

The Role of Mitochondria in the Pathophysiology of Skeletal Muscle Insulin Resistance

Ines Pagel-Langenickel, Jianjun Bao, Liyan Pang, and Michael N. Sack

Translational Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892-1454

Multiple organs contribute to the development of peripheral insulin resistance, with the major contributors being skeletal muscle, liver, and adipose tissue. Because insulin resistance usually precedes the development of type 2 diabetes mellitus (T2DM) by many years, understanding the pathophysiology of insulin resistance should enable development of therapeutic strategies to prevent disease progression. Some subjects with mitochondrial genomic variants/defects and a subset of lean individuals with hereditary predisposition to T2DM exhibit skeletal muscle mitochondrial dysfunction early in the course of insulin resistance. In contrast, in the majority of subjects with T2DM the plurality of evidence implicates skeletal muscle mitochondrial dysfunction as a consequence of perturbations associated with T2DM, and these mitochondrial deficits then contribute to subsequent disease progression. We review the affirmative and contrarian data regarding skeletal muscle mitochondrial biology in the pathogenesis of insulin resistance and explore potential therapeutic options to intrinsically modulate mitochondria as a strategy to combat insulin resistance. Furthermore, an overview of restricted molecular manipulations of skeletal muscle metabolic and mitochondrial biology offers insight into the mitochondrial role in metabolic substrate partitioning and in promoting innate adaptive and maladaptive responses that collectively regulate peripheral insulin sensitivity. We conclude that skeletal muscle mitochondrial dysfunction is not generally a major initiator of the pathophysiology of insulin resistance, although its dysfunction is integral to this pathophysiology and it remains an intriguing target to reverse/delay the progressive perturbations synonymous with T2DM. (*Endocrine Reviews* 31: 25–51, 2010)

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Abbreviations: AIF, Mitochondrial flavoprotein apoptosis-inducing factor; AMPK, AMP kinase; BMI, Body mass index; CoA, coenzyme A; CPT-1, carnitine palmitoyl transferase 1; DAG, diacylglycerol; DGAT, acyl-CoA:DAG acyltransferase; ETC, electron transfer chain; FAO, mitochondrial fatty acid β -oxidation; GLUT4, glucose transporter isoform 4; IMCL, intramyocellular lipid; MIDD, maternally inherited diabetes and deafness (syndrome); MRS, magnetic resonance spectroscopy; mtDNA, mitochondrial genome DNA; PCOS, polycystic ovary syndrome; PGC-1 α , PPAR γ coactivator 1 α ; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; TCA, tricarboxylic acid; T2DM, type 2 diabetes mellitus; UCP, uncoupling protein; VO_{2max}, maximal oxygen consumption.

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I. Introduction

A. The potential role of mitochondria in the pathophysiology of skeletal muscle insulin resistance

Understanding the integrated pathophysiology initiating the development of insulin resistance should broaden our capacity to identify novel therapeutic targets for the prevention and/or treatment of type 2 diabetes mellitus (T2DM). This biology remains incompletely characterized, in part, due to the interaction of multiple organ systems and numerous intracellular perturbations within these organs (including disrupted signaling and metabolic alterations) governing the development of insulin resistance (1). The complexity of this biology is further underscored by the progressive changes in the systemic milieu including the onset of hyperinsulinemia, elevated circulating free fatty acids and triglycerides, hyperglycemia, and the activation of systemic immune system during the development of T2DM (2).

Skeletal muscle is integral to the development of insulin resistance and is a major reservoir for postprandial glucose storage (3–5). In skeletal muscle, disruption of mitochondrial biology is evident in some insulin-resistant subjects years before they develop diabetes (6–8). Furthermore, the disruption in skeletal muscle mitochondria is recognized as a *sine qua non* in established T2DM (9, 10). However, whether perturbations in this organelle are central to the pathophysiology of insulin resistance in skeletal muscle is robustly debated (11–14). Moreover, in light of the lack of macrovascular benefit of exclusively targeting glucose control in T2DM (15, 16), and the strong association of elevated insulin levels with T2DM complications, the need to better understand the pathophysiology of insulin resistance is of immense clinical and therapeutic importance (17).

The fundamental questions that will be addressed in this review are: 1) whether the disruption of skeletal muscle mitochondria is a primary component in the pathophysiology of skeletal muscle insulin resistance; 2) conversely, whether mitochondrial dysfunction is rather a consequence of reduced aerobic activity and/or systemic factors in the pathophysiology of insulin resistance; 3) the potential roles/interactions of metabolic pathways within mitochondria in the development of insulin resistance; and 4) the adaptive local and systemic responses to the disruption of skeletal muscle mitochondrial and metabolic functioning.

B. A brief overview of mitochondrial regulation and function

The mitochondrial biogenesis regulatory program is defined as the intergenomic control of mitochondrial turnover, functional content, and number required to maintain diverse homeostatic demands across tissue types (reviewed in Refs. 18 and 19). In skeletal muscle, the relative abundance of mitochondria mirrors the skeletal muscle subtype with the most mitochondria in slow-twitch (type I fibers) and less mitochondria in fast-twitch (type II) fibers.

Mitochondrial biogenesis requires concordant and integrated regulation between the mitochondrial and nuclear genomes to generate the multisubunit complexes of the respiratory apparatus and the other proteins necessary for homeostatic mitochondrial functions. In skeletal muscle, these include: oxidative phosphorylation, calcium homeostasis, reactive oxygen species (ROS) biology, apoptosis and thermogenesis via mitochondrial uncoupling. The molecular conductors of intergenomic regulation of mitochondrial biogenesis include the nuclear regulatory proteins—nuclear respiratory factors 1 and 2, the cAMP response element binding protein, transcription factor A of mitochondria, and the mitochondrial transcription factor B isoforms, which together coordinately regulate genes encoding for the electron transfer chain (ETC) complexes (20–23). In turn, the upstream transcriptional coactivator peroxisome proliferator-activated receptor (PPAR) γ coactivator 1 (PGC1) α and β and the PGC1-related coactivator modulate these nuclear regulatory proteins to subsequently activate target genes encoding regulatory enzymes of oxidative phosphorylation (24–26) and genes that modulate antioxidant defenses, including nuclear-encoded mitochondrial antioxidant enzymes and the uncoupling proteins (UCPs) (27).

The biochemical/hormonal modulators of this program are not restricted to, but include ROS, nitric oxide, hypoxia, and thyroid hormones (28–31). Furthermore, substrate availability and insulin itself modulate the metabolic and molecular functioning of mitochondria, and these effects will be expanded upon in *Section III. A* (32–34).

Furthermore, autophagy has recently been shown to be integral to the catabolism of intracellular lipid stores (macrolipophagy) (35). Although not yet explored in skeletal muscle, this novel observation may be important in lipid overload-mediated mitochondrial dysfunction in skeletal muscle.

The contribution of mitochondrial dysfunction to the pathophysiology of insulin resistance and diabetes is not only robustly debated but also may be operational at various levels (12, 36, 37). These will be expanded on in context throughout the review. In brief, these include: 1)

changes at the level of the mitochondrial genome (copy number/mutations), which may be instrumental in substrate utilization and ROS generation; 2) perturbation in the content and activity of the oxidative phosphorylation machinery within mitochondria, which may effect similar metabolic pathways; and 3) modifications in the efficiency of ATP production (*i.e.*, mitochondrial coupling *vs.* the utilization of reducing equivalents in the generation of heat).

II. Skeletal Muscle Mitochondrial Biology in Insulin-Resistant and Diabetic Subjects

One hypothesis with respect to the role of the skeletal muscle mitochondria in the development of T2DM states that primary defects in skeletal muscle biogenesis and/or homeostasis disrupt substrate utilization, which leads to the generation of “toxic” fatty acid intermediates that disrupt insulin signaling (9, 38–40). This mitochondrial deficit hypothesis implicates this organelle as primal in the pathophysiology of insulin resistance and T2DM (41) and is explored in *Section II. A–D*.

A. Insulin-resistant offspring of T2DM parents and prediabetic subjects

To evaluate whether perturbations in skeletal muscle mitochondrial biology are evident in subjects at risk for diabetes, investigators have studied offspring of subjects with T2DM. Lean offspring of diabetic patients have been shown to have a significant reduction in insulin-stimulated nonoxidative glucose metabolism in parallel with elevated intramyocellular lipid (IMCL) content (8). Compared with controls, these subjects also show reduced skeletal muscle ATP synthesis in response to insulin stimulation as measured by magnetic resonance spectroscopy (MRS) saturation transfer. These measurements reflect impaired baseline activity of mitochondrial oxidative phosphorylation (8, 42). Insulin-resistant offspring of diabetic parents also show a reduction in skeletal muscle mitochondrial density and content (43). The lean offspring of diabetic parents in these studies may represent a narrowly selected cohort of individuals that do not necessarily reflect the early pathophysiology of T2DM in this heterozygous disease process. In addition, the measurement of basal oxidative phosphorylation flux does not reflect maximal oxidative capacity of skeletal muscle, and whether baseline perturbations are sufficient to initiate the development of skeletal muscle insulin resistance has been questioned (12, 44).

Similarly, employing mRNA array analysis to investigate skeletal muscle gene expression profiles comparing normal control, offspring of diabetic subjects, and estab-

lished T2DM subjects, the expression of nuclear respiratory factor-1, a key regulator of mitochondrial biogenesis, was reduced in diabetic patients, and the expression of the transcriptional coactivators PGC-1 α and PGC-1 β was down-regulated in nondiabetic individuals with a positive family history for diabetes (7). Remarkably, the top-ranked clusters of genes differentially expressed encode for mitochondrial metabolic processes, including proteins of mitochondrial inner membrane, respiratory chain complexes, ATP synthesis, fatty acid oxidation, tricarboxylic acid (TCA) cycle, and pyruvate kinase (7). These are all down-regulated in diabetic subjects compared with normal controls (7).

Interestingly, in subjects with normal cardiopulmonary function, maximal oxygen consumption (VO_{2max}) correlates directly with skeletal muscle mitochondrial function (45, 46). Studies show that insulin-resistant subjects with a family history of diabetes have diminished VO_{2max} (47) and exercise endurance (48) compared with insulin-sensitive control subjects. These findings may even have clinical utility in predicting risk for diabetes as shown in a prospective study of 8633 nondiabetic men where subjects with the lowest cardiopulmonary fitness had a 3.7-fold increased risk of developing diabetes over the 6 yr study compared with the most fit subjects (49). However, the complexity of dissecting the interaction between skeletal muscle bioenergetics and insulin resistance is epitomized by these data in that these physical fitness findings could similarly support the predisposition to insulin resistance in more sedentary individuals (50).

Interestingly, overweight subjects with maintained insulin sensitivity exhibit increased lipid oxidation and the maintenance of normal myocellular lipid content (51). Conversely, nondiabetic extremely obese subjects often do exhibit insulin resistance (52) that is linked, in part, to the accumulation of fatty acid esters in skeletal muscle (53). Interestingly, here the skeletal muscle capacity to oxidize fatty acid substrates is significantly blunted (52, 54). Whether this reduction in mitochondrial fatty acid β -oxidation (FAO) capacity in these individuals is a primary factor in the development of obesity and insulin resistance has not been resolved. However, at least in the short term, FAO perturbations are not restored by weight reduction. Here, after a greater than 45-kg weight reduction, insulin sensitivity was normalized without restoring defects in skeletal muscle FAO (52). Together, these studies may represent a continuum with initial adaptive mitochondrial reprogramming that becomes overwhelmed by progressive weight gain or may reflect distinct underlying genetic predispositions to fat-induced insulin resistance dependent on the adaptive capacity of mitochondrial function.

In summary, these studies show that in distinct subsets of individuals, *i.e.*, subjects with a strong family history of T2DM or some individuals with severe obesity, perturbations in mitochondrial homeostasis and function are associated with insulin resistance. The relative contribution of these perturbations to the pathophysiological progression to established T2DM has not been definitely characterized.

B. Established type 2 diabetes mellitus

The study of skeletal muscle from diabetic individuals also shows the following: coordinate down-regulation of genes encoding oxidative phosphorylation enzymes (55); lower levels of the β -subunit of ATP synthase steady-state protein levels (56); diminished mitochondrial respiration (57); and evidence of reduced bioenergetic capacity, as illustrated by slower postexercise recovery of skeletal muscle high-energy phosphate stores compared with nondiabetic controls (58). These latter data have been confirmed, comparing overweight diabetic and nondiabetic subjects where *in vivo* skeletal muscle phosphocreatine recovery half-time after exercise is blunted in the diabetic subjects (59, 60). Interestingly, in this cohort of obese subjects, the IMCL content was similar, comparing the diabetic and nondiabetic obese controls (60). Furthermore, in an established diabetic cohort we have shown that the $\text{VO}_{2\text{max}}$ is a strong indicator of the duration of diabetes and directly tracks the underlying skeletal muscle mitochondrial genomic content (61). Additional studies show that subjects with insulin resistance or T2DM have diminished type I oxidative muscle fiber content (62) and an increased skeletal muscle glycolytic to oxidative phosphorylation enzyme ratio (63). The analysis of direct respirometry to measure mitochondrial oxygen consumption in skeletal muscle aligns with this concept in that diabetic individuals have diminished complex I and II substrate-driven oxidative capacity in gastrocnemius muscle compared with controls when normalized to muscle mass; however, when normalized to mitochondrial genomic content, these respirometry differences were abolished (11). This study suggests that the function per mitochondria does not differ in diabetes, but rather that the overall skeletal muscle content of mitochondria is diminished. However, respiration in permeabilized isolated muscle mitochondria from obese diabetic subjects was reduced compared with obese nondiabetic controls when pyruvate/malate was used as substrate (57). Similarly, a study comparing basal and maximal stimulated respiration in isolated mitochondria from muscle samples of age- and BMI-matched diabetic patients, their normoglycemic first-degree relatives, and controls showed impaired mitochondrial respiration in diabetic patients compared with controls, thereby demonstrating reduced intrinsic mitochondrial function in diabetic muscle (59). These impairments seem to be

independent of insulin sensitivity because first-degree relatives of diabetic patients showed a similar tendency toward impaired mitochondrial function *in vivo* and *in vitro* without any changes in glucose disposal.

An important factor in the interpretation of mitochondrial regulatory control and bioenergetic assessment in the skeletal muscle of diabetic subjects is the glycemic control and the levels of circulating insulin. Gene array analysis of skeletal muscle from diabetic individuals subjected to treatment withdrawal (poor glycemic control) and subsequent insulin therapy show down-regulation of genes encoding for mitochondrial oxidative phosphorylation when glycemic control is poor, with augmentation of these transcripts following insulin-mediated improved glycemic control (51, 64). These effects of modulating glucose substrate on the measurement of mitochondrial regulatory programs is similarly suggested where differences in lipid availability and insulin sensitivity can modulate the rate of muscle ATP flux (51, 65).

Another confounding factor that may not have been rigorously excluded in numerous human studies of mitochondrial biology comparing diabetic and nondiabetic subjects is body mass index (BMI). Relative adipose mass may have indirect effects on skeletal muscle mitochondrial function, and in some studies the BMI is significantly higher in T2DM subjects compared with controls (55, 56). This potential confounding factor is, however, not uniform in that in other studies control and T2DM subjects have similar BMI ratios with differences in mitochondrial bioenergetic capacity (57, 58, 60).

Although there are numerous studies supporting the concept that a reduction in mitochondrial oxidative function and capacity are operational in subjects with established T2DM, this paradigm is by no means uncontested. Contrarian results are seen in American subjects of Asian Indian ancestry. Despite being more insulin resistant, these Asian Indian T2DM subjects have a substantially higher skeletal muscle mitochondrial genomic content and basal skeletal muscle oxidative phosphorylation capacity compared with nondiabetic Americans of European descent (66). Moreover, in these Americans of Asian Indian descent, the mitochondrial oxidative phosphorylation capacity exceeds their European counterparts irrespective of the insulin sensitivity status of the Indians (66). It is interesting to note that the higher incidence of insulin resistance found in Asian-Indian men is associated with increased hepatic triglyceride content (67). Whether their skeletal muscle mitochondrial capacity represents an adaptation to compensate for altered hepatic lipid metabolism is an intriguing concept that has not been directly explored.

The variability in mitochondrial content and function in these different studies of subjects with established

T2DM may reflect: 1) the polygenic nature of T2DM with varying susceptibility to similar environmental triggers; 2) distinct stages in the progression of this pathophysiology that may range from initial adaptive to more chronic maladaptive changes; and 3) the skeletal muscle mitochondrial biology in the context of the homeostasis of the other peripheral organs that together coordinate systemic insulin sensitivity.

C. The polycystic ovary syndrome and insulin resistance

The polycystic ovary syndrome (PCOS), which affects 5–10% of reproductive-age women, is an endocrine disorder associated with insulin resistance and increased risk for the development of T2DM (68). These subjects have skeletal muscle insulin resistance (69), and disruption in skeletal muscle mitochondrial biology is beginning to be implicated in this disease process (70). Compared with age-matched nondiabetic subjects, women with the PCOS have reduced skeletal muscle expression of nuclear genes encoded for mitochondrial oxidative metabolism as well as reduced expression of the mitochondrial biogenesis regulatory coactivator PGC-1 α (70). Moreover, the administration of the insulin-sensitizing thiazolidinedione pioglitazone, which has been shown to directly activate the mitochondrial biogenesis program in C2C12 myocytes (71), augments skeletal muscle expression of genes encoding for mitochondrial oxidative phosphorylation proteins in PCOS subjects (72). Despite these promising findings, the augmentation of the mitochondrial biogenesis program and the enhancement of skeletal muscle mitochondrial function has not been a uniform response to thiazolidinedione therapy. These discrepancies are reviewed in *Section V*. Furthermore and similarly to the cohort of insulin-resistant offspring of T2DM parents (7), these PCOS subjects exhibit both down-regulation of the mitochondrial biogenesis regulatory program and insulin resistance. However, the interaction between these two observations in this pathophysiology has not been established.

Moreover, as with offspring of subjects with T2DM, young otherwise healthy women with PCOS show a significant reduction in $\text{VO}_{2\text{max}}$ compared with age- and BMI-matched controls (73). This latter observation has, however, been questioned in a smaller study (74).

D. Genetic linkage between mitochondrial biology and diabetes

A genetic link is evident between mitochondrial DNA mutations and the development of T2DM in the maternally inherited diabetes and deafness (MIDD) syndrome (75). Whether mutations in the mitochondrial genome in skeletal muscle contribute toward insulin resistance in these subjects is an intriguing concept that has not been

established. If this were the case, it would be expected in MIDD individuals with a high level of skeletal muscle mitochondrial genomic heteroplasmy. Furthermore, and although not proven to be causative or directly linked to mitochondrial dysfunction, the disruption in skeletal muscle glucose disposal and insulin resistance presaging pancreatic β -cell dysfunction has been shown in a cohort of subjects with MIDD (76).

A recent case report in a 37-yr-old subject with the mitochondrial MELAS syndrome (myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) similarly shows reduced basal and insulin-stimulated skeletal muscle rates of ATP synthesis and recovery of high-energy phosphate stores after exercise as measured by MRS (77). Although these data do not diminish the contribution of pancreatic β -cell dysfunction as a result of mitochondrial abnormalities in this subject, they do support defective skeletal mitochondrial energetic function and capacity as a component of this subject's impaired insulin sensitivity (77).

A relationship between mitochondrial genome DNA (mtDNA) variants and the risk of T2DM has also recently been suggested (78). Combining genetic and computational analysis, it has been calculated that approximately 20% of subjects with T2DM have mtDNA mutations (79), compared with approximately 6% in the general population (80). Although, the direct investigation of these mutations or variants in human disease requires further investigation, the role of the mtDNA in the risk for diabetes has recently been explored using elegant molecular genetic transfer techniques in rats (81). Here, the mitochondrial genome from Brown Norway rats was used to replace the mitochondrial genome of the spontaneously hypertensive rat, thereby generating a conplastic strain (81). Because this strain is genetically identical to the spontaneously hypertensive rat progenitor strain, with the exception of differences in their mtDNA, different phenotypes can be attributed directly to their mitochondrial genome. Using this approach, natural variants in mitochondrial encoded ETC proteins were identified that diminished glucose disposal in response to glucose loading (81). However, whether these variants directly modulate skeletal muscle mitochondrial biology and insulin resistance has not been directly explored.

III. Mitochondrial Disruption in Association with Risk Factors Predisposing to Insulin Resistance

The opposing hypothesis states that mitochondrial dysfunction is predominantly a consequence of risk factors associated with T2DM, rather than a primary initiator of

this pathophysiology (10). This hypothesis does not preclude a subsequent role of mitochondrial dysfunction as a component of a “vicious cycle” exacerbating the consequences of insulin resistance and T2DM. The data supporting a more distal role of mitochondria in the pathophysiology of insulin resistance is discussed here.

A. Nutrient excess as a trigger of mitochondrial dysfunction in the pathophysiology of diabetes

The prevalence of T2DM is increasing rapidly in modern societies and is thought to result, in large part, from excess food consumption (82). In peripheral tissues, this excess influx of nutrients leads to ectopic lipid deposition, ROS damage, and organ dysfunction and is conceptually defined singly or in combination as glucotoxicity in response to hyperglycemia and/or lipotoxicity in response to excess fat (83).

Glucotoxicity itself has been shown to promote cell damage in cells that have a low capacity to resist glucose uptake, such as endothelial and neuronal cells (84). The cellular pathways evoked to explain glucotoxicity in these cell types result in the oversupply of electron carriers NAD(P)H and FADH₂ to the mitochondria, thereby facilitating reverse electron flow at complex III of the ETC, resulting in increased mitochondrial superoxide production (84, 85). Skeletal muscle attenuates excess glucose uptake via down-regulation of glucose transporters, and the role of glucotoxicity *per se* in the disruption of skeletal muscle mitochondrial function has not been well characterized. However, severe hyperglycemia has recently been suggested to reversibly inhibit mitochondrial respiration in skeletal muscle cultures from human subjects (86).

Severe hyperglycemia in association with hypoinsulinemia in rodents with streptozotocin-induced diabetes has been used to investigate skeletal muscle metabolic gene regulation. In these studies, several genes involved in fatty acid uptake including hormone-sensitive lipase, fatty acid binding protein 4, and carnitine palmitoyltransferase 1 (CPT-1), as well as markers of oxidative stress, such as catalase and superoxide dismutase, were induced 2 or 4 wk after the development of diabetes (87, 88). The up-regulation of antioxidant encoding genes is consistent with the prior finding that these gene regulatory programs are induced as an adaptive response to prevent ROS-mediated cell damage and the progression of diabetes (89). Remarkably, whereas genes involved in oxidative stress, lipid transport, and signal transduction were significantly overrepresented among up-regulated genes in hyperglycemic mouse and rat skeletal muscle, several genes involved in oxidative phosphorylation such as cytochrome oxidase and ATP synthase subunits were significantly down-regulated (87, 90). These findings support an emerging concept of a disassociation between various fat catabolic cycles and the

ETC. This idea is expanded upon in *Section IV.C*, where the effects of excess fat on synchronous mitochondrial metabolic cycles are described (91).

The mechanisms whereby excess fat can promote mitochondrial oxidative damage are being extensively investigated. What is clear is that excess fat in the diet results in extraadipocyte fat deposition, including accumulation of IMCLs in skeletal muscle. This process is evident in lean healthy individuals fed a short-term high-fat diet (92). Intracellular fatty acids form lipid peroxides in the presence of ROS, and these in turn have been shown to oxidatively damage mitochondria (93, 94). It is mechanistically feasible that IMCLs may contribute to perturbations in mitochondrial functioning in diabetes with specific relevance in obesity and aging (95). However, IMCL accumulation *per se* is not sufficient to evoke mitochondrial damage because their accumulation is evident in endurance athletes who concurrently have robust oxidative-phosphorylation capacity (96, 97) and are generally insulin sensitive (98). A distinction between these groups is that increased IMCL content is associated with lipid peroxidation in obese subjects, in contrast to skeletal muscle IMCL accumulation without elevated lipid peroxidation in endurance athletes (99). The mechanism explaining this paradox is hypothesized to be that fat-induced obesity is associated with an inability to catabolize the excess skeletal muscle IMCL and therefore predispose to lipid peroxide formation (100). The investigation of this paradox is inferred in studies associating skeletal muscle IMCL accumulation with genes encoding fat metabolism programs in response to endurance training *vs.* high-fat feeding (92, 101). These gene expression profile studies show an induction of genes encoding for FAO in endurance training (101) and an up-regulation of genes encoding for fatty acid storage proteins in sedentary subjects fed a high-fat diet (92). It has also been shown that high endurance athletes uncouple oxidative phosphorylation in skeletal muscle, which would support enhanced FAO and diminished oxidative stress (96, 102). Uncoupling of oxidative phosphorylation in this context is where “exercise-mediated regulatory events” increase proton leak across the inner mitochondrial membrane without generating additional ATP. This reduces the electrochemical gradient across the mitochondrial inner membrane, which results in increased respiration, in an attempt to maintain the membrane potential. A consequence of this could be increased use of substrate, *e.g.*, fatty acids (futile cycling) and a reduction in ROS generation due to the modest reduction in the inner mitochondrial membrane potential (103).

The direct effect of fatty acids on skeletal muscle mitochondrial ATP generating capacity has also been explored in mitochondria isolated from skeletal muscle bi-

opsies from normal healthy volunteers. In an albeit *ex vivo* study, the fatty acid metabolite palmitoyl carnitine itself suppresses mitochondrial ATP production (104). Additionally, primary skeletal muscle cultures from T2DM subjects show decreased insulin-activated citrate synthase activity *vs.* controls (105). In the control subjects, this activation of citrate synthase is abolished by the presence of palmitate (105). In a separate study, volunteers fed an isoenergetic high-fat diet for 3 d showed down-regulation of genes encoding for the mitochondrial biogenesis regulators PGC-1 α and - β and for genes encoding mitochondrial oxidative phosphorylation enzymes (33). Similarly, in healthy volunteers, the acute infusion of lipids promotes insulin resistance with attenuation of glucose uptake/phosphorylation in concert with reduced muscle glycogen synthesis, glucose oxidation, and the ATP synthase flux as measured by MRS (106, 107). Furthermore, the accumulation of IMCL content and toxic intermediates inhibits insulin signaling via the activation of protein kinases (38–40, 43). This inhibition of myocyte insulin signaling may in turn attenuate the mitochondrial biogenesis regulatory program and mitochondrial bioenergetics (71). Together, these studies suggest that multiple mechanisms may be operational whereby increased circulating free fatty acids and IMCL deposition directly modulate mitochondrial regulatory and respiratory functions.

The temporal effects of high dietary fat consumption on skeletal muscle mitochondrial energetics and on glucose tolerance have been investigated in rats (108). Here, after 24 h of high-fat feeding, basal skeletal muscle mitochondrial ATP synthesis rates, as measured by ^{31}P MRS saturation transfer, were markedly depressed. Continued high-fat feeding then resulted in a restoration of the rate of ATP synthesis and a cessation in IMCL accumulation over the next few weeks. However, from approximately 4 wk onward, this dietary intervention results in progressive deterioration in ATP synthesis and a concordant increase in IMCL accumulation. Interestingly, switching back to a chow diet at the 4-wk time-point reverses the mitochondrial bioenergetic defects, IMCL accumulation, and glucose tolerance. This study supports the concept of parallel deterioration in skeletal muscle mitochondrial function with enhanced IMCL content and reduced glucose tolerance. Moreover, this study suggests a degree of plasticity in the system that enables reversibility, at least within an acute time span (108). This reversibility in mitochondrial function may additionally require numerous interventions, as illustrated in a recent study where dietary weight loss alone in human subjects did not restore skeletal muscle respiratory capacity, but the combination of dietary restriction and exercise does have salutary effects on respiratory capacity (109). A potential reason why the com-

bination of exercise and weight loss can be synergistic in improving skeletal muscle mitochondrial functioning may include the finding that dietary restriction alone preferentially diminishes adipose tissue and hepatic lipid stores with a relative paucity of effect on skeletal muscle (110). The concept that elevated lipids can alter the molecular control of mitochondrial biogenesis has also been shown in humans, where muscle biopsies were performed before and either 6 or 48 h into a lipid infusion (111, 112). Here, the acute increase in circulating free fatty acids resulted in down-regulation of transcripts encoding for PGC-1 α and PGC-1 β and for mitochondrial and nuclear-encoded mitochondrial genes. In contrast, a 5-d high-fat diet in healthy volunteers failed to show any effect on mitochondrial function or expression of oxidative phosphorylation gene expression (113). The modulation of mitochondrial regulatory programs and respiratory function in response to high-fat feeding is therefore not unequivocal and may reflect in part the overall dietary content, genetic background, age, and degree of mitochondrial plasticity. This discrepancy is illustrated on the one hand by the significant down-regulation of genes encoding for oxidative phosphorylation proteins in skeletal muscle in association with a modest reduction in mitochondrial enzyme activities in high-fat fed c57BL mice (33). Conversely, fat-fed insulin-resistant C57BL/6J mice, Wistar rats, obese Zucker rats, and db/db mice all show increased fatty acid oxidation and up-regulation of PGC-1 α and oxidative phosphorylation genes in skeletal muscle (32, 114, 115). Similarly, raising free fatty acid levels in rats by a high-fat diet and concomitant heparin injections for 3 wk induced mitochondrial biogenesis that was reflected by an increase in mitochondrial DNA copy number, enzymes of the respiratory chain, and fatty acid oxidation pathway in skeletal muscle (116). Interestingly, an 8-wk high-fat diet in Wistar rats did not change skeletal muscle mitochondrial respiration, ETC protein content, or citrate synthase activity despite significant intramyocellular triglyceride accumulation, but it led to a reduction of mitochondrial copy number and up-regulation of PGC-1 α protein level (117). The thought process here is that these changes may be adaptive, at least in the short term, to compensate for the increased availability of lipids in these models (13, 32) and may reflect a response to the deficit in leptin signaling in the Zucker rats and the db/db mice. The effect of fatty acids on mitochondrial biology and insulin signaling is additionally dependent, in part, on fatty acid saturation and chain length. Experimental data using a reductionist approach demonstrate the presence of polyunsaturated fatty acid channel lipids toward inert triglyceride storage and complete FAO and, conversely, that saturated fats preferentially generate “toxic” diacylglycerol (DAG) and

promote insulin resistance (118–120). Furthermore, long-chain saturated fatty acids, but not medium-chain saturated fats, promote insulin desensitization in skeletal muscle cells (121). In addition, the short-chain fatty acid butyrate was recently demonstrated to prevent the development of diet-induced insulin resistance, possibly via induction of mitochondrial biogenesis (122). In summary, the role of fat in disrupting skeletal muscle mitochondrial regulation and function appears to depend on: 1) an individual's underlying genetic capacity to up-regulate FAO to compensate for excess fat exposure; (2) the chronicity and the composition of the fat exposure; and 3) the activity status of the individual subject spanning from a sedentary lifestyle to one with high-intensity training.

The temporal pathophysiology of combined glucose and lipotoxicity-mediated mitochondrial dysfunction was recently delineated in mice exposed to a high-fat/high-sucrose diet (89). Mice showed skeletal muscle insulin resistance and evidence of oxidative stress 1 month into the diet, but only disrupted mitochondrial biogenesis, loss of mitochondrial structural integrity, and respiratory functioning after more prolonged high-fat/high-sucrose dietary exposure (89). Interestingly, antioxidant therapy administration to streptozotocin-induced hyperglycemic mice shows diminished skeletal muscle ROS generation and the restoration of mitochondrial integrity (89). In parallel, excess palmitate or glucose administration to muscle cells induces ROS generation and disruption of the mitochondrial biogenesis regulatory program and metabolic functioning (89). This phenotype is similarly evoked in muscle cells exposed to the combination of excess glucose and insulin (71).

Overnutrition can also include excess protein intake and excess circulating amino acids (hyperaminoacidemia). Amino acid excess is clearly linked to the development of insulin resistance (123); however, whether this modulates skeletal muscle mitochondrial biology appears not to have been explored to date.

The role of modulating dietary intake in reversing insulin resistance in skeletal muscle has been alluded to with respect to obesity and excess IMCL. Additionally, one dietary intervention that has been shown to up-regulate the skeletal muscle mitochondrial biogenesis regulatory program is caloric restriction (124, 125). The caloric intake from this diet is deficient of caloric requirements, resulting in low-level biological stress (126). This stress is termed hormesis, and stress-activated programs responsive to this up-regulate the mitochondrial biogenesis regulatory program in skeletal muscle (30, 109). Here, the mitochondrial biogenesis program is orchestrated in part by the up-regulation of nutrient-sensing deacetylase SIRT1, which in turn activates skeletal muscle PGC-1 α

(127). Caloric restriction has been shown to improve insulin sensitivity in human subjects (128), although here the direct effects on skeletal muscle mitochondrial metabolism do not appear to have been directly investigated. Furthermore, salutary effects of caloric restriction on adipose tissue, the liver, and pancreas are probably also operational in improving insulin sensitivity.

B. Physical inactivity orchestrated diminution in skeletal muscle oxidative metabolic capacity

Physical inactivity is also a well-established risk factor for the development of insulin resistance and T2DM (50, 129, 130). Physical inactivity is similarly associated with a reduced VO_{2max} (131, 132), diminished skeletal muscle oxidative enzymes (132, 133), and decreased skeletal muscle lipoprotein lipase activities (134, 135). Together, these physical inactivity-mediated reductions in oxidative metabolic capacity may directly limit skeletal muscle metabolism of circulating nutrients. This in turn is a feasible mechanism whereby primary physical inactivity could precede mitochondrial down-regulation in the promotion of insulin resistance. This hypothesis is further supported where increased physical activity restores insulin sensitivity, enhances skeletal muscle mitochondrial bioenergetic capacity, and concomitantly up-regulates the mitochondrial biogenesis regulatory program (136–140). The augmentation of VO_{2max} , skeletal muscle mitochondrial content, and induction of oxidative phosphorylation enzymes is also evident when modest exercise is coupled to dietary restriction and weight loss (141). Increased physical activity has also been shown to delay the onset and/or progression of insulin resistance in large prospective clinical studies (142, 143). These observations are not universal in that skeletal muscle ATP recovery in response to skeletal muscle exertion has been shown to be similar in well-controlled diabetic subjects compared with age- and BMI-matched control subjects that undergo similar and modest daily activity (144). Interestingly, in these same individuals, a modest increase in exercise resulted in improved lipid oxidation without enhancement of skeletal muscle maximal ATP generation as measured by MRS (144).

An interesting study was performed in rats separated by low or high intrinsic aerobic capacity and then bred over 11 generations (145). Rats with low aerobic capacity have diminished skeletal muscle expression of genes encoding for mitochondrial biogenesis regulatory proteins and oxidative phosphorylation enzymes in parallel with features of insulin resistance including hyperglycemia, hyperinsulinemia, and hypertriglyceridemia (145). Moreover, in rats fed a high-fat diet, exercise has been shown to reverse skeletal muscle insulin resistance (146). In mice, endurance training also activates the skeletal muscle mitochondrial biogenesis program, enhances mitochondrial oxida-

tive capacity, and improves glucose tolerance (136). The duration and intensity of exercise may also play a role in modeling glucose tolerance and mitochondrial biology. This is illustrated where high-intensity interval training and continuous moderate-intensity exercise have the same ameliorative effects on weight loss and fat mass in rats. However, the high-intensity training showed greater benefit with respect to improved glucose tolerance and in the induction of skeletal muscle mitochondrial biogenesis (147).

C. Senescence, insulin resistance, and muscle mitochondrial function

Skeletal muscle mass and strength diminish at approximately 5% per decade, starting in the fourth decade of life (140, 148). The loss of muscle mass is termed sarcopenia and is associated with a disproportionate reduction in type II *vs.* type I fibers (148). In parallel, the majority of evidence supports a parallel down-regulation in skeletal muscle mitochondrial oxidative capacity and function (reviewed in Ref. 149). In the context of the data presented to date, these age-associated skeletal muscle changes should associate with progressive insulin resistance. This is indeed supported by epidemiological evidence showing diminished glucose tolerance with aging (150–152) and impaired aging-associated skeletal muscle insulin-stimulated glucose uptake (153). In addition, the study of age-associated mitochondrial biology and glucose tolerance shows a temporal decline in skeletal muscle mitochondrial DNA content and ATP production in parallel with increasing mitochondrial oxidative damage and glucose intolerance (154). These age-associated changes are probably dependent in large part on the level of physical activity because the reduced skeletal muscle mitochondrial genomic copy number and ATP-generating capacity is clearly demonstrated in sedentary elderly individuals compared with young sedentary controls and is less pronounced in trained elderly individuals (155). Finally, comparing lean healthy young and elderly individuals, the elderly subjects are more insulin-resistant and show diminished skeletal muscle insulin-stimulated glucose uptake and mitochondrial oxidative phosphorylation capacity (95). An intriguing and alternative mechanism promoting the rise in skeletal muscle insulin resistance with aging was noted in a rat study in which older rats showed increased mitochondrial efficiency and thermodynamic coupling with a diminution in fatty acid-induced proton leak (156). This in turn is proposed to lead to a decrease in fat utilization, increased IMCL accumulation, and lipotoxicity. Interestingly in this regard, in insulin-resistant elderly human subjects with similar fat mass and BMI to younger subjects, skeletal muscle IMCL content is increased (95). An additional factor that may be opera-

tional in the down-regulation of skeletal muscle adaptive programs with aging is that the activation of the nutrient-sensing mediator of mitochondrial biogenesis, *i.e.*, AMP kinase (AMPK), via either pharmacological treatment or exercise, is blunted in association with impaired mitochondrial biogenesis regulatory programming in old *vs.* young rats (157).

Figure 1 shows a schematic representation of the pathophysiology associated with primary skeletal muscle mitochondrial deficits evident before the onset of T2DM and secondary deficits in response to environmental cues. In light of gene polymorphism studies and genome-wide association studies implicating proteins modulating mitochondrial function as diabetogenic candidates (158–160), it is intriguing to postulate whether individual subjects have distinct genetic susceptibility to environmental cues in the development of insulin resistance.

IV. Molecular Manipulation of Mitochondrial Metabolism and Effects on Skeletal Muscle and Systemic Insulin Resistance

The studies described to date suggest that the role of the mitochondria in the development of insulin resistance can be primary or secondary, depending on the subject's underlying genetic predisposition and environmental factors. Because the integration between cellular and mitochondrial metabolism is complex, the review of both reductionist and *in vivo* animal studies can be used to further dissect out the role of skeletal muscle mitochondria in the pathophysiology of skeletal muscle and systemic insulin sensitivity/resistance. The ability to genetically modulate these programs in mice and, more specifically, in a skeletal muscle-restricted manner, creates a testable biological system to either validate or question the hypotheses generated by clinical studies. It is interesting to note that numerous genetic modulations of mitochondrial metabolic processes have divergent effects when comparing skeletal muscle to systemic insulin sensitivity. To contextualize these, the direct skeletal muscle effects will be initially described and then the systemic effects of these same skeletal muscle-specific perturbations will be examined. The composite of these effects also illustrates the complexity of the interaction between multiple organs in the pathophysiology of insulin resistance.

A. Genetic disruption of the regulatory programs controlling skeletal muscle mitochondrial homeostasis and effects on insulin resistance

Mitochondrial homeostasis is controlled via complex intergenomic regulatory programs as described in *Section I*. Studies in skeletal muscle cell lines show that transient

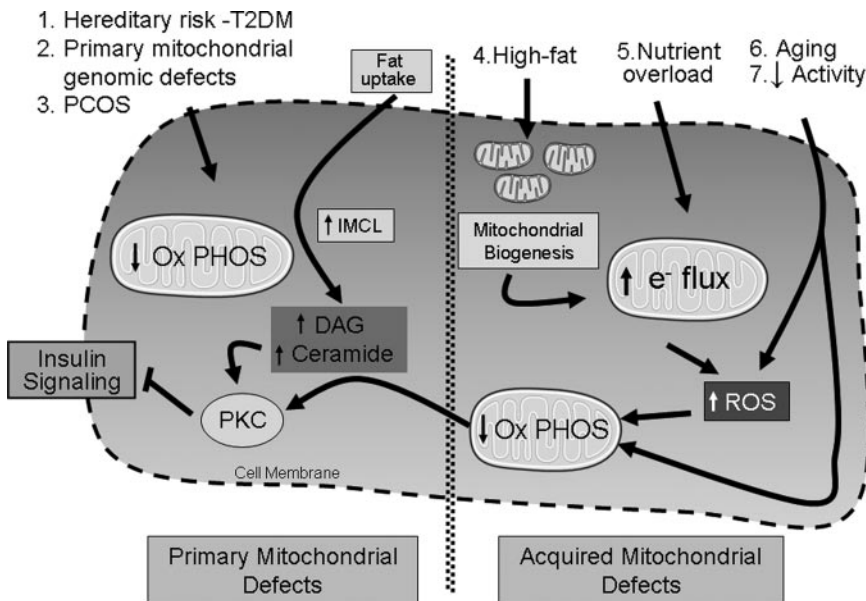


FIG. 1. Mitochondrial role in the development of insulin resistance and T2DM. This schematic shows primary mitochondrial defects to the left of the hatched line in the center of the cell and the development of mitochondrial deficits in response to environmental cues and aging to the right of the hatched line. In primary disruption in the mitochondrial metabolic capacity, FAO is diminished, fat intermediates accumulate and DAG, which appears to be the primary intermediate that then activates protein kinase C isoforms. These in turn phosphorylate and inactivate numerous kinase substrates in the insulin signaling pathway. The reduced insulin sensitivity exacerbates the metabolic perturbations by reducing glucose uptake and possibly by further down-regulation of the mitochondrial biogenesis program. The etiologies of primary mitochondrial defects are labeled 1–3. The etiologies of secondary disruption of mitochondrial dysfunction are labeled 4–7. High-fat diet can promote mitochondrial biogenesis; alternatively nutrient overload, which may include both glucose and fats, enhances both lipid intermediates that facilitate oxidative damage and impair insulin signaling. Furthermore, the nutrient overload presents excess reducing equivalents to the ETC that can result in increased ROS generation. The oxidative damage, in turn, disrupts the mitochondrial oxidative capacity, which then recapitulates the phenotype of primary mitochondrial deficits promoting insulin resistance. Ox PHOS, Mitochondrial oxidative phosphorylation.

overexpression of the “master regulator” of mitochondrial biogenesis, PGC-1 α (26), increases mitochondrial content and respiration (27, 71) in parallel with enhancing glucose uptake and insulin sensitivity (71, 161). The direct transient electrotransfection of PGC-1 α into rat skeletal muscle similarly improves mitochondrial function and insulin sensitivity (162). However, chronic and/or robust restricted overexpression of this transcriptional coactivator in skeletal muscle of transgenic mice has a somewhat complex phenotype including systemic insulin resistance (163). As one would postulate, these mice do have increased expression of genes controlling mitochondrial oxidative metabolism but also show decreased glucose transporter isoform 4 (GLUT4) expression and reduced glucose uptake (163). A second skeletal muscle PGC-1 α transgenic mouse line confirms these effects in parallel with evidence of increased IMCL accumulation that appears to result from increased fatty acid uptake in excess of the capacity for FAO (164). Given this phenotype, mice with skeletal muscle-restricted PGC-1 α depletion were generated (165, 166). These mice show down-regulation of the mitochon-

drial biogenesis transcriptional machinery with parallel down-regulation of TCA cycle and ETC enzyme activities (165) and a shift from oxidative to glycolytic muscle fibers (165, 166). In addition, these mice are resistant to high-fat diet-induced weight gain, and they generate more heat and have higher oxygen consumption—all factors compatible with diminished mitochondrial efficiency (165). The sum of these changes results in the preservation of systemic insulin sensitivity and again shows that it is the summation of adaptive and maladaptive changes after robust modulation of mitochondrial oxidative phosphorylation that determines the local and systemic insulin responsiveness.

An intriguing inflammatory program is also induced in these mice, where IL-6 production is up-regulated in PGC-1 α -depleted skeletal muscle. Interestingly, the direct administration of IL-6 to pancreatic islet cells suppresses glucose-stimulated insulin secretion (165). Similarly in diabetic subjects with known down-regulation of genes encoding for mitochondrial oxidative proteins (55), the transcript levels of IL-6 and the cytokine TNF α are inversely correlated to the levels of

PGC-1 α (165). Together, these findings support an intriguing concept that diabetes-associated disruption in skeletal muscle metabolism may provoke skeletal muscle cytokine-mediated cross-talk with the pancreas as an adaptive process to retard the development of insulin resistance. Skeletal muscle production of cytokines (myokines) to mediate systemic effects is discussed in greater depth in the section reviewing modulation of glucose metabolism in skeletal muscle (Section IV. E).

This insulin responsiveness to chronic skeletal muscle manipulation of PGC-1 α may, in part, reflect the broad array of transcriptional targets under the control of PGC-1 α (26) and additionally reflect the plasticity of tissues to adapt to energy pathway modulations by the utilization of alternate substrates. To dissect this out further, genetic manipulation of distinct downstream transcriptional targets of PGC-1 α has been investigated. The restricted skeletal muscle deletion of the mitochondrial transcriptional activator transcription factor A of mitochondria shows a phenotype similar to PGC-1 α knock-

down. These mice have progressive deterioration of skeletal muscle respiratory chain function in parallel with increasing skeletal muscle glucose uptake and glucose tolerance (167). Another target of PGC-1 α that has been specifically characterized for insulin resistance in skeletal muscle is the overexpression of PPAR α (168). Similarly to the PGC-1 α transgenic mice, the PPAR α transgenics have increased FAO, with repression of GLUT4 and diminished insulin-sensitive skeletal muscle glucose uptake (168). The insulin resistance that develops in these mice is associated with diminution in AMPK activity.

Taken together, these genetic studies directly modulating the transcriptional control of mitochondrial biogenesis and fatty acid oxidation show that chronic manipulation of these programs initiates adaptive and maladaptive responses that, in turn, modulate glucose uptake with a concordant effect on systemic insulin sensitivity.

The complexity of this paradigm is further confirmed in mice with skeletal muscle-restricted ablation of the mitochondrial flavoprotein apoptosis-inducing factor (AIF). This protein functions to maintain the integrity of the mitochondrial respiratory apparatus (169), and its genetic depletion results in a progressive disruption of mitochondrial respiratory function with the longer term disruption in organ integrity (170, 171). Interrogation of insulin sensitivity was therefore investigated relatively early in this temporal progression to mitochondrial dysfunction in skeletal muscle-restricted AIF-ablated mice (172). These mice show significant albeit modest reductions in skeletal muscle mitochondrial respiration, in ETC gene transcripts, and ATP levels. In parallel activation of the metabolic sensor, AMPK and up-regulation of glucose metabolism were evident in association with improved systemic glucose tolerance and insulin sensitivity. Moreover, these mice had no evidence of altered global nutrient utilization (similar respiratory quotient), activity, and energy expenditure (172). In isolation, this metabolic phenotype is inconsistent with a primary contribution of skeletal muscle mitochondrial disruption in the development of insulin resistance. However, the adaptations operational in these mice include enhanced glucose utilization and reduced fat mass (172), features that are not present in the majority of human subjects with T2DM. Another interesting observation in this study is that AIF depletion does not result in the generation of excess ROS. This additionally distinguishes this mitochondrial disruption genetic model from that associated with insulin resistance and diabetes (84, 89).

Collectively, the studies to date targeting nuclear regulatory control of mitochondrial metabolic functioning lend caution to the capacity to directly modulate these regulatory programs in skeletal muscle to alleviate insulin

resistance. However, this paradigm is countered by the effects of skeletal muscle-restricted modulation of PPAR δ (also termed PPAR β). Although expressed ubiquitously, this PPAR isoform is most abundant in skeletal muscle (173) and promotes FAO and skeletal muscle fiber type switching to enhance type I oxidative fiber development (174, 175). Skeletal muscle-restricted genetic ablation of PPAR δ exhibits a functional switch in skeletal muscle fiber type toward lower oxidative capacity with age-associated increase in systemic insulin resistance and glucose intolerance in association with increasing fat mass (176). Interestingly, the absence of PPAR δ in skeletal muscle results in down-regulation of genes encoding for the mitochondrial biogenesis regulatory proteins and of components of the ETC (176). The central role of PPAR δ in skeletal muscle oxidative metabolism and in the control of insulin sensitivity is confirmed after the generation of a skeletal muscle-specific transgenic mouse harboring the PPAR δ transgene (177). These mice show enhanced type I fibers in association with an up-regulation of the mitochondrial biogenesis regulatory program, oxygen consumption, and a markedly enhanced capacity for endurance exercise. Moreover, even in the absence of exercise, these mice are resistant to high-fat-induced weight gain and significantly more glucose tolerant compared with wild-type control mice (177).

B. Skeletal muscle fatty acid uptake and storage as putative mediators of insulin signaling

Excess lipid uptake and impaired disposal may account, in part, for skeletal muscle lipid accumulation and the development of insulin resistance and diabetes. The molecular regulatory programs orchestrating the partitioning of fat into energy production, synthetic pathways, and storage in skeletal muscle are actively being investigated and will be discussed in the context of the modulation of insulin resistance.

The uptake of skeletal muscle fatty acids can be modulated by the overexpression or disruption of transport mechanisms. In skeletal muscle-restricted lipoprotein lipase knockout mice, fat uptake is diminished in parallel with enhanced skeletal muscle insulin sensitivity (178). Interestingly, the contribution of multiple organs in the pathophysiology of insulin resistance is reiterated here in that the diminution in skeletal muscle fat uptake associates with increased liver, heart, and adipose tissue fat uptake, with a resultant exacerbation of overall systemic insulin resistance (178).

The major transport proteins facilitating skeletal muscle fat uptake include the insulin-responsive CD36 and fatty acid transport proteins (179, 180). To our knowledge, skeletal muscle-specific ablation of fatty acid transporters or skeletal muscle-restricted transgenic mice en-

coding for these proteins have not been generated. The whole body knockout of CD36 results in diminished fat uptake in skeletal muscle (181). At baseline, these mice show lower fasting glucose and insulin levels compared with controls and have enhanced glucose tolerance with lower skeletal muscle glycogen and triglyceride content (182). These mice illustrate the complex interaction between molecular defects and diet in that the CD36 null mice had similar susceptibility to high-fat-induced insulin resistance but greater insulin resistance to high fructose compared with control mice (182).

The mechanism whereby accumulation of fatty intermediates in promoting insulin resistance is due to the accumulation of “toxic” lipid intermediates including DAG and ceramide is shown in Fig. 1. The elevated DAG activates protein kinase C ϵ , a serine/threonine kinase that then binds to the insulin receptor and inhibits its tyrosine kinase activity (183). Normally, the neutralization of DAG is orchestrated by the microsomal enzyme acyl-coenzyme A (CoA):DAG acyltransferase (DGAT), which catalyzes the final step in triglyceride synthesis by linking DAG to long-chain acyl-CoA. It is proposed that the exercise-induced “athletes paradox” of increased IMCL stores and improved insulin sensitivity results from the up-regulation of muscle-enriched DGAT1 isoform that converts DAG intermediate into neutral stored triglyceride (184). This hypothesis has been validated in transgenic mice where skeletal muscle-specific induction of DGAT1 increased triglyceride stores and protected these mice from high-fat diet-induced insulin resistance (185). In parallel, using primary myocytes, the knockdown of DGAT1 exacerbated fat-induced insulin resistance, and its overexpression ameliorated this detrimental effect (185). The complex interplay between the multiple peripheral tissues and pathways in regulating whole body insulin sensitivity is again illustrated in that the whole body knockout of DGAT1 resulted in a paradoxical improvement in insulin sensitivity, which is thought to result from these mice having increased energy expenditure in association with the up-regulation of genes encoding for UCPs (186, 187). The role of UCPs is addressed separately in *Section IV. D.*

C. Efficiency of fatty acid oxidation and insulin signaling

As described, after import into myocytes, fatty acids are esterified into fatty acyl-CoA and partitioned into storage, synthetic, or catabolic pathways. To generate energy, fatty acyl-CoA enters the mitochondria. The mitochondria exquisitely coordinate FAO, the TCA cycle, and the ETC to optimize ATP production (188, 189). The synchronization of these fatty acid catabolic pathways in response to high fat has recently been questioned in a series of elegant studies. In skeletal muscle mitochondria isolated from rats exposed to high fat, different metabolic efficiency is noted in skeletal

muscle fiber types with complete FAO in type I oxidative fibers and incomplete FAO in mitochondria isolated from fast-twitch type II fibers (138). Interestingly, it was also shown that high-fat feeding promotes incomplete FAO with a diminution in the generation of CO₂ as the end-product. Conversely, exercise augments complete FAO to generate appropriate amounts of CO₂. In myocyte cultures, these investigators go on to show that the overexpression of PGC-1 α enhances complete FAO (138). In a further study using targeted metabolomic screening to identify these metabolic intermediates, high-fat diet increased the rate of FAO but depleted TCA organic acid intermediates, thereby rendering the TCA cycle limited in the completion of oxidative phosphorylation. This concept is linked to insulin resistance in cultured myocytes, where fat-induced insulin resistance is prevented by the inhibition of fatty acid mitochondrial uptake by the introduction of the CPT-1 inhibitor etomoxir (190). A caveat of this observation is that the reduction in FAO by etomoxir may activate AMPK, which in its own right could enhance insulin sensitivity. Collectively, these studies show that the combination of skeletal muscle fat overload coupled to a relatively diminished mitochondrial oxidative capacity due to limitations in TCA cycle metabolic intermediates appears to coalesce to promote insulin resistance.

Of note, the exact interplay between accumulation of lipid intermediates in the cytosol and the rate and completeness of FAO requires additional investigation. The balance between these programs is further illustrated where the import of fatty acids into the mitochondria via the overexpression of CPT-1 not only increased FAO but also prevented fatty acid-induced insulin resistance in myotubes and after electrotransfer of CPT-1 into rat skeletal muscle *in vivo* (191, 192).

In stark contrast to this, the systemic knockout of malonyl-CoA decarboxylase, the enzyme that degrades the natural CPT-1 inhibitor malonyl-CoA, prevents skeletal muscle mitochondrial fat uptake and FAO in parallel with the accumulation of IMCL (190). In this mouse model in response to a high-fat diet, the knockout mice have lower systemic fasting blood glucose levels and are more glucose and insulin tolerant than the wild-type controls (190). Interestingly, this phenotype may be influenced, in part, by the fact that the malonyl-CoA decarboxylase $-/-$ mice show an enhanced capacity for glucose oxidation, at least in the heart under stress conditions (193). Whether this is operational in other tissues such as the liver and skeletal muscle appears not to have been directly studied at this time.

D. Uncoupling of oxidative phosphorylation

Excess intramyocellular fat appears instrumental in the development of skeletal muscle insulin resistance and in

the disruption of mitochondrial function. A potential strategy to dissipate this program could be via the promotion of “inefficient” oxidative phosphorylation. This counterintuitive process could deplete lipid stores via the generation of heat, and if the change in efficiency is modest, lipid depletion may be achievable without a significant reduction in ATP generation (194, 195). This cellular process results from the leak of protons across the inner mitochondrial membrane into the matrix and is termed uncoupled mitochondrial respiration. The inner mitochondrial membrane UCPs are thought to be major facilitators of uncoupled respiration and may modulate the pathophysiology of diabetes (103, 196, 197).

UCP3 is the skeletal muscle-enriched isoform (198, 199), and the skeletal muscle UCP3 protein levels are down-regulated in T2DM subjects (200) and conversely increased in response to exercise training (201). Furthermore, insulin-sensitizing exercise and lifestyle modifications restore skeletal muscle UCP3 levels in patients with T2DM, supporting a link between insulin sensitivity and regulation of this mitochondrial inner membrane protein (202). Additionally, endurance-trained individuals exhibit increased skeletal muscle uncoupled respiration (96). The role of UCP3 in skeletal muscle biology has not been definitively established, although data show that these proteins modestly uncouple oxidative phosphorylation in parallel with preferential oxidation of fatty acids (203), diminish skeletal muscle ROS generation (102, 204), and are required in pathological thermogenesis (204). To test whether the induction of UCP3 could modulate insulin sensitivity, a skeletal muscle transgenic mouse line harboring the human UCP3 gene was generated (205). These mice are hyperphagic and lean with diminished adipose tissue mass, a phenotype consistent with uncoupled mitochondrial oxidative phosphorylation. Further investigation of these mice shows that they are protected against fat-induced insulin resistance and have lower intracellular DAG levels with improved insulin-responsive signal transduction compared with controls (206). An additional signaling network that probably contributes to the insulin-sensitizing effects of UCP3 overexpression is the resultant activation of AMPK shown to be present after up-regulation of UCP3 in skeletal muscle (207). The long-term response to a high-fat diet has recently been explored in these mice compared with wild-type control and to whole body UCP3 knockout mice (208). The skeletal muscle UCP3 transgenic mice are relatively resistant to weight gain in response to long-term high-fat exposure, and the UCP3^{−/−} mice gain more weight compared with both controls and the transgenic mice. Interestingly, the enhanced glucose tolerance noted in transgenic mice in response to short-term high-fat exposure disappeared after

exposure to this diet for 4 months (208). These data suggest that this adaptive mechanism can be overwhelmed by persistent fat loading. Mice with muscle-specific depletion of UCP3 have to our knowledge not been generated, and the whole body UCP3^{−/−} mice show a paradoxical improvement in glucose tolerance similar to that achieved with the skeletal muscle UCP3 transgene (208). The possible adaptive mechanisms operational in these UCP3^{−/−} mice have not been extensively explored.

Similarly, the ectopic overexpression of the dominant brown adipose tissue thermoregulatory UCP1 in skeletal muscle also evokes resistance to high-fat diet-induced insulin resistance in a dose-dependent manner, resulting in increased sensitivity with higher expression levels (22). In a second study, this group showed that robust overexpression of UCP1 in skeletal muscle perturbs skeletal muscle mitochondrial architecture and content, promotes IMCL accumulation, and increases the activities of AMPK and hexokinase with the concurrent up-regulation of GLUT4 (209).

In addition, when skeletal muscle UCP1 transgenic mice are crossed with genetically obese mice, diabetes and hypertension are reversed (210). Together, these UCP studies further support the concept that partial uncoupling of respiration in skeletal muscle may be useful to improve lipid-induced insulin resistance and even possibly the longer term sequelae of diabetes and vascular pathology. A putative mechanism for this ameliorative effect may stem in part from the activation of AMPK and the enhancement of glucose uptake present in these transgenic mice (211). It has not been established whether additional mechanisms, including altered mitochondrial efficiency and/or attenuation of ROS generation, are also operational.

E. Glucose uptake and utilization defects in disruption of mitochondrial function

As illustrated by prior mouse studies, a systemic adaptive response to disrupted fat metabolism is associated with activation of AMPK, accompanied by increased glucose uptake and metabolism with improved insulin sensitivity. These data suggest interdependence between glucose and fat metabolism in the development of insulin resistance and/or diabetes. Because genetic studies have also been undertaken to modulate skeletal muscle glucose biology, these are briefly explored to focus on the consequences of these perturbations on mitochondrial metabolism and insulin resistance.

GLUT4, the insulin-sensitive glucose transporter, is expressed in skeletal muscle (212), and the translocation of GLUT4 to the skeletal myocyte membrane, which facilitates glucose uptake, is impaired in insulin resistance and diabetes (213, 214). Whole body genetic depletion of

GLUT4 impairs skeletal muscle insulin sensitivity and glucose uptake (215, 216) without altering systemic glucose tolerance (215). Adaptations associated with GLUT4 depletion include improved hepatic triglyceride clearance, increased skeletal muscle mitochondrial mass, and augmented skeletal muscle fat uptake and oxidation (217). In contrast, GLUT4 haploinsufficient mice (GLUT4^{+/-}) show systemic insulin resistance and diabetes (218). Crossing these GLUT4 ^{+/-} mice with a fast-twitch skeletal muscle-restricted GLUT4 transgene prevents insulin resistance and diabetic complications (219). Taken together, these studies show that the direct inhibition of glucose uptake as an initial insult promotes skeletal muscle mitochondrial biogenesis, fat uptake, and oxidation (217) and that restricted rescue of glucose uptake in skeletal muscle can ameliorate the diabetic phenotype of GLUT4 knockout mice.

Augmenting skeletal muscle glucose uptake and utilization via activation of insulin signaling may also promote enhanced insulin sensitivity. This was explored by transient induction of constitutively activated Akt1 in skeletal muscle (220). Before the activation of this transgene, the mice were fed a high-fat/high-sucrose diet for 8 wk and then continued on this diet with or without transgene activation for an additional 4 wk. Transient activation of Akt1 improved glucose tolerance in parallel with an attenuation in hepatic steatosis. Gene expression data support increased skeletal muscle glucose utilization with a concordant induction of genes encoding for FAO in the liver (220). Although the activation of Akt1 did not increase physical activity or change food consumption, the activation of Akt1 was associated with increased $\text{VO}_{2\text{max}}$ and diminished fat mass (220). This adaptive utilization of fat by the liver is reminiscent of extramyocyte adaptations identified in the PGC-1 α and GLUT4 knockout mice (165, 217). In contrast to the PGC-1 α knockout mice, IL-6 was not modified in the skeletal muscle after conditional activation of Akt1 (220). However, the concept that muscle-secreted factors or “myokines” may be operational in response to skeletal muscle stressors to coordinate systemic metabolic activities is beginning to be explored (221). The conditional skeletal muscle Akt1 mouse was therefore employed to identify potential candidate myokines, and the first two identified include fibroblast growth factor 21 and follistatin-like 1 factor (222, 223). Their roles in modulating metabolism and insulin sensitivity are beginning to be explored, and fibroblast growth factor 21 has recently been shown to improve insulin sensitivity in the pancreas, adipocytes, and liver (224–226).

The composite of the studies where skeletal muscle metabolic pathways are genetically manipulated has significantly contributed to our knowledge with respect to met-

abolic interactions, mitochondrial biology, and local and systemic insulin resistance. The broad concepts can be summarized as follows:

1. In general, the long-term genetic modulation of regulatory programs directing mitochondrial biogenesis gives rise to paradoxical findings, in part due to the wide array of targets of these regulatory proteins and due to adaptive systemic responses to counter the disruption of skeletal muscle mitochondrial function.
2. However, specific modulation of PPAR δ is completely consistent with improving glucose tolerance and insulin sensitivity in parallel with enhanced mitochondrial content and higher oxidative muscle fiber type.
3. Disruption in the storage of neutral lipids in the myocyte itself will disrupt insulin signaling and therefore glucose tolerance.
4. Incomplete FAO can arise from excessive fat import into the mitochondria, and this, too, can evoke insulin resistance.
5. Mitochondrial reprogramming to uncouple oxidative phosphorylation, at least in the short term, can improve insulin sensitivity in part by the energy-futile fat catabolism.
6. Enhancing skeletal muscle glucose oxidation either directly, or in response to the disruption of FAO can ameliorate insulin sensitivity.
7. A novel concept identified after the genetic disruption in skeletal muscle metabolic programs is that skeletal muscle itself possesses both innate adaptive programs and endocrine responses capable of secreting myokines that, in turn, modulate whole body fuel disposal, pancreatic islet cell insulin secretion, and systemic insulin sensitivity.
8. Finally, although the knowledge gained from these mouse models has significantly expanded our insight into the interaction of mitochondrial biology with insulin signaling pathways and glucose control, we need to be cognizant of the fact that these are murine models with genetic alterations in orders of magnitude different from the more modest alternations in mitochondrial programs in the insulin-resistant patient.

Figure 2 schematizes the major observations after skeletal muscle-restricted molecular modulation of metabolism and mitochondrial function and illustrates the adaptive and maladaptive sequelae of these distinct perturbations.

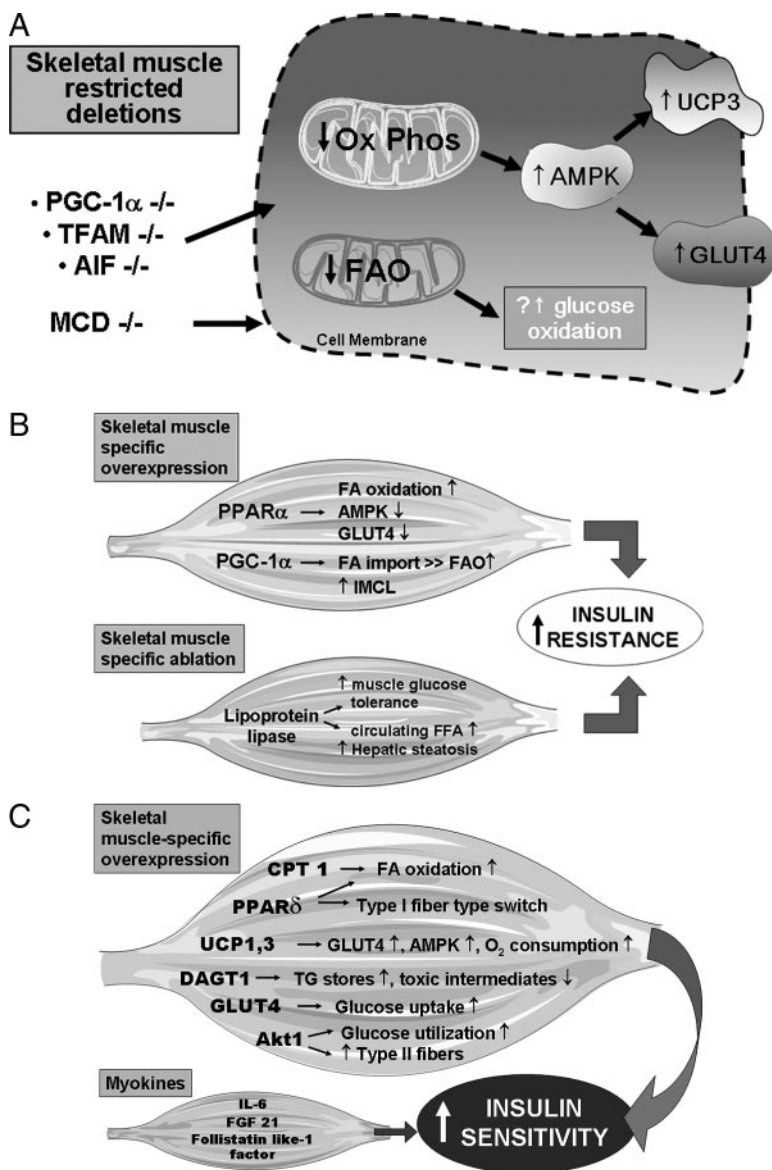


FIG. 2. Adaptive and maladaptive consequences of the molecular modulation of skeletal muscle mitochondrial biology determine overall insulin resistance. A, Skeletal muscle-restricted knockdown of mitochondrial regulatory proteins that exhibit adaptive augmentation of glycolysis, glucose oxidation, and possibly uncoupled respiration resulting in the overall improvement in insulin sensitivity and resilience to fat-induced lipid accumulation. B, Skeletal muscle-restricted overexpression of mitochondrial regulatory proteins and the skeletal muscle-restricted knockdown of a fatty acid transporter that result in the exacerbation of insulin resistance. C, Induction of various regulatory and functional mitochondrial proteins that improve insulin sensitivity. The myokines secreted by skeletal muscle are in response to the skeletal muscle-restricted deletion of PGC-1 α and inducible Akt1, respectively. The myokines then moderate additional peripheral metabolic tissues to enhance insulin sensitivity. TFAM, Transcription factor A of mitochondria; FGF, fibroblast growth factor; FFA, free fatty acid; FA, fatty acid; Ox PHOS, mitochondrial oxidative phosphorylation.

V. Interventions to Improve Skeletal Muscle Mitochondrial Function and Effects on Insulin Signaling

As discussed in this review, specific restricted molecular targeting of skeletal muscle mitochondrial metabolism enhances resistance to dietary-induced insulin resistance and

diabetes (177, 185, 190, 191, 206, 210, 220). However, it is technically challenging to administer lifestyle changes or noninvasive systemic therapeutics to specifically target skeletal muscle. Nevertheless, this limitation is not insurmountable in that the pathophysiology of insulin resistance extends beyond skeletal muscle to incorporate interactions between multiple metabolic tissues. The adaptive and maladaptive metabolic programming within these tissues, on balance, either promotes or prevents the development of systemic insulin resistance and diabetes. Thus, both skeletal muscle weighted interventions (*e.g.*, exercise) and therapeutic strategies that function in part through modulation of skeletal muscle mitochondrial metabolism are probably valid approaches to ameliorate insulin sensitivity. Moreover, in light of the disappointing long-term cardiovascular outcomes from multi-center clinical studies exclusively targeting glucose reduction (15, 16), an emphasis on improving systemic insulin sensitivity through metabolism-based therapy to reduce intracellular lipid accumulation and to enhance glucose utilization may mean more mechanism-directed approaches to therapy and this, in part, can be directed at skeletal muscle. In this section, we explore potential novel pharmacological and biological targets to modulate skeletal muscle mitochondrial bioenergetic capacity as a component of the overall strategy to prevent, reverse, and/or delay T2DM.

A. Pharmacological and biological activation of mitochondrial biogenesis

The biological interplay between skeletal muscle insulin resistance and mitochondria has also been addressed by evaluating whether therapeutic interventions to modulate mitochondrial biology can augment skeletal muscle metabolic capacity and concordantly improve systemic insulin sensitivity.

In adipose tissue, it has clearly been established that the thiazolidinedione class of diabetic agents promotes mitochondrial biogenesis (227, 228). This augmentation of mitochondrial oxidative capacity may play a role in the insulin-sensitizing effects of this class of compound (229). The thiazolidinediones predominantly activate PPAR γ , although they do have varying degrees of activity against other PPAR isoforms (230). In C2C12 muscle cells, the thiazolidinedione pioglitazone directly activates the mi-

tochondrial biogenesis regulatory program, and this induction of mitochondrial energetic capacity appears to be integrally linked to improved insulin sensitivity (71). Furthermore, primary skeletal myocytes grown from T2DM subjects, which show diminished mitochondrial FAO compared with nondiabetic controls, also exhibit reversal of these mitochondrial FAO defects in response to the administration of thiazolidinedione troglitazone (231). An additional study in primary human skeletal muscle from T2DM subjects shows thiazolidinedione-mediated improvement in FAO with enhanced fat uptake in parallel with up-regulation of the fatty acid transporter CD36 (232). In diabetic animal models, the dose of pioglitazone required to enhance skeletal muscle glucose uptake is shown to be higher than required to mediate ameliorative effects in the liver (233). The *in vivo* effects of thiazolidinediones on skeletal muscle have been assessed in numerous human studies. Pioglitazone up-regulates the skeletal muscle mitochondrial oxidative phosphorylation gene expression profile in association with improving insulin sensitivity and glucose tolerance in young women with PCOS (72). Rosiglitazone has been shown to up-regulate skeletal muscle PGC-1 α and PPAR δ with concordant induction of oxidative phosphorylation enzyme activity in parallel with insulin sensitization in diabetic subjects (234), albeit without induction of mitochondrial oxidative phosphorylation protein content or a diminution in IMCL content (234). Furthermore, in newly diagnosed T2DM, the administration of rosiglitazone directly enhances skeletal muscle insulin sensitivity as assessed by glucose uptake (235). In contrast, we and others have demonstrated that in established diabetic subjects, the improvement of glycemic control in response to rosiglitazone appears to be independent of muscle mitochondrial content/activity (61, 236). Interestingly, in our study rosiglitazone-mediated improvement in ETC protein content and citrate synthase activity was evident only in those subjects with relatively preserved $\text{VO}_{2\text{max}}$ and mitochondrial copy numbers (61). One possibility of these divergent findings could be that to enhance skeletal muscle mitochondrial biogenesis, the intervention with a thiazolidinedione is required earlier in the course of insulin resistance. This hypothesis is consistent with the diminished capacity to augment skeletal muscle mitochondrial biogenesis in aging rats (157). Of note, and in keeping with the multiorgan coordination of insulin resistance, thiazolidinediones additionally promote insulin sensitivity by decreasing hepatic steatosis (237, 238). From a clinical perspective, the use of thiazolidinediones to prevent the onset of diabetes in subjects at risk has been demonstrated (239). However, the long-term use of these agents is beginning to be questioned in the light of studies suggesting detrimental effects

of these agents on coronary artery disease and with respect to bone density (240, 241).

In light of the very promising effects in the skeletal muscle-specific PPAR δ transgenic mice, investigators have studied the insulin-sensitizing effects of the PPAR δ agonist GW501516 (242). In wild-type mice placed on a high-fat diet, a 2-month treatment period with GW501516 resulted in reduction in weight gain, increased type 1 skeletal muscle fiber type, and improved glucose tolerance compared with vehicle-treated controls (177). This ameliorative effect has also been shown in db/db mice and is thought to occur at least in part via the promotion of skeletal muscle FAO (175). A recent proof of concept study in human subjects showed that a 2-wk treatment of insulin-resistant subjects with GW501516 did improve insulin sensitivity in parallel with induction of CPT-1 gene expression in skeletal muscle (243). As recently demonstrated in human skeletal muscle cells, the responsible mechanism mediating these beneficial effects of GW501516 involves both PPAR δ - and AMPK-dependent and -independent pathways (244).

Because caloric restriction improves insulin resistance and enhances mitochondrial function, studies have been performed using caloric restriction mimetics to evaluate their efficacy in moderating this pathophysiology. A central mediator of the ameliorative effects of caloric restriction is via activation of the NAD⁺-dependent deacetylase SIRT1 (245), which in turn activates PGC-1 α (127, 246). Resveratrol, a polyphenolic compound extracted from grape skins, increases SIRT1 activity (247) and has been administered to mice in parallel with high-fat feeding (248, 249). Resveratrol markedly attenuated weight gain in association with induction of mitochondrial biogenesis in skeletal muscle and brown adipose tissue in the high-fat-fed mice (249). In parallel, the resveratrol-treated mice show increased endurance capacity, higher oxygen consumption, and improved glucose tolerance (249). The effect of resveratrol extends beyond activation of SIRT1 and includes both antioxidant properties and the activation of AMPK (250–253). These later mechanisms may also be operational in the ameliorative insulin-sensitizing effect of resveratrol in response to fat overload, although these pathways have not been as well characterized in this context. The dose of resveratrol used in mice is not feasible in humans (252, 254), although a study to address the effects of lower doses in humans has been initiated (Clinicaltrials.gov Identifier, NCT00823381).

To directly assess the insulin-sensitizing effect of SIRT1, numerous small molecule activators of this sirtuin have been identified and studied (255, 256). Here, too, these compounds improve insulin sensitivity in parallel with induction of skeletal muscle glucose tolerance and

with the improvement of skeletal muscle mitochondrial capacity (255, 256). Although, the direct link to mitochondria has not been established, it is also important to note that SIRT1 has also been shown to enhance insulin sensitivity in insulin-resistant myotubes via repression of the protein-tyrosine phosphatase 1B (257).

Interestingly, it has recently been observed that levels of MUP-1, a lipocalin-like secreted low molecular weight protein that is predominantly produced by the liver, are diminished in dietary and genetic obese mice (258). Replacement of MUP-1 via osmotic minipump improves glucose tolerance with salutary effects on skeletal muscle without modification of hepatic gluconeogenesis or hepatic insulin signaling (258). The ameliorative effects on skeletal muscle include up-regulation of the mitochondrial biogenesis program, increased mitochondrial oxidative capacity, diminished IMCL accumulation, improved insulin signaling in parallel with enhanced activity, and an elevation in the core temperature (258). Whether this protein represents a “hepatokine” that the liver secretes to enhance skeletal muscle bioenergetic capacity is an intriguing new concept that may have therapeutic potential.

Table 1, highlights the therapeutic strategies showing promise in the parallel improvement in skeletal muscle mitochondrial function and insulin resistance and identifies additional potential salutary effects on other peripheral metabolic tissues.

VI. Conclusions and Considerations

T2DM represents a composite of systemic manifestations and pathological sequelae resulting from a heterogeneous mix of pathophysiological events that modulate skeletal muscle and other organs including the liver, adipocytes, pancreas, brain, and immune system. The disruption of

skeletal muscle mitochondria as an early and probable initiating event in insulin resistance appears to be restricted to: 1) individuals with a strong hereditary predisposition to T2DM that develop insulin resistance at a young age; 2) women with PCOS; 3) some individuals with mitochondrial genomic defects; and 4) in the development of insulin resistance in lean elderly individuals.

In a plurality of T2DM subjects, mitochondrial dysfunction appears to be a direct manifestation of nutrient excess and physical inactivity, environmental factors that initiate a vicious cycle that further exacerbates the metabolic perturbations of T2DM in obese and or sedentary individuals. Whether these individuals have underlying genetic susceptibility to mitochondrial dysfunction is beginning to be explored (158–160). The complexity of the role of skeletal muscle mitochondrial biology in the pathophysiology of diabetes is further suggested, where the induction of skeletal muscle mitochondrial function can represent a more acute adaptive response to nutrient load (32, 115) and/or possibly an adaptive response to metabolic defects that initiate in other metabolic tissues, as may be operational in insulin-resistant individuals of Asian-Indian descent (66, 67).

The complexity of mitochondrial dysfunction incorporates interplay between substrate uptake, storage, and catabolism as well as efficient and balanced interaction of the various metabolic pathways within the mitochondrion itself. At the organ level, the adaptive and maladaptive metabolic interaction of various insulin-responsive tissues has also been shown to be operational in the systemic insulin sensitivity status (259, 260). The summative effect of these intertissue interactions collectively defines the overall systemic tolerance to glucose and insulin sensitivity.

The isolated genetic modulation of skeletal muscle mitochondrial/metabolic functioning in experimental

TABLE 1. Therapeutic insulin-sensitizing interventions that modulate mitochondrial and metabolic functioning in skeletal muscle, adipose tissue, and the liver

Intervention	Skeletal muscle effects	Adipose effects	Hepatic effects
Caloric restriction	Increase mitochondrial biogenesis (125)	Decrease adipocyte mass (261)	Diminish steatosis (262)
Intensive exercise	Increase mitochondrial biogenesis, increase muscle mass—oxidative capacity, increase DGAT1 (136–140, 184)	Decrease adipocyte mass (261)	
Thiazolidinediones	Promote mitochondrial FAO and fat uptake (231, 232)	Increase lipid uptake and metabolism (227)	Diminish steatosis (237, 238)
GW501516	Induction of type I fibers and oxidative capacity (175, 177, 244)		Diminish steatosis (243)
Resveratrol	Increase mitochondrial biogenesis (249)	Increase mitochondrial biogenesis (249)	
SIRT1 activators	Increase mitochondrial oxidative capacity and glucose uptake (255, 256)	No change in mitochondrial oxidative capacity (256)	
MUP-1	Increase mitochondrial biogenesis and oxidative capacity (258)	No weight reduction (258)	

studies shows that the disruption in skeletal muscle mitochondrial/energetic integrity additionally results in the induction of secretory myokines that function to modulate the biology of other organ systems as a component of the adaptive program to prevent perturbations in overall fuel handling by the body.

These emerging data support a hypothesis that multiple adaptive metabolic and mitochondrial programs in peripheral metabolically active tissues need to be overwhelmed to progress to systemic insulin resistance. Although this review has focused on the metabolic and mitochondrial functioning in skeletal muscle, in all probability, the homeostatic control across all metabolic tissues integrates in this pathophysiology.

In conclusion, we propose that understanding the regulatory control of mitochondrial homeostasis may generate therapeutic options to modify the development of and reversal of insulin resistance. Moreover, in light of the capacity to overwhelm adaptive modulations by persistent overnutrition, the global strategy to reverse insulin resistance would appear to require a multipronged approach including tackling the underlying deficits and to prevent the environmental stressors promoting progression to diabetes.

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Address all correspondence and requests for reprints to: Michael N. Sack, Building 10-CRC, Room 5-3150, 10 Center Drive, MSC 1454, Bethesda, Maryland 20892-1454. E-mail: sackm@nhlbi.nih.gov.

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