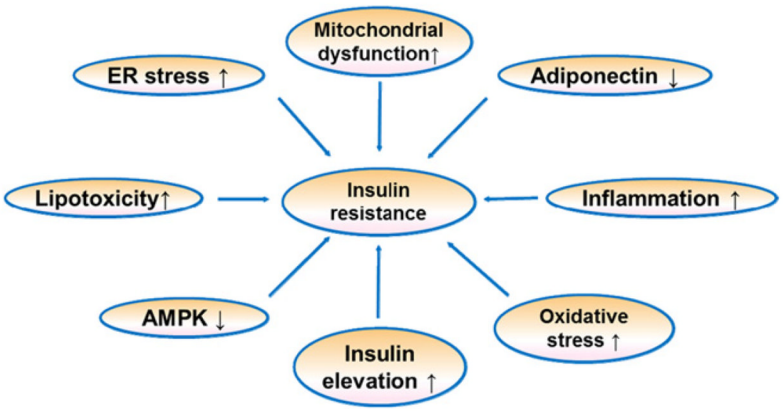

Figure 1.
Mechanism of insulin resistance. There are several hypotheses about insulin resistance. These include mitochondrial dysfunction, endoplasmic reticulum (ER) stress, adiponectin reduction, inflammation, lipotoxicity, AMPK inactivation, oxidative stress, and insulin elevation.

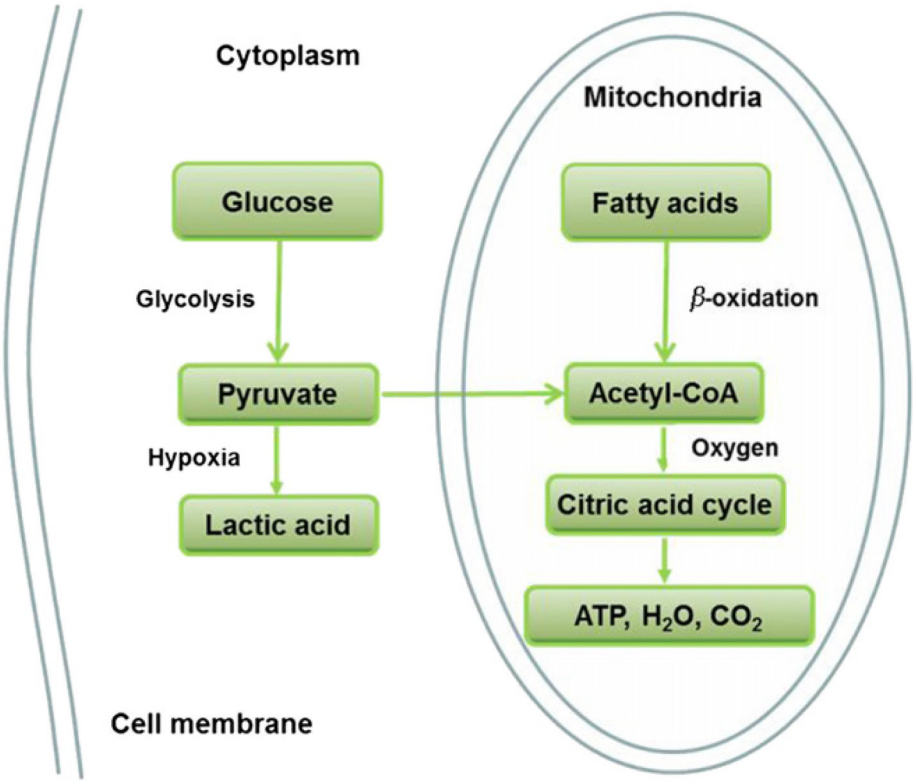

Figure 2.
Glucose and fatty acids catabolism in cells. Glucose breaks down into pyruvate in the process of glycolysis. In the hypoxia condition, pyruvate becomes lactic acid in the cytoplasm and released out of cells. In the presence of oxygen, pyruvate is converted into acetyl-CoA by PDH in the mitochondria, and then used in TAC cycle for ATP production. The byproducts are water and carbon dioxide. Fatty acids breaks down into acetyl-CoA through β-oxidation in mitochondria, and then used in ATP production through TCA cycle. In hypoxia condition, fatty acid cannot be used in ATP production.

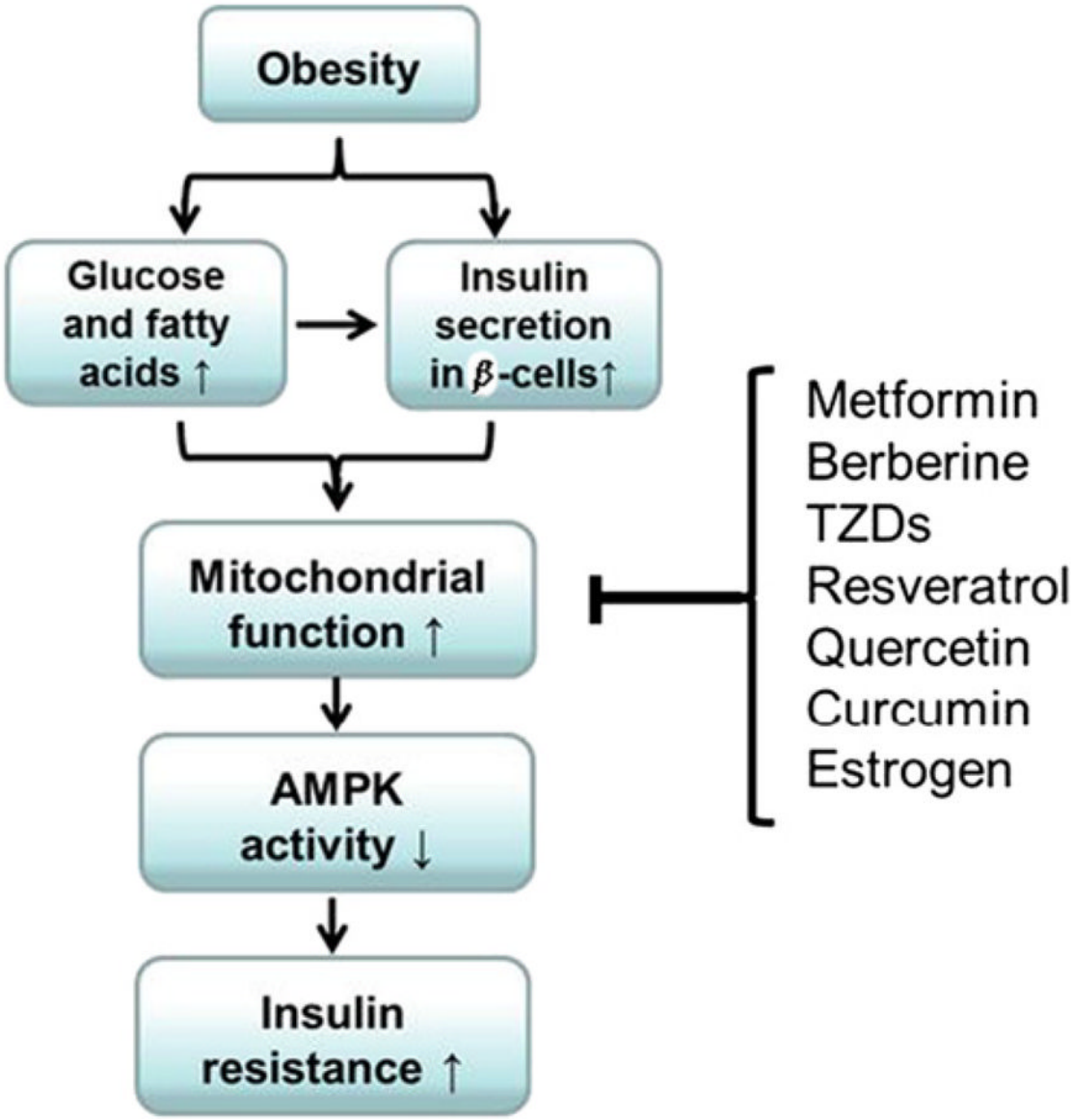

Figure 3.
Mitochondrial inhibition as a new approach for insulin sensitization. In obesity, mitochondria over-activation by substrates and insulin leads to reduction in AMPK activity. Insulin signaling activity is inhibited in the presence of AMPK inactivation. Inhibition of mitochondrial function is an alternative pathway in AMPK activation.

Table 1

Mitochondrial inhibitors and their function.

Mitochondrial inhibitor	Function
Berberine	Inhibit mitochondrial respiration
Metformin	Inhibit mitochondrial respiration, uncoupling oxidative phosphorylation
TZDs	Inhibit respiratory chain complex I
Resveratrol	Inhibit mitochondrial ATP synthase
Piceatannol	Inhibit ATPase activity of mitochondrial ATP synthase
Diethylstilbestrol (DES)	Inhibit proton translocation activities of mitochondria
Quercetin	Inhibit ATPase activity of mitochondria
Genistein	Inhibit ATP hydrolysis and ATP synthesis activities of mitochondrial ATP synthase
Biochanin A	Inhibit ATPase activity of mitochondria
Epicatechin gallate (ECG) Epigallocatechin gallate (EGCG)	Inhibit ATP hydrolysis activity of ATP synthase
Curcumin	Inhibit ATPase activity of mitochondria
Phloretin, theaflavin, tannic acid	Inhibit ATPase activity of mitochondria
Estrogen	Inhibit ATPase activity of mitochondria
Oligomycin	Inhibit ATP synthase
Ossamycin	Inhibit both ATPase and oxidative phosphorylation activities of mitochondrial ATP synthase
Propranolol	Inhibit mitochondrial ATPase activity
Atrazine	Inhibit ATP synthesis activity of ATP synthase
Azide	Inhibit ATPase activity of mitochondria