

Mitochondrial Deficiency Is Associated With Insulin Resistance

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The specific cellular underpinnings or mechanisms of insulin resistance (IR) are not clear. Here I present evidence to support a causal association between mitochondrial energetics and IR. A large body of literature indicates that mitochondrial capacity for oxidative metabolism is lower in human obesity and type 2 diabetes. Whether or not mitochondria play a causal role in IR is hotly debated. First, IR can be caused by many factors, many of which may or may not involve mitochondria. These include lipid overload, oxidative stress, and inflammation. Thus the first tenet of an argument supporting a role for mitochondria in IR is that mitochondria derangements can cause IR, but IR does not have to involve mitochondria. The second tenet of this argument is that animal models in which oxidative metabolism are completely abolished are not always physiologically or pathologically relevant to human IR, in which small metabolic perturbations can have profound effects over a prolonged period. Lastly, mitochondria are complex organelles, with diverse functions, including links with cell signaling, oxidative stress, and inflammation, which in turn can be connected with IR. In summary, mitochondrial “deficiency” is not merely a reduced energy generation or low fatty acid oxidation; this concept should be expanded to numerous additional important functions, many of which can cause IR if perturbed. *Diabetes* 62:1032–1035, 2013

The most common forms of human skeletal muscle insulin resistance (IR) are associated with 1) obesity, particularly abdominal obesity and excess accumulation of lipids in non-adipose tissues such as liver and skeletal muscle; and 2) physical inactivity. Identifying a common cellular basis for these conditions, however, remains elusive. Impairments in mitochondrial energetics have been linked to each of these conditions. Obesity has been reported to be associated with reduced mitochondria content and altered mitochondrial performance (1). Physical inactivity is associated with lower mitochondrial biogenesis and content (2). Conversely, exercise is a potent inducer of mitochondria biogenesis (3). Thus it is not surprising that considerable attention has been given to the possibility that mitochondria play a role in IR. But of course associations do not infer that derangements in mitochondria cause IR. Although many of these arguments can be made for other insulin-sensitive tissues such as liver, this line of reasoning to support a role for mitochondria in IR will focus on skeletal muscle.

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DEVELOPING THE ARGUMENT

While there is little debate that low mitochondria content or poor mitochondrial capacity or performance is associated with IR in muscle, the current debate is centered on whether or not some aspect(s) of mitochondrial biogenesis, content, or energetics actually cause IR. I will make the case that the majority of evidence supports the claim that mitochondrial derangements can cause IR in humans. In order to set the stage for this argument, it is important to outline some fundamental truisms that support this view.

First, we must first accept that IR can be defined as a decreased insulin-stimulated glucose uptake by muscle cells or tissues, as this provides a quantitative integrated measurement of insulin action. At first this seems obvious; let's not, however, allow that defects in insulin-signaling, GLUT4 translocation or content, or other parameters upstream of glucose uptake be held up as direct evidence. That is because—and the second consideration—impairment in insulin-stimulated glucose uptake can occur without alterations in classical insulin signaling pathways. For example, IR induced by palmitate or oxidative stress can occur without diminished Akt phosphorylation (4). In other words, there are multiple ways of inducing IR (Fig. 1).

We must agree that mitochondria deficiency should not be defined as merely inadequate ATP generation or reduced fatty acid oxidation, as has been argued in previous commentaries (5,6). To the contrary, mitochondria are very dynamic organelles with many roles, including mediating oxidative stress, cellular redox state, and generating signaling molecules (Fig. 1). We must also put a heavy emphasis on human IR. While appreciating that mechanistic, causal studies are difficult—if not impossible—to perform in human subjects, we must also, however, eventually be able to translate mechanistic studies to human health and disease.

I will also argue that we are just now beginning to understand some of the other potential roles that mitochondria may have in governing metabolism, including IR. Now, although a dearth of information or poor understanding is itself not a good basis for an argument that the phenomenon is true, arguing against a causal role for mitochondria in IR without acknowledging the possibility that mitochondria can cause IR is a dangerous position to hold. I will present emerging evidence that expands the outdated simplistic view of mitochondria as merely being involved in energy generation to include a broader role for mitochondria in cell signaling, redox, oxidative stress, and likely IR.

EARLY EVIDENCE LEADING TO THE “MITOCHONDRIA HYPOTHESIS”

The landmark studies by Randle et al. (7) supported a model in which increased mitochondrial fatty acid oxidation

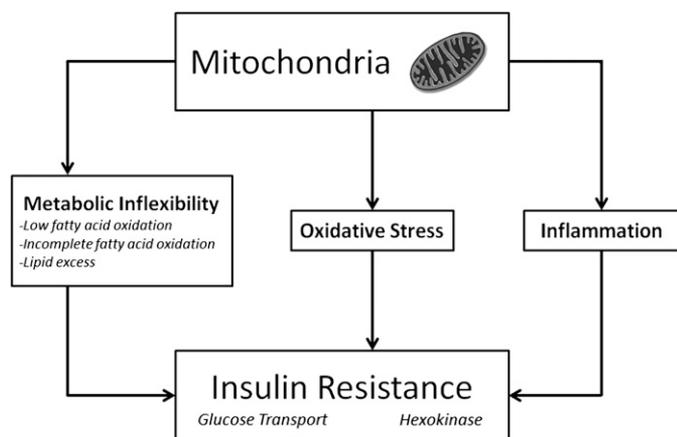


FIG. 1. Mitochondria are now widely recognized to have numerous complex functions, many of which have been implicated in skeletal muscle insulin resistance.

in muscle leads to an accumulation of acetyl-CoA and citrate, thereby inhibiting pyruvate dehydrogenase and phosphofructokinase, respectively. This inhibition then increases glucose-6-phosphate concentrations, inhibiting hexokinase and resulting in reduced glucose uptake and oxidation. Although some cast doubt on whether this glucose–fatty acid cycle was operative in all tissues under all circumstances (8), these early studies spawned a series of subsequent investigations examining the biochemical mechanisms of reciprocal glucose and fatty acid oxidation.

Several groups of investigators in the 1990s led resurgence in the field to investigate the link between fatty acid oxidation and skeletal muscle IR. Kelley and Mandarino (9) reported that, in contrast to the predictions of the original glucose–fatty acid cycle, during conditions of hyperglycemia and high glucose oxidation, fat oxidation was concomitantly lower in muscle of individuals with type 2 diabetes, and this effect was exacerbated by obesity (10,11). Other studies also reported that glucose inhibits fat oxidation (12), calling it the reverse Randle cycle. These findings were also consistent with observations that skeletal muscle in obesity-induced IR has increased levels of malonyl-CoA (13), thereby inhibiting carnitine palmitoyl transferase (CPT) and thus fatty acid oxidation (14).

MITOCHONDRIA, FATTY ACID OXIDATION, AND INTRAMYOCYELLULAR LIPIDS

More recently, a prevalent hypothesis has been put forth to link mitochondria with IR, whereby lower mitochondrial content or impairments in mitochondrial fatty acid oxidation lead to excess accumulation of intramyocellular lipids, most notably diacylglycerol and ceramides (15). Although attractive, there is considerable debate about whether or not this “lipotoxicity” or excess nonadipose tissue (ectopic) lipid is mechanistically linked with IR, and further, whether this can be tied to altered mitochondrial capacity for fatty acid oxidation. Human studies reported that skeletal muscle in obese subjects either without or with type 2 diabetes was inherently poor at oxidizing fatty acids (11), or had reduced mitochondrial capacity for oxidative metabolism (1).

It has been argued that mitochondrial capacity for fatty acid oxidation is not the root cause of IR because, even in IR of obesity and type 2 diabetes, mitochondria have an

excess capacity to maintain adequate fatty acid oxidation and provide energy during resting conditions in which energy demand is low, for example during resting (5,6). There is no question that total energy flux is driven by energy demand. Hence the muscle mitochondria in IR and diabetes have more than adequate capacity for energy production during resting conditions. Substrate selection, on the other hand, can be independent of energy demand. For example, chronic exercise training results in higher proportion of energy generated from fatty acid oxidation at the same absolute energy demand (16). I would also argue that—at the same energy demand—reduced mitochondria content and capacity results in a proportionate shift away from fatty acid oxidation during noninsulin stimulated conditions, leading over time to lipid accumulation and IR. This metabolic inflexibility that occurs in IR muscle is characterized by both lower fatty acid oxidation in the basal, fasting state and a lower glucose uptake in the insulin-stimulated state (17).

One of the arguments made that mitochondrial deficiency is disconnected from IR derives from either pathological conditions or genetic animal models of severe mitochondrial deficiency (18). In these extreme examples, energy derived from oxidation of fatty acids is essentially abolished, so that glucose is practically the only substrate available—in both basal and insulin-stimulated conditions. Therefore, these models in which glucose is necessarily the default substrate do not provide convincing evidence against the mitochondrial link with IR.

Another argument that altered mitochondrial fatty acid oxidation may play a role in IR is suggested by genetic models in which shifting substrate selection toward fat oxidation protects against diet-induced IR. Acetyl-CoA carboxylase deficient mice exhibit the expected increase in fatty acid oxidation (presumably because of lower malonyl CoA levels to inhibit CPT-1) and protection against obesity-induced IR (19). Other studies using muscle-specific acetyl-CoA carboxylase deficient animals have not observed a similar metabolic phenotype (20), perhaps because of a compensatory decrease in overall glucose oxidation and increase in de novo lipogenesis. However, in model systems in which CPT-1 is overexpressed in skeletal muscle, fat oxidation is increased together with improved insulin sensitivity (21). Taken together, genetic manipulation in animal models or in cell systems has often led to contradictory and confusing results. Again, although these model systems have been useful to demonstrate potential causes of IR, we must look at the human data to support or refute these model systems.

Another line of evidence used to refute a causal role for mitochondria in IR is from studies in rodents in which high-fat feeding causes IR while increasing mitochondria in muscle (22). Moreover, overexpression of lipoprotein lipase in muscle provides greater free fatty acid exposure to stimulate peroxisome proliferator-activated receptor- δ and stimulate mitochondrial biogenesis (23). A counter to this is a study by Sparks et al. (24) who found that a short-term high-fat diet downregulates mitochondrial genes. This evidence can also be countered by evidence in humans. First, chronic high-fat feeding or obesity is not associated with an increase in mitochondria or oxidative capacity in humans (25). On the contrary, mitochondria capacity is decreased with obesity in humans (25). We need to consider many confounders when comparing the data from rodents fed high-fat diets with cross sectional studies of obese humans (e.g., physical activity, chronic vs. acute

effects of fat overload). Second, Morino et al. (26) recently reported that reductions in lipoprotein lipase play a key role in reduced mitochondrial content in human IR through decreased peroxisome proliferator-activated receptor- δ activation by polyunsaturated fatty acids, eicosapentaenoic acid specifically. Thus we need to be careful in how we interpret the animal model data used to refute a role for mitochondria in IR.

Human evidence to support a link between mitochondria and IR may also appear to be inconsistent. It should follow from the mitochondrial-lipotoxicity hypothesis, that lower mitochondrial capacity in IR states still maintains an adequate reserve capacity to oxidize fatty acids and cannot lead to excess accumulation of intramuscular lipids. This contention can be countered by acknowledging that higher mitochondria content and capacity are associated with a higher proportion of fatty acid oxidation during resting conditions (17). Thus, although mitochondrial capacity in IR conditions should be adequate to oxidize fatty acids and maintain lower intramuscular lipids, it is likely that a lower total mitochondrial capacity can lead to a shift in substrate selection in the fasting or exercise condition away from fatty acid oxidation, which would lead to lipid accumulation and IR. Of course, although poor mitochondrial performance can lead to IR, IR can develop in the setting of lipid oversupply without low mitochondrial content or function (27). In other words, IR can develop because of lower mitochondrial capacity, but does not require it because there are many other possible causes of IR.

We have used energy restriction weight loss and exercise programs to examine how intervention-induced changes in mitochondria, muscular lipids, and IR track with one another. These interventions are reasonably good models to distinguish between increases in energy demand and reductions in energy supply. To summarize these studies: exercise increases mitochondria content and capacity for fatty acid oxidation and improves IR (28). Diet-induced weight loss, however, improves IR without increasing mitochondrial capacity (29). One interpretation is that this is solid proof that mitochondria are not in a causal pathway to IR. It is quite likely, however, that just as there are many paths to develop IR, there are several means to improve IR. Exercise improves mitochondria content and capacity, decreases ceramides (30), and improves IR. Although it is possible that these are simply phenomena all occurring as an adaptation to exercise, this leaves the door ajar to the possibility that exercise improves IR by increasing the proportion of energy derived from mitochondrial fatty acid oxidation during resting conditions, thereby partitioning fatty acids away from lipotoxic moieties. An alternative compatible explanation linking mitochondria to IR is that reductions in oxidative stress with either exercise or energy restriction can improve IR. In this next section, I will argue that this and other facets of mitochondrial energetics are likely causally linked with IR.

MITOCHONDRIA, METABOLIC OVERLOAD, OXIDATIVE STRESS, AND INFLAMMATION

During the past few years, interesting insights have come to light from genetic models of IR in which high rates of incomplete fat oxidation and by-products of fatty acid catabolism are associated with IR (31). In these models, IR was associated with elevated β -oxidation with no change in overall mitochondrial respiration. This might suggest that in the setting of low energy demand, e.g., a sedentary

lifestyle, a metabolic overload stress on the mitochondria contributes to IR. It remains to be determined whether an increase in mitochondria content or capacity—even in low energy demand states—may act as a buffer to reduce this metabolic stress and maintain insulin sensitivity.

Glucose transport is a key defect in systemic glucose disposal, and some have argued that this is the primary derangement governing IR in muscle (32). Recent studies in animal models have challenged the concept by demonstrating a more distributed control of glucose flux involving hexokinase during conditions in which glucose transport is maximized (33). This should be viewed in the context of studies demonstrating hexokinase binding to mitochondria (34) and its link to thioredoxin-interacting protein (TXNIP) as well as TXNIP effects to inhibit glucose uptake in humans (35). Thus it is quite possible that reduced mitochondrial content or remodeling in IR states results in decreased overall hexokinase binding and subsequently a reduced phosphorylation of glucose and diminished glucose uptake.

Another attractive paradigm gaining acceptance is that mitochondria-derived reactive oxygen species (ROS) act as signaling molecules (36). Anderson et al. (37) demonstrated that in obese rodents and humans that elevated mitochondrial-mediated oxidative stress was related to IR, and that antioxidant scavenging improved IR. In support of a role of mitochondrial oxidative stress in IR, Lee et al. (38) reported that overexpression of a mitochondria-targeted catalase resulted in a reduction in diacylglycerol and protection against aging-induced IR. Moreover, it is well accepted that ROS activate several aspects of cell signaling related to insulin action (39). An interesting study by Shi and colleagues (40) reported that remodeling of cardiolipin, a phospholipid specific to mitochondria, plays a key role in mediating mitochondrial O_2 consumption, oxidative stress and IR.

Inflammation has steadily gained acceptance as a likely cause of IR. The links among inflammation, mitochondria and IR have only recently begun to be appreciated. Mitochondrial TXNIP may also be an important link between mitochondria and inflammation. TXNIP has also been implicated in type 2 diabetes, and is associated with ROS-dependent Nod-like receptor 3 inflammasome activation after its detachment from thioredoxin (41). Zhou et al. (41) found that inhibition of mitochondrial respiratory chain activity, which causes ROS generation, activates the Nod-like receptor 3 inflammasome, and further, that the mitochondria voltage-dependent anion channels are crucial for inflammasome activation. These data place mitochondrial signaling squarely in the path of inflammation. This inflammatory response has also been shown to contribute to changes in the extracellular matrix, an altered mechanosignal transduction, and reduced mitochondrial content and function associated with IR. Taken together, these studies indicate that oxidative stress and inflammation are important aspects of mitochondrial energetics and play a role in IR.

In summary, human type 2 diabetes and obesity are associated with a reduced mitochondrial capacity and IR. The etiology and underlying mechanisms of skeletal muscle IR are not clear, in part because there are many cellular causes of IR. Lipid overload, derangements in fatty acid oxidation, oxidative stress, and inflammation have all been shown to cause IR. Further, the majority of the available evidence indicates that mitochondria play a role in these forms of human skeletal muscle IR. Mitochondrial deficiency, as articulated previously from the perspective of

reduced oxidative capacity or low fatty acid oxidation, should be more broadly defined to include mitochondria's various complex roles in health and disease. By accepting that mitochondria are complex organelles with many functions and that there are multiple pathways leading to IR, we will more fully appreciate that mitochondria may cause IR. Then we will be able to advance a clearer understanding of this complex pathology.

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