Targeting mitochondrial biogenesis for preventing and treating insulin resistance in diabetes and obesity: Hope from natural mitochondrial nutrients

Jiankang Liu a,f,*, Weili Shen b, Baolu Zhao c, Ying Wang d, Karin Wertz d, Peter Weber d, Peifang Zhang e

a Institute of Mitochondrial Biology and Medicine, Department of Biology and Engineering, The Key Laboratory of Biomedical Information Engineering of Ministry of Education, Xi'an Jiaotong University School of Life Science and Technology, Xi'an, 710049, China
b Institute for Nutritional Science, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai
c State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Academia Sinica, Beijing 100101, China
d DSM Nutritional Products, R&D Human Nutrition and Health, Basel, Switzerland
e Nestle Beijing R&D Centre, Beijing 100095, China
f Graduate Center for Toxicology, University of Kentucky College of Medicine, Lexington, KY 40536, USA

A B S T R A C T

Insulin resistance is an important feature of type 2 diabetes and obesity. The underlying mechanisms of insulin resistance are still unclear and may involve pathological changes in multiple tissues. Mitochondrial dysfunction, including mitochondrial loss and over-production of oxidants, has been suggested to be involved in the development of insulin resistance. Increasing evidence suggests that targeting mitochondria to protect mitochondrial function as a unique measure, i.e. mitochondrial medicine, could prevent and ameliorate various diseases associated with mitochondrial dysfunction. In this review, we have summarized recent progress in pharmaceutical and nutritional studies of drugs and nutrients to targeting mitochondria by stimulating mitochondrial metabolism (biogenesis and degradation) to improve mitochondrial function and decrease oxidative stress for preventing and ameliorating insulin resistance. We have focused on nutrients from natural sources to stimulating mitochondrial biogenesis in cellular systems and in animal models. The in vitro and in vivo studies, especially our own work on the effects and mechanisms of mitochondrial targeting nutrients or their combinations, may help us to understand the importance and mechanisms of mitochondrial biogenesis in insulin resistance, and provide hope for developing mitochondria-targeting agents for preventing and treating insulin resistance in type 2 diabetes and obesity.

© 2009 Elsevier B.V. All rights reserved.
1. Introduction

Insulin resistance is an important feature of type 2 diabetes and obesity. The underlying mechanisms of insulin resistance are still unclear. Adipose, skeletal muscle and liver are major organs involved in the glucose metabolism and therefore, play important roles in insulin resistance. Oxidative stress has been suggested to be involved in the pathology of insulin resistance. Studies have shown that insulin resistance is associated with mitochondrial (mt) dysfunction, such as reduced mitochondrial number and ATP production [1,2]. In prediabetic and diabetic humans, the expression of genes involved in oxidative phosphorylation (OXPHOS) is significantly reduced in the skeletal muscle [3]. Mitochondria are the major sites of reactive oxygen species (ROS) production in the body. If the efficiency of OXPHOS is reduced (e.g. by deletions of energy metabolism genes from the mt genome), more O$_2^{-}$ is generated at the expense of ATP. Therefore, reducing oxidative damage by improving mitochondrial function seems a rational way to prevent and treat insulin resistance. In this review, we summarized the available evidence with a focus on our recent studies of natural nutritional products on stimulating mitochondrial biogenesis as a strategy to improve mitochondrial function and reducing oxidative stress, leading to prevention and amelioration of insulin resistance.

2. Mitochondrial dysfunction due to oxidative damage may play an essential role in insulin resistance

2.1. Reactive oxygen species, oxidative damage and insulin resistance

Normal metabolism uses oxygen; however, excess oxygen is also toxic to all life forms. The theoretical reduction of oxygen to water by the electron transport chain involves a coordinated four-electron transfer. During this process, reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, hydroxyl radical, and nitric oxide, are generated and may cause oxidative damage to biomolecules, such as lipids, proteins, and nucleic acids [4]. This dark side of oxygen has been extensively studied in the past two decades under the influence of the Free Radical Theory of Aging [5,6].

Type 2 diabetes is considered a result of derangement of homeostatic systems of metabolic control and immune defense and oxidative stress. Insulin resistance is a major feature of the pathophysiology of type 2 diabetes. A growing body of evidence indicates that increased ROS levels, i.e., a significant imbalance between the production of ROS and antioxidant defenses, plays a major role in leading to alterations in stress-signaling pathways and potentially end-organ damage [7]. For example, we have shown that immune dysfunction in Goto–Kakizaki diabetic rats is associated with increased oxidative damage and mitochondrial dysfunction [8]. A close association between ROS level and insulin resistance, and reduced insulin resistance by antioxidant treatment has been demonstrated [9–12]. Houstis et al [13] conducted a genomic analysis of two cellular models of insulin resistance, induced either by cytokine tumor-necrosis factor-α or by glucocorticoid dexamethasone, two stimuli that produce insulin resistance but act through very different signaling mechanisms. They found that 18% of the genes, which were similarly regulated in response to both treatments, encoded products that in response to insulin stimulation of its target cells and these observations have led to the hypothesis that ROS may serve as second messengers in the insulin action cascade [9].

Mitochondrial dysfunction plays a central role in a wide range of age-associated disorders and various forms of cancer [17]. Emerging evidence supports the hypothesis that type 2 diabetes is associated with mitochondrial dysfunction [18]. Mitochondria are the major sites of cellular ROS production, and also are targets of ROS. Oxidative DNA damage of mt DNA includes the so-called common deletion, which deletes, among others, several genes involved in oxidative phosphorylation. Indeed, Fukagawa et al found heterogeneous mutations between base pair 8468 and 13446 in mtDNA, the region known as the “common” deletion, in the muscle of older humans with impaired glucose tolerance or diabetes mellitus. They also demonstrated that rats with insulin resistance have increased susceptibility to mtDNA deletions in vivo and that high glucose concentrations with ROS induce mtDNA mutations in vitro, suggesting that glucose-related oxidative stress and possibly hyperinsulinemia may contribute to alterations in mitochondrial gene integrity [19]. Accordingly, a transcrcriptomic approach demonstrated that a major difference between prediabetic and diabetic patients to healthy individuals is that genes involved in oxidative phosphorylation are down-regulated [3].

Therefore, we propose that ROS-induced oxidative damage contributes to mitochondrial dysfunction (including decreased mitochondrial biogenesis) due to the vicious cycle of ROS and mitochondrial dysfunction may play a key role in the onset and development of insulin resistance and type 2 diabetes (Fig. 1).

3. Mitochondrial biogenesis plays an important role in improving insulin resistance

3.1. Mitochondrial metabolism: biogenesis, degradation, and dynamics

Mitochondrial biogenesis in mammalian tissues is modulated through control of peroxisome proliferator-activated receptor-α (PPAR-γ) coactivator-1α (PGC-1α) expression. Though many pathways are not well understood, it is known that the critical factors involved in mitochondrial biogenesis include: 1) those that stimulate PGC-1α gene transcription, such as calcium/calmodulin-dependent protein kinase IV (CaMKIV), AMP-activated protein kinase (AMPK), and nitric oxide; 2) those that are stimulated by PGC-1α, such as nuclear respiratory factors (NRFs) and radicals in ethanol oxidation in vivo, the reduction of ribonucleosides, their role in oxidation, carboxylation and hydroxylation reactions, their roles in phagocytosis, in the actions of peroxidase and NADH oxidase enzymes, and in the synthesis of eicosanoids (prostaglandins and leukotrienes) by oxidation of polyunsaturated fatty acids [14]. Endogenous nitric oxide has been shown to act as a messenger molecule to stimulate mitochondrial biogenesis [15]. Moreover, it has been known for more than 30 years that oxidants can facilitate or mimic insulin action and that hydrogen peroxide is generated in response to insulin stimulation of its target cells and these observations have led to the hypothesis that ROS may serve as second messengers in the insulin action cascade [9]. In addition, glucose restriction has been shown to extend Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress [11]. Also, it has also been reported that overexpression of glutathione peroxidase 1 caused a development of insulin resistance in mice, suggesting that increased glutathione peroxidase 1 activity may interfere with insulin function by overquenching intracellular reactive oxygen species required for insulin sensitization [10].

On the other hand, if excess amounts of ROS are present, or too low levels of antioxidants are available to counterbalance, the resulted redox imbalance will contribute to aging and disease development. Accordingly, antioxidants for therapeutic and preventive approaches are only effective when and where ROS are overproduced [16].
PPARs, to which PGC-1α also binds and activates; and 3) mitochondrial transcription factor A (Tfam) expression and initiation of mitochondrial DNA replication. The NRFs also induce OXPHOS gene transcription, following which the resulting nuclear-encoded proteins travel to the mitochondria [15,20–22]. The signal transduction pathways that modulate mitochondrial biogenesis are diagrammed in Fig. 2.

Recent studies have suggested that the dynamics (fusion and fission) of mitochondria are important in development and disease [23,24]. Chen et al. [25] showed that mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion, and that the cooperation between mitochondria has protective effects on the mitochondrial population. Hood et al. [26] have pointed out that the expansion of muscle mitochondria involves the assembly of an interconnected network system (i.e. a mitochondrial reticulum) and the expansion of the membrane size is influenced by the balance between mitochondrial fusion and fission. This means that mitochondrial biogenesis requires the coordination of multiple cellular events, including transcription of two genomes, the synthesis of lipids and proteins and the stoichiometric assembly of multisubunit protein complexes into a functional respiratory chain. Impairment at any step can lead to defective electron transport, a subsequent failure of ATP production, and an inability to maintain energy homeostasis [26]. In addition, the mitochondrial assembly also involves molecular chaperones such as mitochondrial import stimulating factors and cytosolic/mitochondrial heat-shock protein 70 (Hsp70) [26].

3.2. Mitochondrial metabolism and insulin resistance

Increasing evidence shows that mitochondrial number and function decrease in various tissues in human aging and diseases, such as diabetes and obesity [27,28]. The endocrine and non-endocrine roles of adipose tissue with regard to energy intake and expenditure play important roles in the development of insulin resistance.

In terms of endocrine functions, adipocytes from visceral adipose tissue of obese subjects release (among other adipokines) reduced amounts of adiponectin, which leads to reduced activation of the AMPK/PGC-1α pathway in muscle tissue (Kadowaki and Yamaguchi Endocrine Reviews 2005 26, 439–451). Since PGC-1α is a key regulator of mitochondrial biogenesis, its downregulation by reduced adiponectin levels may be crucial for the reduced capacity for oxidative phosphorylation. Indeed, reduced PGC-1α expression seems to be involved in obesity and the prediabetic state in skeletal muscle [29–32]. Adipocytes and skeletal muscle mitochondria in patients with type 2 diabetes and obesity exhibit impaired bioenergetic capacity [1,18]. Impaired mitochondrial activity also occurs in the skeletal muscle of insulin-resistant offspring of patients with type 2 diabetes [33].

It has been suggested that the decrease in PGC-1α expression observed in the (pre)diabetic state may be the result, rather than the cause of lipid-induced insulin resistance [32]. On the other hand, exercise is shown to enhance mitochondrial biogenesis in both animals and humans and to rescue insulin resistance-induced mitochondrial dysfunction [34–37].

In addition to adipokine secretion, FFA are mobilized from adipose tissue in obesity. The FFA are then ectopically accumulated in non-adipose tissue — including liver, skeletal, and β cells —, resulting in inflammation and insulin resistance [38,39]. Skeletal muscle is insulin resistant in type 2 diabetes and obesity patients, and displays dysregulated carbohydrate metabolism and lipid fuel oxidation. In addition to contributing directly to insulin resistance, the ectopic FFA accumulation [40] and the dysregulated glucose metabolism (utilization by muscles and gluconeogenesis in liver) also contribute...
to increased ROS production in mitochondria and thus further reduce mitochondrial function [40]. The cellular capacity for oxidative phosphorylation is connected to the capacity for fatty acid burning. The reduced mitochondrial function described above should considerably contribute to the risk for developing insulin resistance and diabetes in obese subjects. Since intramuscular lipid accumulation is linked to insulin resistance, a vicious cycle may ensue.

3.3. The PI3/Akt pathway may provide upstream signaling for mitochondrial biogenesis

Akt is a serine/threonine kinase that requires functional PI3K to be stimulated by insulin and other growth factors. Akt can regulate glucose uptake and metabolism in 3T3-L1 adipocytes [41]. The relationship between the PI3K/Akt signaling pathway and mitochondrial biogenesis is unclear. PI3K/Akt is a possible upstream signaling pathway for mt biogenesis. For example, it is known that PI3K/Akt activation targets eNOS and CREB, which are related to PGC-1α activation as shown in Fig. 2. Activated Akt/PKB deactivates glycogen synthase kinase-3beta (GSK3), which permits Nrf2 nuclear translocation and occupancy of the 4 antioxidant response elements (AREs) in the nuclear respiratory factor (NRF)-1 promoter; the ensuing accumulation of nuclear NRF-1 protein leads to gene activation for mt biogenesis [42]. Moreover, the PI3K/Akt pathway is well known to regulate the Sirt1/FOXO system [43]. Sirt1 activates PGC-1α by deacetylation [44].

4. Agents for preventing/treating insulin resistance stimulate mitochondrial metabolism

Because mitochondria are the main site of ROS generation and calcium overload, which consequently lead to mitochondrial dysfunction, DNA damage, cell death, mitochondrial disease and resulting in mitochondria-related diseases, including diabetes and obesity, the main aims of mitochondrial medicine are to develop agents and drugs that target mitochondria to protect mitochondrial function and inhibit mitochondrial damage and cell death associated with various diseases [45–48]. The delivery of agents and drugs to the mitochondria has been the key to mitochondrial medicine [49]. The major strategies to deliver agents/drugs to target the mitochondria include synthesizing lipophilic cations, using the mitochondrial protein import machinery and mitochondrial gene therapy [49]. Several drugs and a few of natural and nutritional compounds are able directly or indirectly to target mitochondria to improve mitochondrial function by stimulating the mitochondrial biogenesis pathway and therefore, provide a promising option for prevention and treatment of insulin resistance in type 2 diabetes and obese patients.

4.1. Drugs that target mitochondrial biogenesis

Colca [50] suggests that insulin resistance is a physiological compensation for inappropriate oxidative metabolism that induces a metabolic inflammatory response, and that insulin sensitizers, such as thiazolidinediones, exert their pharmacology through modifications
of mitochondrial metabolism, preventing metabolic inflammation and allowing the up-regulation of mitochondrial biogenesis. Thiazolidinediones, such as pioglitazone, increase PGC-1α expression and mitochondrial DNA copy number, and enhance the oxidative capacity of white adipose tissue, leading to insulin sensitization [38,39,51]. Forskolin increases mitochondrial copy number and the expression of genes involved in mitochondrial biogenesis and fatty acid oxidation [52].

Forskolin and 5-amino-imidazole-4-carboxamide ribonucleoside (AICAR), in human umbilical vein endothelial cells, inhibited hyperglycemia-induced intracellular and mitochondrial oxidant production, stimulated AMPK activity, increased the mRNA expression of NRF1 and Tfam, stimulated mitochondrial proliferation, and increased expression of PGC-1α and manganese superoxide dismutase (MnSOD) mRNAs [53,54]. Adiponectin exerts anti-diabetic effects, possibly via the mechanism of increasing mitochondrial number and function [55].

4.2. Natural compounds that target mitochondrial biogenesis

4.2.1. Mitochondrial nutrients

We have published a review paper [56] focusing on a group of micronutrients, termed mitochondrial nutrients, that either are mitochondrial components or whose metabolites influence the structure and function of mitochondria.

We define mitochondrial nutrients as those which protect the mitochondria from oxidative damage and improve mitochondrial function [57]. Well-known mitochondrial nutrients or prosthetic groups are R-alpha-lipoic acid (LA), acetyl-l-carnitine (ALCAR), and coenzyme Q10. These are mitochondrial components, all of which can enter the cells and the mitochondria following exogenous treatment [57].

Such nutrients can perform a number of beneficial functions: 1) prevent oxidant production or scavenge free radicals to eliminate oxidative stress in mitochondria; these micronutrients include iron chelating agents and enzymatic and non-enzymatic radical scavengers, e.g. α-tocopherol and lipoic acid [56]; 2) act as phase-2 enzyme inducers, can enhance antioxidant defenses e.g. sulphoraphane [58]; 3) enhance mitochondrial metabolism, by repairing and degrading mitochondria, and by increasing mitochondrial biogenesis; e.g. lipoic acid and acetyl-l-carnitine [59,60]; and 4) protect mitochondrial enzymes and/or stimulate mitochondrial enzyme activity by elevating substrate and cofactor levels e.g. B vitamins [56]. We have classified mitochondrial nutrients into the following three groups: 1) antioxidants, such as coenzyme Q, lipoic acid (LA), glutathione, and α-tocopherol; 2) energy enhancers and others, such as carnitine/acetyl-l-carnitine, creatine, pyruvate, and choline; and 3) cofactors and their precursors, such as lipoic acid, coenzyme Q, and the B vitamins.

Some nutrients may have multiple functions and some combinations may possess unique functions different from the individual nutrients. We have emphasized that these nutrients also have other additional functions and are not necessarily located in mitochondria.

Since mitochondria are the major sites of cellular ROS production and also targets of ROS [6,61], deficiency of mitochondrial nutrients may cause ROS increase and oxidative stress to the mitochondria, leading to mitochondrial dysfunction and age-associated diseases, including metabolic syndrome. Thus, keeping sufficient mitochondrial nutrients in the mitochondria may be effective in reducing oxidative stress and mitochondrial dysfunction, and to prevent onset and slow the development of metabolic syndrome (Fig. 3). Friedly and Philipson [62] found that the cellular mechanisms that protect against oxidative stress per se are capable of creating an oxidant-dependent insulin-resistant state, and oxidant-induced mitochondrial dysfunction can lead to disruptions of lipid metabolism, increasing the intracellular lipid content, and, in addition, contribute to lipid-dependent insulin resistance in myocytes. They suggest that therapeutic strategies should, therefore, be directed towards reducing insulin resistance without an increase in oxidant production, and the activation of mitochondrial biogenesis in particular could be highly beneficial in the prevention or treatment of both insulin resistance and type 2 diabetes [62]. Especially strategies ensuring a physiological redox balance in mitochondria, such as thioredoxin reductase and thioredoxin [63], may prove promising.

A disturbed redox balance also occurs in conditions other than metabolic syndrome. A deficiency of the antioxidants vitamin E or selenium, can cause an impairment of immune function, leading to increased susceptibility to infectious disease [64]. Alzheimer’s disease prevention and treatment may also benefit from appropriate levels and types of micronutrients in addition to administration of non-steroidal anti-inflammatory drugs (NSAIDs) [65].

Based on epidemiologic, laboratory and clinical studies, we propose that using optimal combinations of mitochondrial nutrients, to target mitochondrial dysfunction may provide an effective strategy in delaying aging, and in preventing and treating cognitive dysfunction and metabolic syndrome. Combinations of a number of nutritional cofactors have been tested in different mitochondrial disorders for additive or synergistic effects. Combinations that have been studied include riboflavin plus carnitine to improve muscle weakness and exercise capacity in complex I-deficient myopathy; riboflavin plus nicotinamide to improve encephalopathic symptoms and nerve conduction; vitamin K₃ plus ascorbate to clinically improve exercise capacity in patients with a complex III defect; CoQ plus vitamin K₃, ascorbate, thiamin, riboflavin, and niacin to reduce mortality in mitochondrial myopathy and encephalomyopathies [66]; and carnitine plus choline and caffeine to reduce body fat and serum leptin concentrations [67]. We observed that acetyl l-carnitine (ALCAR) and lipoic acid (LA) are more effective in combination than when used individually to ameliorate the decay of mitochondria in old rats. This is the case because each plays a different role in restoring mitochondrial function, including the complementary effect of LA on ALCAR in inhibiting oxidative stress [68–70]. Therefore, we have also tested a few combinations of mitochondrial nutrients on insulin resistance in various cellular and animal systems as described below.

4.2.1.1. Lipoic acid (LA). Lipoic acid (LA) is a coenzyme involved in mitochondrial metabolism. The reduced form of LA, dihydrolipoic acid, is a powerful mitochondrial antioxidant [71–74]. It recycles vitamins C and E, raises intracellular glutathione and ascorbic acid levels, and chelates iron and copper [71–74]. In addition, LA induces phase II antioxidant enzymes [75,76]. LA readily crosses the blood–brain barrier and is absorbed by human cells as substrate, where it is reduced to dihydrolipoic acid [71]. LA enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle [77], activates the insulin signaling pathway, and exerts insulin-like actions in adipose and muscle cells [71]. LA has long been used to treat or
improve glucose transport and metabolism [98]. ALCAR also has been shown to reduce diabetic neuropathy and has pancreatic and peripheral effects that involve mitochondrial biogenesis [83]. We have recently shown that LA and ALCAR synergistically stimulate expression of PPAR-γ and factors involved in mitochondrial biogenesis, including PGC-1α, Tfam, and NRFs, leading to increases in mitochondrial number, protein levels, and function in 3T3L1 adipocytes.

4.2.1.2. Acetyl-l-carnitine (ALCAR). Acetyl-l-carnitine (ALCAR) is the acetyl derivative of l-carnitine, which is the carrier that transports long-chain fatty acids into the mitochondria for fuel. ALCAR is better absorbed than l-carnitine and crosses the blood–brain barrier more efficiently [84]. Tissue levels of carnitines in animals, including humans, decrease with age [85–87], leading to a decrease in mitochondrial membrane integrity. Animal studies with rats, mice, and dogs have shown that ALCAR remedies age-associated cognitive dysfunction and nerve degeneration, increases cardiolipin content, elevates mitochondrial enzyme activity, and improves mitochondrial function [87]. ALCAR has been shown to lower cholesterol in brain [88] and blood [89], possibly by acting as an inhibitor of acyl-CoA:cholesterol acyltransferase [90,91], a not well-studied mechanism for ALCAR. ALCAR has been tested in several small-scale clinical trials for preventing or treating diabetes and its complications, and requires further follow-up in large-scale clinical trials [92,93].

4.2.1.3. A combination of LA and ALCAR on beta cells. Mitochondrial oxidant production associated with hyperlipemia disrupts glucose-stimulated insulin secretion by pancreatic β-cells, which are particularly susceptible to oxidative damage. LA is reduced in the mitochondria to dihydroxyacid (DHLA), a potent antioxidant which scavenges free radicals and recycles other antioxidants to reduce the oxidative stress in the mitochondria [70,72,94–96]. ALCAR is a betaine required for the transport of long-chain fatty acids into the mitochondria for α-oxidation, ATP production, and for the removal of excess short and medium-chain fatty acids [96,97]. LA has been used in the treatment of diabetic neuropathy and has pancreatic and peripheral effects that improve glucose transport and metabolism [98]. ALCAR also has been used as treatment for diabetes and chronic diabetic neuropathy in animal experiments [99] and in clinical studies [100]. LA and ALCAR improve mitochondrial function in aging and degenerative diseases and in combination seem more potent owing to complementary effects [70,101]. Thus, optimal doses of a combination of mitochondrial nutrients could be a strategy for delaying and treating cellular dysfunction. We have shown that chronic exposure of pancreatic β-cells to sublethal oleic acid levels causes a suppression of glucose-stimulated insulin secretion accompanied by an increase in intracellular oxidant formation, a decrease in mitochondrial membrane potential (MMP), enhancement of UCP-2 protein expression and decreased glucose-induced ATP production. Uncoupling protein 2 (UCP-2) may act as an important link to impaired insulin secretion [102]. Pretreatment with LA and ALCAR reduced oxidant formation, increased MMP, regulated UCP-2 mRNA and protein expression, increased glucose-induced ATP production, and restored glucose-stimulated insulin secretion. The key findings on ATP production and insulin secretion were verified with isolated rat islets [103]. The beneficial effects of supplementation with LA and ALCAR that tend to ameliorate free fatty acid– (FFA)–related insulin secretion in β-cells may be attributed to their ability to act either as direct mitochondrial antioxidants, phase 2 antioxidant enzyme inducers, energy enhancers, or as enzyme cofactors [56].

4.2.1.4. A combination of LA and ALCAR on adipocytes. White adipose tissue is an important endocrine organ involved in the control of whole-body metabolism and insulin sensitivity. Thus, mitochondrial biogenesis could in part underlie the central role of adipose tissue in the control of whole-body metabolism and the actions of some insulin sensitizers [51]. Indeed, it has been reported that mitochondrial loss in adipose tissue is correlated with the development of type 2 diabetes [104]. Hence it is possible that stimulation of mitochondrial biogenesis may reduce the effects of mitochondrial loss of function. We found that treatment with the combination of LA and ALCAR at concentrations of 0.1, 1 and 10 μmol/l for 24 h significantly increased mitochondrial mass, expression of mitochondrial DNA and mitochondrial complexes, oxygen consumption, and fatty acid oxidation in 3T3L1 adipocytes. These changes were accompanied by an increase in expression of Pparγ, Ppara and Cpt1a mRNA, as well as increased expression of PGC-1α, Tfam and Nrf1. However, the treatments with either LA or ALCAR alone at the same concentrations showed little effect on mitochondrial function and biogenesis. The strong synergistic effects of the combination of LA and ALCAR in 3T3L1 adipocytes suggest that these two nutrients complement each other’s functions in mitochondrial biogenesis. The combination of relatively low doses of LA and ALCAR improved mitochondrial function and may provide a possible therapeutic intervention for preventing and treating insulin resistance and type 2 diabetes [105].

4.2.1.5. B vitamins. B vitamins are especially important for protecting mitochondrial and other enzymes because they are cofactors or precursors of mitochondrial enzyme cofactors [106]. High doses of B vitamins stimulate defective enzymes with decreased coenzyme-binding affinity and ameliorate genetic diseases and deleterious polymorphisms [107]. One possible mechanism of this stimulation may derive from the fact that high B vitamin doses elevate the corresponding coenzymes’ levels. About 50 human genetic diseases caused by defective enzymes, including 14 in the mitochondria, can be ameliorated by ingesting high levels of the corresponding coenzyme’s vitamin component [107]. However, our knowledge of the biochemical and genetic mechanisms relating B vitamin deficiency to disease risk and elevated vitamin intake to disease prevention, is greatly limited, even for the much studied folate [108] and biotin [109].

Nicotinamide, the amide derivative of nicotinic acid, has over the past forty years been given at high doses in various therapeutic applications. Niacin was shown to be an antilipolytic agent because it, similar to the actions of the adenosine receptor agonist phenylisopropyladenosine, lowers plasma glucose, plasma FFA and hepatic glucose production, and enhances insulin-stimulated glucose uptake in streptozotocin–induced diabetic rats [110]. It is currently in trial as a potential means of preventing the onset of type I (insulin-dependent) diabetes mellitus in high-risk, first-degree relatives.

Biotin has been shown to improve glucose and insulin tolerances in genetically-diabetic KK mice [111], long-term spontaneously hyperglycemic rats with non-insulin-dependent diabetes mellitus [112], streptozotocin–induced diabetic Wistar rats [113], and patients with non-insulin-dependent diabetes mellitus [112]. Biotin is known to promote cGMP production by directly binding and activating soluble guanylate cyclase [114,115], suggesting that biotin can stimulate PGC-1α to enhance mitochondrial biogenesis just as nitric oxide does [15]. Therefore, it is likely that mitochondrial nutrients can regulate mitochondrial biogenesis to prevent and improve insulin resistance.
A combination of LA, ALCAR and B vitamins on Goto–Kakizaki (GK) rats. The GK rat is a model of non-obese, spontaneous type 2 diabetes. The pathogenesis of diabetes in the GK rat involves impaired insulin secretion, insulin resistance, abnormal glucose metabolism, impaired ontogenetic development of pancreatic islet cells [116,117], and mitochondrial dysfunction in the liver [118,119] and heart [119]. We observed that defects in glucose and lipid metabolism are associated with low mitochondrial content and reduced mitochondrial enzyme activity in skeletal muscle of GK rats. We investigated the effects of a combination of nutrients on insulin resistance and mitochondrial biogenesis/function in skeletal muscle of these rats. The combination contained LA, ALCAR, biotin and niacin. These nutrients were chosen for the following reasons. First, LA has been shown to mitigate insulin resistance in GK rats [120], and it has also been shown that this improvement in insulin sensitivity is mediated by activation of AMPK and reduced triglyceride accumulation in skeletal muscle [121]. Second, ALCAR plays an important role in lipid metabolism, in which it acts as an obligatory cofactor for beta-oxidation of fatty acids by facilitating the transport of long-chain fatty acids — in the form of acylcarnitine esters — across the mitochondrial membrane. Both LA and ALCAR improve insulin-mediated glucose disposal in both healthy subjects and in type 2 diabetic patients, by two possible mechanisms: (1) by regulating acetyl and acyl cellular trafficking so as to correctly meet energy demand, and (2) by controlling the synthesis of key glycolytic and gluconeogenic enzymes [93,122].

Third, biotin was selected because biotin-dependent carboxylases play an important role in mitochondrial function, given that four out of five of these enzymes are present in mitochondria. A high intake of biotin may exert effects that favor good glucose tolerance in beta cells, liver and skeletal muscle [123]. Moreover, while LA alone can reduce the activities of the biotin-dependent carboxylases pyruvate carboxylase and b-methylcrotonyl-CoA carboxylase in rat liver, biotin co-treatment with LA can normalize these carboxylase activities [124]. Although the mild decrease in carboxylase activities caused by LA would presumably not cause pathology, it is always essential to maintain homeostasis and avoid side-effects by simply co-administering LA with biotin.

We have demonstrated that treatment with a combination of these four mitochondrial nutrients produced effects comparable to the anti-diabetic drug pioglitazone, ameliorated the symptoms of diabetes including β-cell dysfunction, enhanced mitochondrial biogenesis, improved mitochondrial function, and normalized fatty acid and glucose metabolism in diabetic GK rats [59]. In addition, although effective, the pioglitazone causes gain of body weight, a known clinical drawback of this treatment. In contrast, the nutrient treatment did not cause any significant change in body weight, suggesting this to be an advantage of nutrients over anti-diabetic drugs.

In addition, the development of type 2 diabetes is accompanied by decreased immune function and the mechanisms are unclear. We hypothesize that oxidative damage and mitochondrial dysfunction may play an important role in the immune dysfunction in diabetes. Therefore, we have investigated this hypothesis in diabetic Goto–Kakizaki rats by the treatment with the combination of four nutrients [8]. We first studied the effects of the combination of these four nutrients on immune function by examining cell proliferation in immune organs (spleen and thymus) and immunomodulating factors in the plasma. We then examined, in the plasma and thymus, oxidative damage biomarkers, including lipid peroxidation, protein oxidation, reactive oxygen species, calcium, and antioxidant defense systems, mitochondrial potential, and apoptosis-inducing factors (caspase 3, p53 and p21). We found that immune dysfunction in these animals is associated with increased oxidative damage and mitochondrial dysfunction and that the combined nutrient treatment effectively elevated immune function, decreased oxidative damage, enhanced mitochondrial function, and inhibited the elevation of apoptosis factors. As in the muscle mitochondrial biogenesis, these effects are comparable to, or greater than, those of the anti-diabetic drug pioglitazone [8]. These results suggest that the combination of the four mitochondrial targeting nutrients is effective in improving immune function in type 2 diabetes through enhancement of mitochondrial function, decreased oxidative damage, and delayed cell death in the immune organs and blood.

Other natural and nutritional compounds

Berberine. Berberine is a natural product with anti-diabetic properties that reduces body weight and significantly improves glucose tolerance in db/db mice, downregulates the expression of genes involved in lipogenesis and upregulates those involved in energy expenditure in adipose tissue and muscle, and increases AMPK activity in 3T3-L1 adipocytes and L6 myotubes [126].

Hydroxytyrosol. Hydroxytyrosol (HT) is the most potent and abundant antioxidant polyphenol in olives and virgin olive oil, which are important attributes of the Mediterranean diet. Olive polyphenols and HT were shown to have beneficial effects in the context of heart health and inflammation. Most of the health effects of hydroxytyrosol studied so far, are connected with its potent activity to directly scavenge ROS, but also to activate endogenous antioxidant systems [127]; [133, Zhu, submitted] [128,129]. We [130] have recently shown that HT significantly protected ARPE cells from decreases in mitochondrial DNA synthesis and transcription factors Nrf1 and Tiam, and therefore may prevent age-related macular degeneration. This suggests that HT may be an effective mitochondrial biogenesis protector or stimulator, comparable to metformin, or LA/ALC combinations. In adipocytes, at physiological concentration, hydroxytyrosol treatment resulted in an enhancement of mitochondrial function: it produced (i) increases in the activity and protein expression of mitochondrial complexes I, II, III, IV and V, (ii) increased oxygen consumption, and (iii) a decrease in free fatty acids. The mechanistic study of the signaling pathway for PGC-1α activation demonstrated that hydroxytyrosol is a potent activator of 5’AMP-activated protein kinase (AMPK) and its target enzyme acetyl-CoA carboxylase; in fact, HT is roughly hundreds of times more efficient than the AMPK activator 5-amino-imidazole-4-carboxamide-riboside (AICAR). Thus, HT is an effective nutrient for stimulating mitochondrial biogenesis and function via the PGC-1α pathway. This mitochondria-targeting property may provide a possible mechanism for the efficacy of HT in lowering the risk of obesity and even type 2 diabetes.

Epigallocatechin gallate (EGCG). Many of the beneficial effects of green tea have been attributed to its most abundant catechin, EGCG [131–133]. EGCG exerts potent anti-obesity effects in mouse and rat models of diet-induced obesity, which are at least partly mediated via a direct impact of EGCG on adipose tissue [134]. EGCG causes a dose-dependent decrease of in vitro adipocyte differentiation and down-regulates the mRNA expression of several lipogenic genes in adipose tissue. We (Yan, Shen, Liu, Tian, Zeng, and Zhao, unpublished) have recently found that green tea catechins (GTCs) supplementation to the obesity rats significantly attenuated the increase in body and liver weights and serum and liver triglyceride contents, increased the PPARY level in the subcutaneous white adipocyte tissue while decreased the PPARY level in the visceral white adipocyte tissue, and up-regulated the levels of in PPARY in white adipocyte tissue and brown adipose tissue and fat β-oxidation related enzymes. The in vitro study showed that EGCG, the major component of GTCs, improved the lipids storage and oxidation functions of 3T3-L1-adipocytes by promoting lipid oxidation through increasing of the expression of PPARY and its downstream genes and by decreasing C/EBP-homologous protein-10 and up-regulating CCAAT enhancer-binding proteinα (C/EBPα), C/EBPα and PPARY. We (Yan, Shen, Liu, Tian, Zeng, and Zhao, unpublished) also found that EGCG increased mitochondrial biogenesis by enhancing mitochondrial mass, oxygen consumption, and expression of complex I, II, V, mtDNA, and PPARY cofactor-1α (PGC-1α) in 3T3-L1 adipocytes.
These results suggest that EGCG might control obesity-associated adipose tissue through mitochondrial remodeling. In the absence of EGCG, PPARα activation causes a net flux of fatty acids from the circulation and other organs into adipocytes, resulting in adipocyte hypertrophy. However, use of EGCG leads to an increase in fatty acid oxidation that protects against such hypertrophy.

5. Perspectives and future directions

Mitochondrial dysfunction appears to be a key contributor to insulin resistance. Preventing mitochondrial dysfunction by enhancing mitochondrial biogenesis seems a plausible strategy for preventing or treating insulin resistance. We have identified natural compounds and nutrients which can target mitochondrial biogenesis and function. We hypothesized that these mitochondrial nutrients or their combinations may be effective in regulating PGC-1α activity so as to enhance mitochondrial biogenesis, and that such regulation may lead to the prevention and improvement of insulin resistance. Therefore, targeting mitochondria, such as by stimulating mitochondrial biogenesis, may be an effective strategy for developing effective new agents for preventing and treating insulin resistance in conditions involving metabolic syndrome, including diabetes and obesity. In fact, stimulating mitochondrial biogenesis may be the underlying mechanism of the anti-diabetic effects observed in interventions with a wide variety of drugs, antioxidants, and nutrients. So far this has not been shown for many compounds. The promising effects of natural products and nutrients provide great hope for preventing and treating diabetes and obesity by use of simple nutritional interventions, and especially, of combinations of nutrients with complementary benefit for mitochondria. Therefore, the future directions include 1) identify more potent mitochondrial targeting nutrients from food and herbs; 2) study their specific mitochondrial biogenesis targeting sites and signaling pathways, 3) investigate the effects of mitochondrial nutrients on mitochondrial degradation targeting the sites and signal pathways, an area that has not been well studied, and 4) use modern technology of nutrigenomics, proteomics, and metabolomics to identify the combinations of various mitochondrial targeting nutrients for optimal effects on preventing and treating insulin resistance in diabetes and obesity.

In this review, we have focused on the detrimental effect of oxidative stress on mitochondrial function and its consequences for insulin sensitivity. The role of ROS in aging and the development of chronic diseases very much depends on their amount. While a certain level of ROS is required for normal body function, excess ROS exposure can contribute to aging, inflammation and chronic degenerative diseases. Mitochondria are clearly the cellular sites where the ROS exposure is highest, and excess ROS endanger mitochondrial function and integrity. We have shown for the antioxidants LA, EGCG and hydroxyprolytol is a very promising effect on mitochondrial biogenesis and energy metabolism. However, it appears that not all antioxidants support mitochondrial biogenesis, rather oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance, Am. J. Clin. Nutr. 87 (2008) 142–149.


D.A. Hood, I. Irrcher, V. Ljubicic, A.M. Joseph, Coordination of metabolic plasticity
M.F. McCarty, Chronic activation of AMP-activated kinase as a strategy for
V.B. Ritov, E.V. Menshikova, J. He, R.E. Ferrell, B.H. Goodpaster, D.E. Kelley,
A.E. Civitarese, B. Ukropcova, S. Carling, M. Hulver, R.A. DeFronzo, L. Mandarino,
M. Roden, Muscle triglyceride and mitochondrial function: possible mechanis-
G. Trubiani, N. Hongu, D.S. Sachan, Caffeine, carnitine and choline supplementation of rats
E. Ravussin, R.C. Parkinson, J. Lindgren, G. Shademan, D. Trivedi, C. Bajaj, E. Jha,
A. Dall’Asta, L. Janssens, P. van ‘t Veer, D. Griendling, J.C. Milne, P.J. Elliott, J.
S. Corvera, Mitochondrial biogenesis and remodeling during adipogenesis and in
P.H. Reddy, Mitochondrial medicine for aging and neurodegenerative diseases,
M. Suwa, T. Egashira, H. Nakano, H. Sasaki, S. Kumagai, Metformin increases the
W. Shen, J. Hao, C. Tian, J. Ren, L. Yang, X. Li, C. Luo, C.W. Cotman, J. Liu, A
M. Hagen, J. Liu, J. Lykkefskild, C.M. Wehr, R.T. Ingersoll, V. Vinarsky, J.C.
L. Wilson-Fritch, S. Nicoloro, M. Chouinard, M.A. Lazar, P.C. Chui, J. Leszyk, J.
C. P. Earnest, Exercise interval training: An improved stimulus for improving the
V. P. Revzin, V. Petrov, S. V. Shenvi, J. M. R. Luz, T. M. Hagen, Decline in PGC-1{alpha} protein and oxidative enzyme activities possibly via AMPK
M. Suwa, T. Egashira, H. Nakano, H. Sasaki, S. Kumagai, Metformin increases the
D. Ziegler, F.A. Gries, Alpha-lipoic acid in the treatment of diabetic peripheral and
A. Civitarese, B. Ukropcova, S. Carling, M. Hulver, R.A. DeFronzo, L. Mandarino, E.
A. Civitarese, B. Ukropcova, S. Carling, M. Hulver, R.A. DeFronzo, L. Mandarino, E.
A. Civitarese, B. Ukropcova, S. Carling, M. Hulver, R.A. DeFronzo, L. Mandarino, E.


