

This Review is part of a thematic series on the **Role of Mitochondria in Cardiovascular Diseases**, which includes the following articles:

Free Radicals, Mitochondria, and Oxidized Lipids: The Emerging Role in Signal Transduction in Vascular Cells

Mitochondrial Dysfunction in Atherosclerosis

Defective Mitochondrial Biogenesis: A Hallmark of the High Cardiovascular Risk in the Metabolic Syndrome?

Endothelial Mitochondria: Contributing to Vascular Function and Disease

Role of Mitochondrial Dysfunction in Insulin Resistance

*Marshall S. Runge, Guest Editor*

## Role of Mitochondrial Dysfunction in Insulin Resistance

Jeong-a Kim, Yongzhong Wei, James R. Sowers

**Abstract**—Insulin resistance is characteristic of obesity, type 2 diabetes, and components of the cardiometabolic syndrome, including hypertension and dyslipidemia, that collectively contribute to a substantial risk for cardiovascular disease. Metabolic actions of insulin in classic insulin target tissues (eg, skeletal muscle, fat, and liver), as well as actions in nonclassic targets (eg, cardiovascular tissue), help to explain why insulin resistance and metabolic dysregulation are central in the pathogenesis of the cardiometabolic syndrome and cardiovascular disease. Glucose and lipid metabolism are largely dependent on mitochondria to generate energy in cells. Thereby, when nutrient oxidation is inefficient, the ratio of ATP production/oxygen consumption is low, leading to an increased production of superoxide anions. Reactive oxygen species formation may have maladaptive consequences that increase the rate of mutagenesis and stimulate proinflammatory processes. In addition to reactive oxygen species formation, genetic factors, aging, and reduced mitochondrial biogenesis all contribute to mitochondrial dysfunction. These factors also contribute to insulin resistance in classic and nonclassic insulin target tissues. Insulin resistance emanating from mitochondrial dysfunction may contribute to metabolic and cardiovascular abnormalities and subsequent increases in cardiovascular disease. Furthermore, interventions that improve mitochondrial function also improve insulin resistance. Collectively, these observations suggest that mitochondrial dysfunction may be a central cause of insulin resistance and associated complications. In this review, we discuss mechanisms of mitochondrial dysfunction related to the pathophysiology of insulin resistance in classic insulin-responsive tissue, as well as cardiovascular tissue. (*Circ Res.* 2008;102:401-414.)

**Key Words:** mitochondrial dysfunction ■ insulin resistance ■ cardiovascular disease

There are at least 47 million people in the United States who have the cardiometabolic syndrome, a precursor to diabetes and subsequent cardiovascular complications.<sup>1</sup> Furthermore, the development of insulin resistance, the cardinal feature of the cardiometabolic syndrome, is associated with increased tissue renin-angiotensin system activity and increasingly appears to be a nexus between components of the

syndrome.<sup>2,3</sup> The metabolic actions of insulin maintain glucose homeostasis by promoting glucose uptake in skeletal muscle and suppressing glucose production in the liver. Insulin resistance is typically defined as decreased sensitivity to these metabolic actions of insulin. Insulin-resistant individuals are at higher risk of developing type 2 diabetes mellitus (T2DM) and cardiovascular disease compared with

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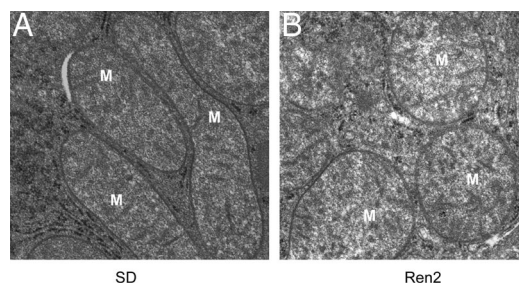
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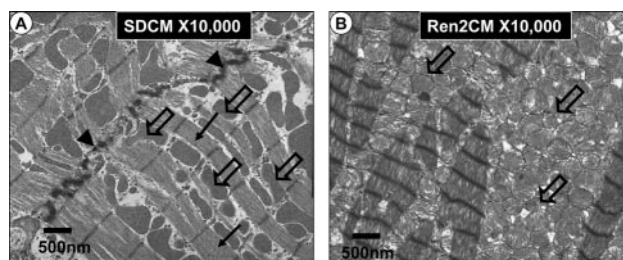
**Figure 1.** Mitochondrial abnormality in the liver of transgenic Ren2 male rat at 12 weeks of age. A, The normal hepatic mitochondrial morphology in the Sprague-Dawley control male rat model at 12 weeks of age. B, Mitochondrial abnormality in morphology in the 12-week-old transgenic Ren2 male rat model of hypertension and insulin resistance. Note the swollen and decreased matrix density by transmission electronic microscopy. Magnification,  $\times 25\,000$ .

subjects with normal insulin sensitivity.<sup>2,4</sup> Studies from our group have shown that increased tissue expression of angiotensin II via stimulation of the angiotensin II type 1 receptor (AT<sub>1</sub>R) can cause mitochondrial morphological and functional abnormalities in skeletal muscle, and liver (Figure 1) as well as cardiovascular tissue (Figure 2).<sup>5–7</sup> Furthermore, we and others have shown that blockade of the AT<sub>1</sub>R reduces oxidative stress and mitochondrial structure and functional abnormalities in rodent models of excessive tissue renin-angiotensin system activity.<sup>7,8</sup>

Metabolic regulation is largely dependent on mitochondria, which play an important role in energy homeostasis by metabolizing nutrients and producing ATP and heat. Imbalance between energy intake and expenditure leads to mitochondrial dysfunction, characterized by a reduced ratio of energy production (ATP production) to respiration.<sup>9</sup> Genetic and environmental factors including exercise, diet, aging, and stress affect both mitochondrial function and insulin sensitivity.<sup>10,11</sup> Importantly, it has been shown that mitochondrial dysfunction is associated with insulin resistance in skeletal muscle,<sup>12–14</sup> as well as in other tissues, including liver, fat, heart, vessels, and pancreas.<sup>15–18</sup> Thus, insulin resistance caused in part by mitochondrial dysfunction may contribute to a common pathophysiologic etiology for many chronic diseases.

### Mitochondrial Function

Aerobic organisms consume oxygen to produce energy from nutrients. In eukaryotic cells, energy production, mostly in the form of ATP, is controlled by mitochondria that link oxidative respiration with metabolism of nutrients (Figure 3). Mitochondria are compartmentalized by outer and inner membranes, and the mitochondrial respiratory chain is located in the inner membrane. Production of ATP requires 2 major steps, oxidation of NADH (or FADH<sub>2</sub>) and phosphorylation of ADP to form ATP (oxidative phosphorylation [OXPHOS]). These 2 reactions are coupled in mitochondria, and OXPHOS is an efficient and energy-conserving way of producing energy in aerobic organisms. NADH or FADH<sub>2</sub> are generated during glucose metabolism via glycolysis and the tricarboxylic acid cycle or  $\beta$ -oxidation of fatty acids. NADH or FADH<sub>2</sub> are oxidized to NAD<sup>+</sup> or FAD while protons are



**Figure 2.** Mitochondrial biogenesis in the transgenic Ren2 male rat at 10 weeks of age. A, The longitudinal normal myocardial mitochondrial morphology in the Sprague-Dawley control male rat model at 10 weeks of age. Note the orderly and linearly arranged sarcomeres (closed arrows) and subsarcolemmal (sarcolemmal) mitochondria (open arrows). Normal intercalated disc (arrowheads). Magnification,  $\times 10\,000$ . Bar=500 nm. B, Mitochondrial biogenesis in the 10-week-old transgenic Ren2 untreated control (Ren2C) rats, which display insulin resistance and abnormalities in both systolic and diastolic cardiac functions. Note the biogenesis of increased myocardial interdigitating mitochondria. Also note the loss of the orderly and linearly arranged sarcomeres. Magnification,  $\times 10\,000$ . Bar=500 nm.

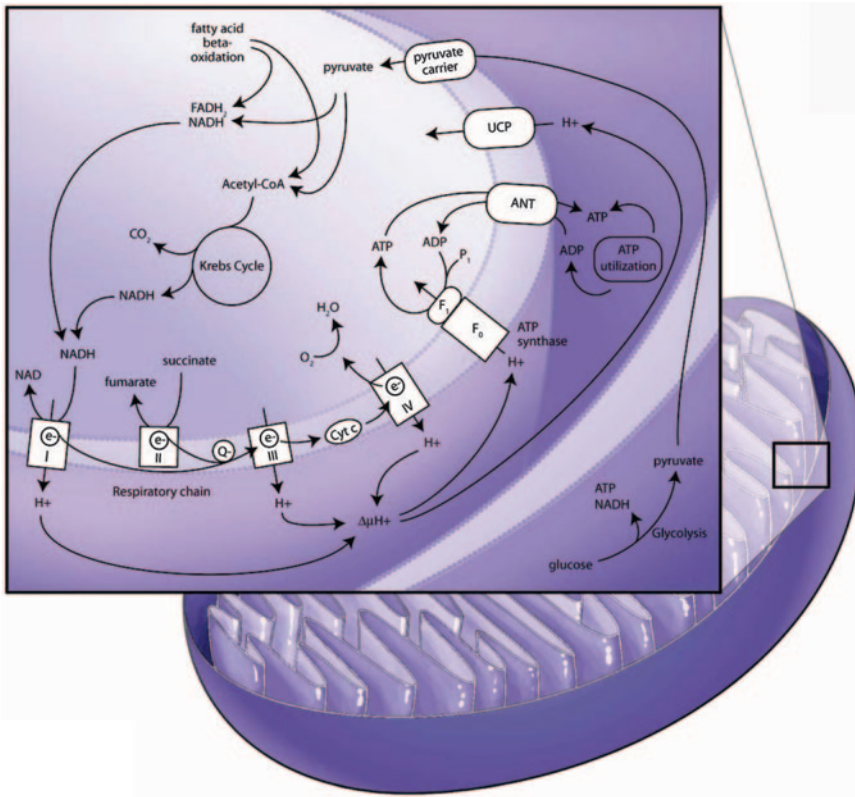
pumped to the intermitochondrial membrane through respiratory complexes I, III, and IV. Electrons from NADH or FADH<sub>2</sub> are then transferred through a series of respiratory chain complexes to O<sub>2</sub>, which finally generates H<sub>2</sub>O. A proton gradient across the membrane is the driving force of F<sub>0</sub>F<sub>1</sub>-ATPase (ATP synthase) to produce ATP from ADP. ATP is transported to the cytosol by exchanging with ADP through adenine nucleotide translocator and used for various biological events that require energy. On the other hand, mitochondria generate heat by a mechanism called “proton leak.” Proton leak from the intermembrane space to matrix (uncoupling) reduces proton-motive force and generates heat instead ATP. Uncoupling proteins (UCPs) play a major role in reducing the proton gradient.<sup>19</sup> UCP1 is expressed almost exclusively in brown adipose tissue. UCP2 is ubiquitously expressed, and UCP3 is expressed in skeletal muscle. UCP1, up to 10% of membrane protein, regulates adaptive thermogenesis, whereas UCP2 and -3 do not appear to play a major role in thermogenesis; mice with genetic ablation of UCP2 and -3 display a normal response to cold, normal basal proton conductance, and normal body weight.<sup>20,21</sup> Indeed, overexpression of UCP2 or -3 lowers reactive oxygen species (ROS) production,<sup>22</sup> stimulates the metabolic rate, and protects against weight gain and insulin resistance.<sup>23</sup> Moreover, UCP3 knockout mice show severe oxidative damage.<sup>24</sup> Collectively, these results suggest that UCPs play an important role in mitochondrial function by regulating both heat and ROS generation. Mitochondrial function with regard to energy balance is important in normal physiology and cellular function.

### Mitochondrial Dysfunction

There is evidence that mitochondrial dysfunction is associated with T2DM and age-related insulin resistance (Figure 4).<sup>25,26</sup> Genetic factors, oxidative stress, mitochondrial biogenesis, and aging may affect mitochondrial function, leading to insulin resistance and various pathological conditions.

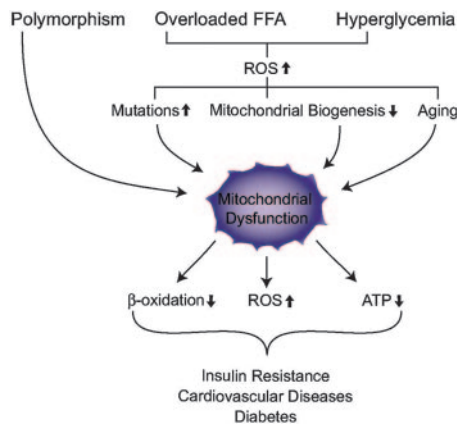
### Genetic Factors

Mitochondrial proteins are encoded by both nuclear and mitochondrial genes. Mitochondrial genes encode 13 protein



**Figure 3.** Mitochondrial respiratory chain and nutrient metabolism. Reducing agents (NADH or FADH<sub>2</sub>) are generated from glycolysis and Krebs cycle of glucose metabolism and β-oxidation of fatty acids. While NADH or FADH<sub>2</sub> are oxidized to NAD<sup>+</sup> or FAD, the electrons are carried to complex I (NADH–ubiquinone reductase), complex II (succinated ubiquinone reductase), complex III (ubiquinone–cytochrome c reductase), and finally to O<sub>2</sub>, which produces H<sub>2</sub>O. Oxidation of NADH or FADH<sub>2</sub> generates protons that are pumped to intermembrane space through complex I, III, and IV. The pumped protons increase electrochemical gradient across the membrane. This proton gradient is the driving force for F<sub>0</sub>F<sub>1</sub>-ATPase (ATP synthase) to produce ATP, which is used as an energy source in the body. On the other hand, the pumped protons can be leaked to matrix of mitochondria by UCP, which reduces proton gradient and in turn generates heat. Producing ATP or heat is controlled by energy needs in the body. ANT indicates adenine nucleotide translocator.

subunits of the oxidative phosphorylation complex as well as mitochondrial specific ribosomal and transfer (t)RNAs. The oxidative capacity of mitochondria is determined by the expression level of OXPHOS subunits and by the number and size of mitochondria.<sup>10</sup> Because mitochondrial dysfunction and gene expression of mitochondrial OXPHOS genes are related to insulin resistance,<sup>26</sup> mutations in mitochondrial genes caused by aging or cellular stress conditions may be one of the mechanisms underlying insulin resistance and other features of cardiometabolic syndrome.



**Figure 4.** Mechanism of mitochondrial dysfunction. Excess intake of nutrients, including overloaded FFAs or hyperglycemia conditions, increases ROS production and reduces mitochondrial biogenesis, causing mitochondrial dysfunction. Mitochondrial dysfunction leads to decreased β-oxidation and ATP production and increased ROS production, resulting in insulin resistance, diabetes, and cardiovascular disease.

It has been hypothesized that the mitochondrial genome is more susceptible to various mutagenic stressors because mitochondrial genes are more proximal to the ROS source and are not protected by histones.<sup>27</sup> Moreover, the mitochondrial genome constitutes only coding sequences, whereas nuclear DNA contains noncoding sequences.<sup>19</sup> Indeed, a naturally occurring thymidine-to-cytidine mutation in the mitochondrial tRNA<sup>Leu</sup> gene is associated with phenotypes of hypertension, hypercholesterolemia, and hypomagnesemia.<sup>28</sup>

Another mutation, A3243G, on mitochondrial DNA that encodes tRNA (LeuUUR) causes impaired insulin secretion.<sup>29</sup> In addition, patients with defects in acyl-coenzyme A (COA) dehydrogenase have phenotypes of cardiomyopathy, liver dysfunction, and neurological disorders.<sup>30</sup> Furthermore, polymorphisms in the promoter of UCP2 are associated with decreased incidence of obesity, reduced insulin secretion, and a high prevalence of T2DM.<sup>31,32</sup> Nuclear genes encoding mitochondrial proteins are also involved with insulin resistance.<sup>33</sup> Thus, genetic factors that are inherited through nuclear or mitochondrial genes may influence the pathogenesis of the cardiometabolic syndrome and cardiovascular disease through functional impairment of mitochondria.

### Mitochondrial Biogenesis

Fewer and smaller-sized mitochondria are found in skeletal muscle of insulin-resistant, obese, or T2DM subjects.<sup>34–36</sup> The number and size of mitochondria are correlated with mitochondrial oxidative capacity.<sup>10</sup> The decreased mitochondrial oxidative capacity accompanies the reduction in expression of mitochondrial proteins encoded by both the mitochondrial genome (cytochrome c oxidase 1) and nucleus (succinate



dehydrogenase and pyruvate dehydrogenase).<sup>34</sup> The molecular mechanism of mitochondrial biogenesis is driven, in part, through peroxisome proliferator-activated receptor (PPAR) coactivator (PGC)-1. PGC-1 $\alpha$  was discovered as a transcriptional regulator of UCP that plays a role in thermogenesis in adipose tissue.<sup>37</sup> The expression of PGC-1 $\alpha$  is increased on cellular ATP demand, including exercise, cold exposure, and fasting.<sup>38–41</sup> PGC-1 is a coactivator of nuclear transcription factors including nuclear respiratory factor (NRF)-1 and PPAR- $\gamma$  and - $\alpha$ .<sup>37,42,43</sup> NRF-1 regulates expression of many mitochondrial genes, including OXPHOS genes and mitochondrial transcription factor A (TFAM), that are crucial for mitochondrial gene expression and replication of the mitochondrial genome.<sup>43</sup> Expression of PGC-1 is decreased in insulin-resistant and diabetic human subjects, and NRF-1 expression is reduced in diabetic subjects.<sup>44</sup> Moreover, the reduction of PGC-1 expression is age dependent,<sup>45</sup> and PGC-1 $\alpha$ -null mice exhibit serious defects in contractility in both skeletal and cardiac muscle.<sup>42,46</sup> Thus, insulin-resistant subjects have fewer mitochondria in their muscle, possibly because of decreased expression of PGC-1 $\alpha$  and PGC-1 $\beta$ .<sup>43,47</sup>

Because expression of PGC-1 is regulated by the endothelial NO synthase (eNOS)/NO/cGMP/PGC-1 activation axis, eNOS plays an important role in mitochondria biogenesis.<sup>16,48</sup> In fact, eNOS-deficient mice are insulin resistant and hypertensive and have defects in fatty acid metabolism and fewer mitochondria.<sup>48–50</sup> Furthermore, exogenous NO or cGMP increase mitochondrial biogenesis.<sup>51</sup> However, the mechanism by which cGMP activates PGC-1 is unknown.

Another important factor that regulates mitochondrial biogenesis is AMP-activated protein kinase (AMPK).<sup>52</sup> Pharmacological drugs ( $\beta$ -guanidinopropionic acid [ $\beta$ GPA] or 5'-D-aminimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside [AICAR]) that activate AMPK promote mitochondrial biogenesis through PGC-1 $\alpha$  and NRFs.<sup>53,54</sup>  $\beta$ GPA increases mitochondrial DNA content and expression of PGC-1 $\alpha$  and cytochrome *c* in wild-type mice. However, application of  $\beta$ GPA to a transgenic mouse overexpressing dominant negative mutant AMPK does not have this effect. Exercise stimulates AMPK, leading to activation of PGC-1 by direct phosphorylation on threonine and serine residues. This phosphorylation event may ultimately promote mitochondrial biogenesis.<sup>55,56</sup>

Boushel et al observed that mitochondrial function that has been normalized for mitochondrial DNA content in T2DM patients is not significantly different compared with normal healthy subjects.<sup>57</sup> Moreover, DNA microarray studies have shown that expression of PGC-1 with regard to mitochondrial biogenesis may be responsible for metabolic disorders, including T2DM and insulin resistance.<sup>44,58</sup> These results suggest that decreased mitochondrial function is mainly attributable to the reduced number of mitochondria. Others observed that subsarcolemmal mitochondrial electron transport activity is lower in T2DM and obese subjects compared to lean active adults.<sup>36</sup> This diminished mitochondrial electron transport activity is partly attributable to the reduced mitochondrial content, but the decrement in mitochondrial function is greater than can be explained by mitochondrial content. Moreover, mRNA expression of PGC-1 $\alpha$ , PGC-1 $\beta$ , NRFs,

and TFAM is not different in insulin-resistant offspring of T2DM parents compared with control groups, but the mitochondrial function is significantly decreased.<sup>34</sup> These results suggest that reduced mitochondrial biogenesis cannot fully account for the mitochondrial dysfunction. Thus, both abnormalities in mitochondrial function, including ATP production and oxidative respiration, and mitochondrial biogenesis are associated with energy metabolism and insulin resistance.

### Oxidative Stress

Extramitochondrial oxygen consumption can occur by non-enzymatic and other enzymatic reactions, including NADPH oxidase, xanthine oxidase, uncoupled NO synthase, D-aminooxidase, p450 cytochromes, and proline hydroxylases<sup>19</sup>; however, mitochondria are the major sites of ROS production (0.2% to 2% of total oxygen taken by cells). ROS production occurs mainly at complex I (NADH CoQ reductase) and complex III (*bc<sub>1</sub>* complex) in mitochondria. ROS production is increased when excess electrons are provided to mitochondrial respiratory chains. The excess electrons are transferred to oxygen, which is converted to superoxide and subsequently to hydrogen peroxide either by spontaneously or via superoxide dismutase. The highest rate of ROS production occurs when the proton gradient is high and oxygen consumption (ATP demand) is low. Excess calorie intake and low energy expenditure can cause high proton-motive force and less ATP demand. Therefore, most electron carriers are occupied by electrons, and excess electrons are transferred to oxygen without ATP production.<sup>59</sup> When exercise increases ATP demand, electron transfers are coupled to ATP production and reduce proton-motive force. Despite intracellular protective mechanisms, including superoxide dismutase, catalase, and reduced glutathione, excess ROS is detrimental to cellular physiology. ROS generated from mitochondria damages proteins, DNA, and lipid in membrane components, which results in mitochondrial dysfunction.<sup>60,61</sup>

### Aging

Aging is a process of irreversible decline in physiological function over time. Several postulated mechanisms for aging include cumulative DNA damage, mitochondrial dysfunction, telomere loss, altered gene expression, and oxidative damages.<sup>62</sup> However, the precise molecular mechanisms of aging remain largely unknown. With increasing age, fat mass tends to gradually increase, especially visceral fat, and daily energy expenditure and physical activity tend to decrease. Because regulation of energy production is dependent on ATP needs, reduced energy expenditure with age decreases ATP needs. This leads to decreased oxidative capacity in aged skeletal muscle and heart in both animals and humans.<sup>63,64</sup> Old mitochondria have changes in morphology in addition to increased ROS production and decreased ATP production and respiration.<sup>65</sup> Furthermore, respiration is decreased in isolated mitochondria from elderly human subjects who have reduced mitochondrial number and function.<sup>14</sup> Gene expression-profiling studies demonstrate that genes related to fatty acid oxidation are altered and mitochondrial density and oxidative capacity are decreased with age.<sup>63,66</sup> In addition, mitochondrial biogenesis may be impaired by age-dependent accumu-

lations of point mutations in human mitochondrial (mt)DNA at specific regions that control replication of mtDNA.<sup>67</sup> Furthermore, age-dependent reduction in PGC-1 expression may account for reduced oxidative phosphorylation in elderly humans.<sup>45</sup> This is consistent with the observation that elderly human subjects have  $\approx 40\%$  reduction in oxidative phosphorylation with insulin resistance.<sup>26</sup> In addition, activation of AMPK- $\alpha_2$  is blunted in aged rats.<sup>55,68</sup> Because AMPK- $\alpha_2$  plays an important role in mitochondrial biogenesis by activating PGC-1 $\alpha$ ,<sup>55,68</sup> this is an additional mechanism by which the aging process may contribute to decreased mitochondrial biogenesis and insulin resistance. Thus, genetic factors, altered mitochondrial biogenesis, increased ROS production, and aging all contribute to mitochondrial dysfunction that is associated with insulin resistance.

### Insulin Resistance

Insulin maintains glucose homeostasis in a dynamic relationship with both feeding (glucose uptake) and fasting (gluconeogenesis) states. Additionally, nonclassic roles of insulin associated with cardiovascular, renal, and neural functions may explain why insulin resistance is associated with the risk factors for hypertension, cardiovascular disease, nephropathy, retinopathy, and neuropathy, etc.<sup>2,3,69</sup> Perturbation of insulin signaling by various metabolites and crosstalk with other signaling pathways leads to insulin resistance.

### Pathophysiology of Insulin Resistance

Insulin resistance is characterized by a diminished ability of cells or tissues to respond to physiological levels of insulin. Genetic and environmental factors, including aging, obesity, lack of exercise, and stress, contribute to insulin resistance. Disorders of glucose and lipid metabolism cause defects in insulin signaling that are linked to various pathological conditions.<sup>3,70,71</sup> Thus, molecular and cellular mechanisms of insulin resistance are relevant to understanding the pathogenesis of various diseases associated with insulin resistance.

Circulating free fatty acids (FFAs) are elevated by stress, lipodystrophy, or excess energy intake. Elevated plasma FFA levels lead to accumulation of FFAs, diacylglycerol (DG), and triglycerides in nonadipose tissue, including skeletal muscle, liver, heart, and  $\beta$ -cells. In fact, lipid infusions and high-fat feeding in human subjects and rodents reduces insulin-stimulated glucose disposal.<sup>72–74</sup> These data suggest that defects in lipid metabolism leading to impairment of insulin signaling seem to be a major mechanism for insulin resistance.<sup>75</sup> Impaired insulin signaling not only affects insulin-stimulated glucose metabolism in skeletal muscle but also impairs other actions of insulin in diverse tissues including, liver, adipose tissue, heart, and the vasculature.<sup>76–80</sup>

### Metabolic Tissues

Insulin resistance is associated with decreased mitochondrial number, abnormal morphology, lower levels of mitochondrial oxidative enzymes, and lower ATP synthesis both in vivo<sup>36,81</sup> and ex vivo in human muscle biopsies.<sup>82</sup> Notably, either acute lipid infusion or chronic elevation of plasma FFAs causes hepatic insulin resistance.<sup>83,84</sup> Elevated FFAs in the plasma leads to intracellular lipid accumulation that is associated

with insulin resistance in muscle and liver.<sup>85–87</sup> Intramyocellular lipid accumulation leads to reduction in the ratio of glycolytic to oxidative enzyme activities, which is negatively correlated with insulin sensitivity in T2DM when compared with nondiabetic subjects.<sup>35,81,88</sup> Thus, intracellular lipid accumulation causes reduced mitochondrial oxidative capacity in skeletal muscle of T2DM subjects and offspring of patients with T2DM. Mitochondrial abnormalities including ultrastructural lesions, depletion of mtDNA, decreased activity of respiratory chain complexes,<sup>89</sup> and impaired mitochondrial  $\beta$ -oxidation are also found in patients with nonalcoholic fatty liver disease (NAFLD).<sup>90</sup> These mitochondrial abnormalities are associated with NAFLD, which leads to hepatic steatosis and other liver injury.<sup>91</sup> Mitochondria with abnormal morphology are also observed in the liver of the Ren2, with transgenic overexpression of murine rennin, the insulin-resistant and hypertensive rat model (Figure 1). This suggests that increased tissue expression of renin causes abnormalities in mitochondria that may lead to NAFLD. Although the detailed mechanism for renin-induced mitochondrial abnormality is not understood, ROS produced through NADPH oxidase could be one potential mechanism that causes mitochondrial abnormality and hepatic dysfunction in Ren2 model (unpublished observation).

Adipose tissue that is overloaded with triglycerides in obesity, and dyslipidemia increases lipolysis and the release of FFAs that cause defects in glucose metabolism and insulin resistance in nonadipose tissues. Conversely, excess loss of adipose tissue, lipodystrophy, also leads to insulin resistance and T2DM.<sup>92</sup> Having adipocytes that are impaired in lipid storage creates lipotoxicity in other tissues and metabolic abnormalities associated with intracellular accumulation of lipid. Moreover adipocytes release adipokines, including leptin, adiponectin, resistin, and tumor necrosis factor- $\alpha$ , that positively or negatively regulate metabolic pathways.<sup>93–95</sup> Endocrine and nonendocrine roles of adipose tissue with regard to energy intake and expenditure play important roles in insulin resistance.<sup>96,97</sup> The number of mitochondria and the expression of genes that are involved in mitochondrial biogenesis are significantly decreased in adipocytes from patients with T2DM or morbidly obese human subjects.<sup>98,99</sup> Thus, decreased number of mitochondria, decreased mitochondrial gene expression, abnormal morphology of mitochondria, and abnormal functions in oxidative phosphorylation are commonly found in insulin-resistant metabolic tissues, including skeletal muscle, liver, and fat. These abnormalities in mitochondria are associated with intracellular lipid accumulation, insulin resistance, and pathophysiology of T2DM and NAFLD.

### Cardiovascular Tissues

Cardiovascular diseases, including coronary artery disease, hypertension, heart failure, and stroke, are associated with insulin resistance and endothelial dysfunction.<sup>4,100</sup> As described above, FFAs, but not glucose infusion, contributes to insulin resistance and reduces mitochondrial oxidative capacity, cardiac efficiency, and ATP production and increases myocardial oxygen consumption in obese and insulin-resistant *ob/ob* mice.<sup>101</sup> In addition, intramyocardial lipid accu-

mulation induces lipotoxic injury and cardiac dysfunction, including diastolic dysfunction, left ventricular hypertrophy, and impaired septal contractility in rodent and human obesity.<sup>102,103</sup> These results suggest that reduced mitochondrial oxidative capacity contribute to cardiac dysfunction. Mitochondria occupy 20% to 30% of the cell volume in cardiac myocytes, compared with  $\approx 67\%$  in oxidative skeletal muscle such as the soleus and only 2% to 3% in glycolytic muscle such as the gastrocnemius muscle.<sup>104</sup> Depressed mitochondrial transcription factors and oxidative capacity in rat contribute to failing cardiac function. The heart possesses a relatively low endogenous antioxidant capacity, as contributed by both enzymatic and nonenzymatic free radical scavengers and antioxidants, making it susceptible to oxidative stress with attendant structural and functional abnormalities.<sup>7</sup> Increased oxidative stress in the heart has been linked to ventricular hypertrophy, systolic and diastolic functional abnormalities, and abnormal insulin metabolic signaling.<sup>7,105</sup> Several studies have shown an association between mitochondrial oxidative energy and alterations in mitochondrial morphology and function, including decreased production of ATP and impaired activation of mitochondrial ATP-activated potassium channels.<sup>106–108</sup> Transmission electron microscopic analysis of myocardial tissue in insulin-resistant rodent models has demonstrated the presence of increased numbers of morphologically abnormal mitochondria (Figure 2).<sup>5,7,42,109</sup> Increased numbers of mitochondria have been associated with hypertrophied hearts displaying oxidative stress.<sup>5,7,110</sup> Accordingly, these changes may represent an adaptive response to greater energy requirements as well as oxidative stress. However, in pathological hypertrophy, which can be observed at the later stage of pathogenesis of cardiac dysfunction, the number of mitochondria and DNA contents are reduced.<sup>111</sup>

Another important insulin action in the heart is to increase myocardial blood flow and reduce coronary vascular resistance.<sup>112</sup> Impairment of endothelium-dependent vasodilation and glucose intolerance accompany intramyocardial lipid accumulation, and this precedes T2DM and heart failure.<sup>113</sup> Thus, the role of mitochondria in cardiac function is important, and cardiac mitochondrial dysfunction may contribute to various cardiovascular diseases, including coronary heart disease, hypertension, cardiomyopathy, and heart failure.

Although the association between insulin resistance and endothelial dysfunction has been emphasized,<sup>114</sup> the role of mitochondrial function in endothelium is not clearly understood. Endothelial cells produce 75% of ATP from glycolysis<sup>115</sup>; thus, oxidative metabolism does not seem to be important in vascular endothelial cells. However, mitochondria in endothelial cells may play an important role in cellular signaling as sensors for local oxygen concentration and as regulators of intracellular  $[Ca^{2+}]$  and NO production.<sup>116</sup> Moreover, Brownlee suggested that mitochondrial dysfunction occurs as a “unifying mechanism” for microvascular and macrovascular complications through ROS production.<sup>18</sup> Hyperglycemia induced by endothelial dysfunction is inhibited by blocking ROS production from mitochondria and by overexpression of UCPI, or manganese superoxide dismutase.<sup>18,117</sup> Furthermore, eNOS in vascular endothelial cells

seems to play an important role in insulin-stimulated NO production and vasodilation,<sup>118</sup> as well as in mitochondrial biogenesis.<sup>48,51</sup> Indeed, eNOS-null mice are insulin resistant and hypertensive and have dyslipidemia.<sup>48,119,120</sup> Thus, insulin resistance decreases eNOS activity, and associated mitochondrial dysfunction impairs various cardiac functions that may contribute to heart failure, cardiomyopathy, and coronary artery disease.

### Pancreatic $\beta$ -Cells

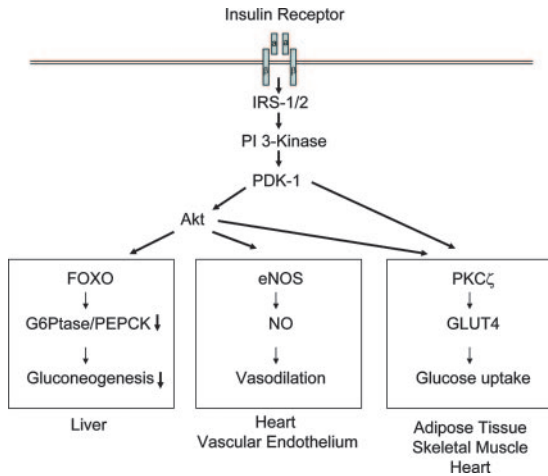
Insulin-resistant patients can develop overt T2DM when pancreatic  $\beta$ -cells cannot produce enough insulin to maintain euglycemia. Pancreatic  $\beta$ -cells in T2DM cannot sense glucose properly, contributing to impaired insulin secretion. Glucose oxidation by mitochondria produces ATP, which increases the ATP/ADP ratio. ATP/ADP ratio is primarily regulated by mitochondrial function, and the increased ATP/ADP ratio contributes to inhibition of potassium channel ( $K_{ATP}$ ), which leads to plasma membrane depolarization, voltage-gated calcium channel opening, calcium influx, and secretion of insulin. Thus, mitochondrial function may be correlated with  $\beta$ -cell function because of the importance of the ATP/ADP ratio.<sup>121</sup> In addition, when mitochondrial genes are removed from  $\beta$ -cells, insulin secretion is impaired, and pancreatic  $\beta$ -cell function is restored when the cells are replenished with normal mitochondria.<sup>122</sup> Furthermore, tissue-specific knockout of TFAM, a nuclear encoded mitochondrial protein, results in reduced  $\beta$ -cell mass, impaired insulin secretion, and development of overt diabetes with severe mtDNA depletion by 5 weeks after birth.<sup>123</sup> The results of studies support the notion that mitochondrial function is important for healthy  $\beta$ -cell function, whereas mitochondrial dysfunction may contribute to the pathogenesis of T2DM by affecting insulin secretion as well as insulin action.

## Signaling Between Mitochondrial Dysfunction and Insulin Resistance

### Insulin Signaling

Canonical insulin signaling pathways are initiated by insulin binding to the extracellular  $\alpha$  subunit of the insulin receptor (IR) (Figure 5).<sup>71,124</sup> This causes a conformational change in the  $\beta$  subunit of IR, which has intrinsic tyrosine kinase activity, resulting in autophosphorylation of IR tyrosine residues (eg, NPEY motif of the receptor) and enhanced tyrosine kinase activity of the receptor. The activated receptor phosphorylates insulin receptor substrate (IRS) family members and activates phosphatidylinositol 3-kinase (PI3K), a lipid kinase that phosphorylates phosphatidylinositol 4,5-bisphosphate generating phosphatidylinositol 3,4,5-triphosphate. Receptor tyrosine kinases including insulin receptor activate class IA isotypes of PI3K that are composed of heterodimer with catalytic (p110 $\alpha$ , p110 $\beta$ , p110 $\delta$ ) and regulatory (p85 $\alpha$ , p50 $\alpha$ , p55 $\alpha$ , p55 $\gamma$ , p85 $\beta$ ) subunits.<sup>125,126</sup> Activation of PI3K leads to stimulation of various downstream serine kinases, including phosphoinositide dependent kinase-1, protein kinase B (Akt), atypical protein kinase C (PKC), and other serine kinases, which culminates in the





**Figure 5.** Insulin signaling pathway. The metabolic PI3K branch of insulin signaling pathway and tissue-specific actions of insulin are shown. PI3K branch of insulin signaling pathway plays a major role in gluconeogenesis in the liver, enhances NO production in the endothelium and heart, and glucose uptake in skeletal muscle, adipose tissue, and heart.

pleiotropic biological actions and metabolic functions of insulin. Similarly, SH2 domain of Grb-2 binds to Shc and activates GTP exchange factor Sos, which subsequently activates the small GTP protein Ras. Activation of Ras leads to the activation of downstream signaling that activates Raf and mitogen-activated protein (MAP)/extracellular signal-regulated kinase kinase (MEK), and MAP kinase (MAPK). This MAPK branch of the insulin signaling pathway regulates growth, mitogenesis, and differentiation. Insulin signaling constitutes a highly complex network with multiple feedback loops and crosstalk between major signaling branches, as well as signaling from heterologous receptors.<sup>127</sup> It is noteworthy that in addition to the classic role of insulin signaling, the roles of insulin signaling in cardiac growth, vasodilation, and maintenance of vascular tone is similar but the biological responses are distinct.<sup>2,3,114,128</sup> Thus, the complexity of insulin signaling gives rise to the specificities of insulin signaling and actions based on the context, ie, the tissue type and the particular physiological or pathophysiological conditions.

### Molecular Mechanism of Insulin Resistance

Defects at multiple sites in insulin signaling pathway have been suggested as mechanisms underlying insulin resistance<sup>71</sup>: (1) increased serine phosphorylation of IRS proteins<sup>129</sup>; (2) increased degradation of IRS proteins<sup>130</sup>; (3) increased activity of phosphatases including (src homology 2 domain containing inositol 5'-phosphatase 2 [SHIP2], phosphatase tensin homolog deleted on chromosome ten [PTEN], and phospho-tyrosine phosphatase 1B [PTP-1B])<sup>131–133</sup>; (4) decreased activation of insulin receptor downstream signaling molecules including Akt and atypical PKC.<sup>134</sup>

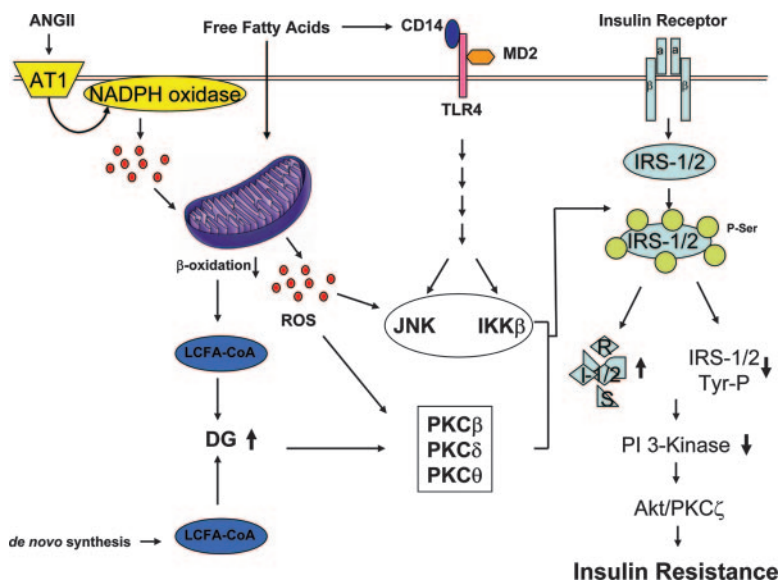
Reduction in tyrosine phosphorylation of IRS family members has been observed in insulin-resistant animal models and human subjects, including those who are obese or made insulin resistant by lipid infusion.<sup>135–139</sup> One possible mechanism to explain decreased tyrosine phosphorylation of IRS-1 and -2 is activation of serine/threonine kinases that can

phosphorylate IRS family members at multiple serine sites.<sup>70,129</sup> Phosphorylation of IRS proteins at particular serine residues inhibits the interaction of IRS proteins with the IR. This leads to reduction in tyrosine phosphorylation of IRS and subsequently decreases activation of PI3K.<sup>129</sup> Interestingly, high-fat diet-induced insulin resistance is ameliorated when specific serine/threonine kinases are genetically ablated or pharmacologically inhibited.<sup>140–143</sup> Increased proinflammatory signaling is another potential mechanism underlying insulin resistance. FFAs stimulate receptor (Toll-like receptor)-mediated proinflammatory signaling, which activates I $\kappa$ B kinase (IKK) $\beta$  and c-Jun N-terminal kinase (JNK) and stimulates production of other cytokines, including tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$ , and interleukin-6.<sup>144–146</sup> IKK $\beta$  and JNK are well-known serine kinases that phosphorylate IRS-1 at serine residues, leading to decreased metabolic signaling. Inhibition of IKK $\beta$  or JNK with antiinflammatory drugs or gene knockout improves insulin sensitivity contemporaneously with reductions in serine phosphorylation of IRS proteins.<sup>142,143,147,148</sup> Endoplasmic reticulum (ER) stress is another mechanism contributing to insulin resistance through activation of serine kinases. JNK activation, as a consequence of ER stress, increases serine phosphorylation of IRS proteins. Chemical chaperones including 4-phenyl butyric acid and taurine-conjugated ursodeoxycholic acids (TUDCA) significantly reduce ER stress, resulting in improved insulin sensitivity.<sup>149</sup> This treatment also decreases fatty liver in animal models and is associated with a reduction in hepatic JNK activity as well as IRS-1 serine phosphorylation.<sup>149</sup> Lastly, mitochondrial dysfunction and consequent increases in ROS, in turn, activate various serine kinases that phosphorylate IRS proteins, leading to insulin resistance.<sup>34</sup> Furthermore, ROS stimulates proinflammatory signaling by activation of IKK $\beta$  that phosphorylates IRS-1 at serine residues.<sup>150</sup> Although, the detailed mechanism for serine kinase activation mediated by ROS is not clearly understood, decreased ROS production by antioxidants or increased expression of UCP2/3 improves both mitochondrial function and insulin sensitivity. Mitochondrial dysfunction results in accumulation of fatty acid metabolites, DG, and long-chain fatty acyl-CoA (LCFA-CoA).<sup>151</sup> Intracellular accumulation of DG, allosteric activator of PKCs, activates PKCs, including PKC- $\beta$ ,  $\delta$ , and  $\theta$ , that increase serine phosphorylation of IRS proteins, leading to inhibition of insulin signaling and insulin resistance (Figure 6).<sup>139,151,152</sup> In fact, the PKC $\theta$ -deficient mouse is protected from fat-induced insulin resistance.<sup>140</sup> This suggests that activation of PKCs attributable to the mitochondrial dysfunction may cause insulin resistance. Thus, lipid-induced mitochondrial dysfunction impairs insulin signaling both directly and indirectly through generation of excess ROS.

### Therapeutic Intervention

#### Pharmacological Intervention

Because mitochondrial function is associated with mitochondrial biogenesis, stimulation of mitochondrial biogenesis may have beneficial effects in both metabolic and cardiovascular diseases. In fact, beneficial effects of thiazolidinediones (synthetic PPAR- $\gamma$  ligands) have been reported to improve



**Figure 6.** Proposed molecular mechanism for insulin resistance caused by mitochondrial dysfunction. FFAs activate inflammatory signaling and reduce ATP production that contributes to mitochondrial dysfunction and accumulation of LCFA-CoA and DG. Accumulation of lipid metabolite activates PKCs ( $\beta$ ,  $\delta$ , and  $\theta$ ). ROS produced by NADPH oxidase by angiotensin II causes mitochondrial dysfunction. Conversely, mitochondrial dysfunction increases ROS production, which causes activation of serine/threonine kinases, including IKK $\beta$ , JNK, and PKCs, which increases serine phosphorylation of IRS proteins and subsequently results in insulin resistance. Increased serine phosphorylation of IRS-1/2 leads to decreased activity of insulin downstream signaling pathways, including PI3K, Akt, and PKC $\zeta$ , which culminates in decreased glucose uptake, increased glucose production, and reduced vasodilation and insulin secretion. The reduced insulin responsiveness (insulin resistance) causes diabetes and cardiovascular diseases. PDK-1 indicates 3'-phosphoinositide-dependent protein kinase 1.

insulin resistance in liver, adipocytes, and heart, as well as  $\beta$ -cell function and endothelial dysfunction in studies using animal models and human intervention studies.<sup>153–158</sup> The molecular mechanism of insulin-sensitizing activity for thiazolidinediones may be, in part, through increased mitochondrial biogenesis.<sup>98</sup> Metformin is used as another insulin sensitizer, which is reported to reduce ROS production, increase expression of PGC-1 $\alpha$ , and stimulate AMPK.<sup>68,159,160</sup> Metformin may improve mitochondrial function by reducing oxidative stress and stimulating mitochondrial biogenesis through activation of AMPK/PGC-1/NRFs axis pathway. Studies from our group have demonstrated that increases in tissue angiotensin II increase NADPH oxidase activity and tissue ROS and that this is associated with abnormalities in mitochondrial structure and function. Thus, drugs that reduce actions of angiotensin II also increase insulin sensitivity, reduce ROS production, and improve mitochondrial function. For example, treatment with angiotensin-converting enzyme inhibitor has been reported to increase insulin sensitivity, reduce ROS production, and increase mitochondrial biogenesis.<sup>8,158,161</sup> Angiotensin receptor blockers also significantly improve insulin resistance and block angiotensin-induced oxidative stress in both human and animal models.<sup>6,162–164</sup> Excessive tissue angiotensin causes insulin resistance, cardiomyopathy, hypertension, and renal failure, possibly because of mitochondrial dysfunction; thus, angiotensin receptor blockers or angiotensin-converting enzyme inhibitors may also exert beneficial effects to various pathological conditions by enhancing mitochondrial biogenesis and function.<sup>3,5–7,164–168</sup> The detailed molecular mechanism for the actions of these drugs targeting excess tissue angiotensin in mitochondrial dysfunction is not well defined. Reducing angiotensin receptor-mediated ROS production may improve mitochondrial function and improve insulin-mediated metabolic actions. However, further investigation is needed to better understand the detailed mechanisms of these and other therapeutic drugs with regard to mitochondrial functions and biogenesis.

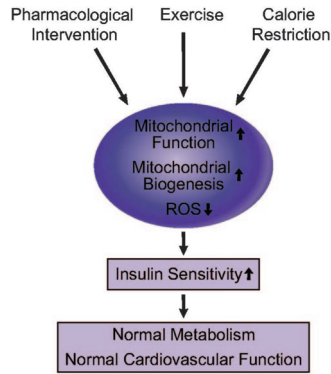
Mitochondrial dysfunction is induced by increased ROS, whereas impairment of mitochondrial function, in turn, pro-

duces more ROS and lipid byproducts, including LCFA-CoA and DG. Thus, therapeutic intervention using antioxidant supplements may be beneficial to interrupt this vicious cycle. In fact,  $\alpha$ -lipoic acid reduces hyperglycemia and increased GLUT4 content in rat skeletal muscle tissue.<sup>169</sup> Moreover,  $\alpha$ -lipoic acid opposes ROS-induced inhibition of insulin signaling.<sup>170</sup> Although oral vitamin C supplementation does not significantly affect insulin resistance or endothelial function in T2DM,<sup>171</sup> intraarterial vitamin C improves endothelial-dependent vasodilation in T2DM.<sup>172</sup> Tempol, a superoxide scavenger, is able to ameliorate cardiac and vascular dysfunction, normalize angiotensin II-induced insulin resistance, and improve mitochondrial morphology and function.<sup>6,7,164</sup> Pharmacological targets that can stimulate mitochondrial biogenesis (eg, thiazolidinediones) or reducing ROS production (eg, metformin, angiotensin receptor blockers, and antioxidants) may have beneficial effects on cardiometabolic syndrome partly by improving mitochondrial function.

### Exercise

Large-scale epidemiological studies demonstrate that low aerobic exercise correlates with increased mortality and cardiovascular disease.<sup>173,174</sup> Impaired mitochondrial function may be an important mechanism for low aerobic capacity and cardiovascular risk factors that accompany the cardiometabolic syndrome.<sup>175</sup> Exercise improves insulin action and glucose tolerance in insulin-resistant subjects and animal models.<sup>176,177</sup> Substantial evidence indicates that aerobic exercise stimulates mitochondrial biogenesis by increasing gene expression of PGC-1, NRF-1, and TFAM.<sup>178,179</sup> Endurance exercise training increases mitochondrial size, number, and oxidative activity contributing to improved whole-body glucose metabolism.<sup>180</sup> Increased expression of eNOS by physical activity may stimulate mitochondrial biogenesis.<sup>49,181</sup> Moderate-intensity physical activity combined with weight loss improves insulin sensitivity through increasing skeletal muscle electron transport chain activity and increasing mitochondrial cristae (without changing mtDNA content).<sup>182</sup> Age-associated reduction in expression of mitochon-





**Figure 7.** Improvement of mitochondrial function by pharmacological intervention, exercise, and calorie restriction can improve insulin sensitivity, which leads to normal metabolism and cardiovascular function.

drial genes and mitochondrial biogenesis is restored with aerobic exercise.<sup>63,183</sup> Thus, exercise can improve glucose and lipid metabolism by activation of AMPK and PGC-1 $\alpha$  that increase mitochondrial biogenesis and function.

### Calorie Restriction

Calorie restriction increases lifespan in organisms ranging from yeast to mammals.<sup>184,185</sup> Calorie restriction ameliorates many of the pathophysiological conditions associated with the cardiometabolic syndrome related to glucose and lipid metabolism.<sup>186</sup> One study using oligonucleotide-based array techniques showed that expression of stress response genes and oxidative stress-inducible genes are enhanced, whereas gene expression of energy metabolism is decreased with aging. Interestingly, in the same study, calorie restriction reversed these changes in gene expression patterns.<sup>66</sup> Calorie restriction increases expression of UCP2 and -3 in human subjects.<sup>187</sup> Calorie restriction increases mitochondrial biogenesis, oxygen consumption, ATP production, and expression of SIRT1 (NAD<sup>+</sup>-dependent deacetylase) through eNOS expression. SIRT family members play a central role in the physiological effect of calorie restriction. The specific roles of SIRT1, -3, and -4 in metabolism have been described in various tissues.<sup>186</sup> SIRT1 activates PGC-1 $\alpha$ , which may lead to mitochondrial biogenesis. Furthermore, eNOS-null mice do not respond appropriately to calorie restriction,<sup>50</sup> suggesting that calorie restriction improves mitochondrial biogenesis and function, in part, through increases in NO production. Another study has shown that calorie restriction induces mitochondrial biogenesis, reduced ROS production.<sup>188</sup> Mitochondrial biogenesis or improvement of mitochondrial function may be a major mechanism for the beneficial effects of calorie restriction. Thus, the beneficial effects of calorie restriction mediated by enhancement of mitochondrial biogenesis and function may lead to improvement of glucose and lipid metabolism, as well as insulin resistance. Therapeutic interventions to improve mitochondrial function or to stimulate mitochondrial biogenesis may ameliorate insulin resistance and other components of the cardiometabolic syndrome and improve cardiovascular function and outcomes (Figure 7).

### Summary and Perspectives

Insulin resistance plays a central role in the pathogenesis of the cardiometabolic syndrome, T2DM, and attendant cardio-

vascular complications. Excess nutrients or sedentary lifestyle cause various pathological conditions that are associated with mitochondrial dysfunction. Genetic factors, reduced mitochondrial biogenesis, increased oxidative stress, and aging may be causal factors for abnormalities in mitochondrial dysfunction. The resultant mitochondrial dysfunction, in turn, increases ROS production, resulting in a vicious cycle. Antioxidants protect from both mitochondrial dysfunction and insulin resistance by scavenging free radicals. However, both lipid accumulation and excess ROS further stimulate various serine/threonine kinases and inflammatory signaling pathways that inhibit insulin signaling. Although several serine/threonine kinases that are responsible for insulin resistance have been identified, the detailed molecular mechanisms by which ROS can activate these kinases are unknown.

Blockade of the AT<sub>1</sub>R and scavengers of ROS prevent cardiac, vascular, and hepatic dysfunction as well as mitochondrial dysfunction in rodents with tissue overexpression of angiotensin II.<sup>2,7</sup> These observations suggest that angiotensin II contributes to mitochondrial dysfunction through a mechanism that is distinct from elevated FFAs. Studies from our laboratory suggest that excess tissue angiotensin II results in enhanced NADPH oxidase activity, leading to increased ROS and mitochondrial abnormalities associated with insulin resistance, cardiac dysfunction, vascular inflammation, and reduced NO-mediated relaxation.<sup>6,7,165,168,189</sup> ROS produced by angiotensin II activation of NADPH oxidase may damage cellular proteins or transduce signaling that influences mitochondrial biogenesis and function. However, the precise mechanism by which ROS can affect mitochondrial biogenesis and function remains to be clarified.

Intracellular lipid accumulation is observed in insulin-resistant skeletal muscle.<sup>13</sup> Lipid infusion decreases glucose transport and ATP synthesis.<sup>9</sup> It is not clear whether reduced ATP synthesis causes insulin resistance or whether reduced insulin sensitivity leads to reduction in generation of ATP. The intramyocellular triglyceride contents in lipid-infused skeletal muscle are not different from controls.<sup>9</sup> Thus, the accumulation of lipid metabolites, including LCFA-CoA and DG, may not be attributable to the increased de novo synthesis of lipid but attributable to reduction in  $\beta$ -oxidation of fatty acid in the mitochondria. The cause-and-effect relationship between reduced mitochondrial function and excess intramyocellular lipid content is an area ripe for investigation.

Decreased number of mitochondria may be a mechanism for insulin resistance. Regulatory mechanisms for mitochondrial biogenesis and expression of OXPHOS genes related to energy status are not completely defined and require further investigation. Nonetheless, mitochondrial dysfunction seems to play a central role in metabolic and cardiovascular disorders. Thus, new therapeutic strategies that regulate mitochondrial function and mitochondrial biogenesis may have therapeutic potential for decreased insulin action and pancreatic  $\beta$ -cell production, lipid accumulation in liver (eg, NAFLD), skeletal muscle impairments, endothelial-mediated vasorelaxation, and both systolic and diastolic myocardial function, all components of the cardiometabolic syndrome.

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## Disclosures

None.

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