Mitochondrial dysfunction and insulin resistance from the outside in: extracellular matrix, the cytoskeleton, and mitochondria

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Coletta DK, Mandarino LJ. Mitochondrial dysfunction and insulin resistance from the outside in: extracellular matrix, the cytoskeleton, and mitochondria. Am J Physiol Endocrinol Metab 301: E749–E755, 2011. First published August 23, 2011; doi:10.1152/ajpendo.00363.2011.—Insulin resistance in skeletal muscle is a prominent feature of obesity and type 2 diabetes. The association between mitochondrial changes and insulin resistance is well known. More recently, there is growing evidence of a relationship between inflammation, extracellular remodeling, cytoskeletal interactions, mitochondrial function, and insulin resistance in human skeletal muscle. Several sources of inflammation, including expansion of adipose tissue resulting in increased lipolysis and alterations in pro- and anti-inflammatory cytokines, contribute to the insulin resistance observed in obesity and type 2 diabetes. In the experimental model of lipid oversupply, an inflammatory response in skeletal muscle leads to altered expression extracellular matrix-related genes as well as nuclear encoded mitochondrial genes. A similar pattern also is observed in “naturally” occurring insulin resistance in muscle of obese nondiabetic individuals and patients with type 2 diabetes mellitus. More recently, alterations in proteins (including α-actinin-2, desmin, proteasomes, and chaperones) involved in muscle structure and function have been observed in insulin-resistant muscle. Some of these cytoskeletal proteins are mechanosignal transducers that allow muscle fibers to sense contractile activity and respond appropriately. The ensuing alterations in expression of genes coding for mitochondrial proteins and cytoskeletal proteins may contribute to the mitochondrial changes observed in insulin-resistant muscle. These changes in turn may lead to a reduction in fat oxidation and an increase in intramyocellular lipid, which contributes to the defects in insulin signaling in insulin resistance.

skeletal muscle; inflammation

INSULIN RESISTANCE IN SKELETAL MUSCLE is considered to be a prominent feature of obesity, type 2 diabetes, and other diseases associated with cardiometabolic risk. In point of fact, there is a broad range of insulin action in skeletal muscle, with many apparently healthy individuals having insulin action on the lower end of the distribution (Fig. 1). The position of an individual on the standard curve of insulin action, that is, their insulin action phenotype, is likely governed by familial, presumably genetic factors and the individual’s environment, including variables such as physical activity and diet. Lower insulin action has been associated with lower insulin-stimulated activities of enzymes such as glycogen synthase and hexokinase (35, 45) and reduced ability of insulin to activate a variety of elements of the insulin signaling system, including tyrosine phosphorylation of the insulin receptor and insulin receptor substrate (IRS-1) (13, 30). More recently, lower insulin action has been associated with reduced content, and perhaps elements of function, of mitochondria in skeletal muscle that has more characteristics of fast-twitch, glycolytic, type II muscle fibers (11, 26, 46). As such, muscle exhibiting lower insulin action displays many characteristics of muscle subjected to “insufficient” physical activity, although the interplay between insulin action and exercise is complex (40).

Related to these changes have been a number of gene expression differences between muscle from individuals with higher and lower levels of insulin action. For example, peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), a transcriptional activator with an important role in mitochondrial biogenesis and formation of type I muscle fibers (33), exhibits reduced expression in individuals with a family history of type 2 diabetes, obesity, or type 2 diabetes mellitus itself (37, 42). This lower expression of PGC-1α is accompanied by lower expression of mRNAs (23, 32) and protein abundance of a large number of nuclear-encoded mitochondrial genes (23, 32).
Recent evidence suggests that inflammation is associated with lower insulin action as well (19). It is not clear at this point whether inflammation precedes or merely coincides with lower insulin action, whether individuals who reside on the lower end of the insulin action curve have greater propensity for inflammatory processes, or whether inflammation itself moves an individual leftward along the insulin action curve to a state of reduced insulin action that eventually leads to disease propensity. At this point, the mechanistic or causal relationship between lower insulin action in skeletal muscle and disease is far from clear. Here, we review data from our laboratory that we believe point to a novel potential mechanism for the development of insulin resistance in skeletal muscle. This is not intended to be an exhaustive review of this subject, and the reader is referred to several excellent reviews in this area (1, 7, 17, 24, 38, 50, 51).

Sources of Inflammation

Several sources of inflammation have been associated with insulin resistance in muscle (Fig. 2). The recognition that adipose tissue can be a major source of proinflammatory cytokines has been a major advance in understanding the sources of inflammation associated with lower insulin action. Expansion of adipose tissue has been associated with increases in proinflammatory cytokines, including tumor necrosis factor-α (TNFα), interleukin 6 (IL-6), resistin, and retinol-binding protein, and insulin resistance is associated with reductions in plasma levels of the anti-inflammatory cytokine adiponectin (34, 57). It is conceivable that expansion of adipose tissue, accompanied by increased release of inflammatory cytokines, especially in individuals already on the lower end of the insulin action distribution, could tip the balance toward disease.

These inflammatory cytokines can act on a variety of receptors in skeletal muscle to activate Toll-like receptor signaling, interleukin receptor signaling, NF-κB, and IκB kinase signaling, as well as a number of other inflammatory kinases (39, 47, 52, 54). It is believed, albeit far from demonstrated in humans, that many of these inflammatory kinases can reduce insulin
signaling by phosphorylating IRS-1 on several serine residues that might lower the ability of IRS-1 to transduce the insulin signal (39, 52). It is not clear whether this is the only or primary mechanism responsible for lower insulin action in states of chronic inflammation, and much work remains to establish the validity of this model.

Expansion of adipose tissue is an inevitable outcome of a positive energy balance. In Western societies, a common route to a positive energy balance is a combination of low physical activity and a high-fat diet. A diet high in fat content not only increases adipose tissue mass but also increases plasma free fatty acid and triglyceride concentrations. In vitro, elevated free fatty acids can inhibit insulin action in a variety of ways (20, 55, 58). In vivo, higher free fatty acids and triglycerides induced by an infusion of lipid emulsions, with or without heparin to activate lipase, have been shown to reduce insulin action and insulin signaling, as measured by a decreased ability of insulin to stimulate glucose disposal (8, 9, 12, 15, 27, 28, 48). Infusion of lipids itself increases inflammatory cytokines and activates inflammatory signaling in muscle (4, 49).

Toll-like receptors (TLRs) play an important role in the innate immune system by activating inflammatory pathways. TLR4, the best-characterized TLR, functions as the receptor for lipopolysaccharide (LPS) of gram-negative bacterial cell walls (36). In addition, there is evidence that saturated and polyunsaturated fatty acids signal through TLR, particularly TLR4 (22). Activation of the TLR4 pathway induces the expression of a large number of proinflammatory genes (47). It does so by regulating the activities of signal-dependent transcription factors that include NF-κB, activator protein-1, and interferon regulatory factors family members (39). Obese and type 2 diabetic subjects had significantly elevated TLR4 gene expression and protein content in muscle, which correlated with the severity of insulin resistance. Obese and type 2 diabetic subjects also had lower IκBα content, an indication of elevated IκB/NF-κB signaling. The increase in TLR4 and NF-κB signaling was accompanied by elevated expression of the NF-κB-regulated proinflammatory genes IL-6 and superoxide dismutase 2 (47).

Changes in Plasma Lipid Levels Alter Gene Expression in Skeletal Muscle

The decrease in expression of PGC-1α referred to above was related to plasma free fatty acid concentrations in vivo in humans (42). This finding led to a test of the hypothesis that raising plasma lipid levels in healthy individuals itself was sufficient to lower PGC-1α expression (49). In this experiment, a lipid emulsion was infused for 48 h, followed by a euglycemic clamp experiment to assess insulin action along with percutaneous needle muscle biopsies (49). A 48-h saline infusion served as the control experiment. Using a combination of quantitative PCR and oligonucleotide microarrays, the lipid infusion was found to lower PGC-1α expression as well as suppress the mRNA levels for a number of genes coding for proteins involved in mitochondrial function (49). However, the use of global gene expression analysis revealed that unexpected and more profound changes were occurring simultaneously. The mRNA levels for a number of extracellular matrix-related genes were increased profoundly, including various collagens, fibronectin, proteoglycans, matrix metallopro-
A study in human adipose tissue demonstrated that collagen VI-α3 (COL6A3)-subunit mRNA expression was increased significantly in obese and type 2 diabetic patients (41). The obese subjects with high COL6A3 mRNA had greater visceral adipose tissue mass, lower size of small and medium adipocytes, more CD68+ and CD163/MAC2+ macrophages, and increased macrophage inflammatory protein-1α and macrophage chemoattractant protein-1α mRNA (41). These results suggest that adipose tissue fibrosis is present in human obesity.

Similar to the human studies described above, high-fat-fed mice had insulin resistance and increased muscle collagen III and collagen IV protein (25). Rescue of muscle insulin resistance by genetic, muscle-specific, mitochondria-targeted catalase overexpression or by the phosphodiesterase 5a inhibitor sildenafil reversed high-fat feeding effects on extracellular matrix remodeling and increased muscle vascularity (25). These results in mice further demonstrate that extracellular matrix collagen expansion is tightly associated with muscle insulin resistance.

Taking all these results together, we predicted that decreasing plasma free fatty acids would decrease the expression of genes encoding for these extracellular proteins. Although a reduction in lipid supply by acipimox treatment reduced free fatty acids and improved insulin sensitivity (2), which was concomitant with a decrease in intramuscular fatty acyl-CoA, it did not reverse the molecular changes associated with lipid oversupply in muscle. Paradoxically, the expression of collagen genes and other extracellular matrix genes such as connective tissue growth factor and TGFβ1 was increased following treatment with acipimox (2). This suggests that changes in insulin sensitivity in either direction, induced by changes in plasma free fatty acids, are accompanied by a remodeling of the extracellular matrix.

Global Proteomics Analysis Leads to Potentially Novel Mechanisms of Insulin Resistance

On the basis of these surprising and sizeable changes in extracellular matrix protein abundance found in insulin-resistant muscle, a global mass spectrometry-based proteomics discovery experiment was undertaken to find other unexpected changes in insulin-resistant muscle. As before, we used lean, healthy controls, obese nondiabetics, and patients with type 2 diabetes and subjected muscle biopsies to mass spectrometry analysis using a normalized spectrum abundance factor method (44, 60, 61) for quantification (23).

On the basis of earlier studies (37, 42, 53), we expected that we would find decreases in mitochondrial proteins. Mitochondrial proteins make up more than 20% of the human muscle proteome (18). In all, 1,218 proteins were assigned in the study, and 400 could be quantified in at least one-half of all subjects. Muscle from patients with type 2 diabetes demonstrated the largest decreases in abundance of a variety of mitochondrial proteins in a wide range of functional categories, including electron transport proteins (23). Other unexpected proteins and groups of proteins also emerged from this analysis as being potentially dysregulated in insulin-resistant muscle. For example, chaperone proteins, including the TCP1 family of cytosolic protein chaperones, were increased dramatically in insulin-resistant muscle (23). The TCP1 proteins comprise the major cytosolic chaperone complex that is responsible for folding proteins such as actin (31, 59). In addition, a large number of proteasome subunits were increased in abundance, suggesting increased protein degradation in insulin-resistant muscle. The combination of increased chaperone and proteasome proteins in insulin resistance suggests a generalized increase in protein turnover. This, for example, could reflect inflammation or endoplasmic reticulum stress (5, 6, 21), although specific proteins involved in the unfolded protein response to endoplasmic reticulum stress were not represented in the proteins assigned or quantified in this experiment, perhaps due to low abundance.

Perhaps most dramatic of all were decreases in insulin-resistant muscle in proteins involved in muscle structure and function. Both α-actinin-2 and desmin were decreased profoundly in insulin-resistant muscle (23). Desmin is the major component of intermediate filaments in skeletal muscle and heart. Intermediate filaments tie together the sarcomere, the fundamental contractile unit, with the extracellular matrix.
through dystroglycan and sarcoglycan complexes in the sarcolemma. These intermediate filaments can be thought of as “cables” that transmit force from the sarcomere to the extracellular matrix and then eventually to tendons to apply force for movement. In addition, intermediate filaments are mechanosignal transducers that allow muscle fibers to sense contractile activity and respond appropriately with changes in gene expression that can accommodate increases or decreases in activity (10). Of potential import in this regard are findings that insulin-resistant muscle is also “exercise resistant” in its response to a bout of exercise or muscle contraction (14). Lack of desmin produces a variety of “desminopathies” that affect both heart and skeletal muscle (43).

The other major structural protein that is altered in abundance in insulin resistance is α-actinin-2. Actinin-2 is the major isoform of actinin in skeletal muscle and heart, with actinin 3 being a minor isoform that is not expressed in about 20% of humans without adverse consequences (3). Actinin-2 binds to and anchors α-actin at the Z-disk of the sarcomere and is the major component of Z-disks (10). Additionally, actinin-2 participates in protein-protein interactions of a variety of cellular proteins with the actin cytoskeleton and participates in a variety of other functions in addition to skeletal muscle contraction via α-actin (10, 29). We found actinin-2 to be decreased by nearly 50% by both mass spectrometry analysis and immunoblot analysis in insulin-resistant muscle (23). At this point, the decrease has not been localized to Z-disk or cytoskeletal actinin-2, but the magnitude of the decrease makes it likely that the Z-disk actinin is decreased because it comprises the majority of cellular actinin in skeletal muscle. The extent of a decrease in cytoskeletal-associated actinin-2 could not be determined from these studies.

With respect to actinin in insulin resistance, it may be significant that, in L6 myotubes, α-actinin-4, which binds filamentous cytosolic actin, associates with glucose transporter 4 (GLUT4) either directly or indirectly (16), and even more significantly, knockdown of actinin-4 abrogates insulin-stimulated GLUT4 translocation in these cells (56). Actinin-4 abundance is much lower than actinin-2 in human skeletal muscle. In fact, in our proteomics analysis, only a single spectrum from a peptide unique to actinin-4 was assigned in only one of 24 total biopsies analyzed (23). In contrast, hundreds of actinin-2 spectra were assigned in human skeletal muscle; in all, there were 24 biopsies. It is tempting to speculate that, although the direct applicability of the actinin-4 results in L6 myotubes to human muscle can be questioned, it is possible that actinin-2 serves the same function in human muscle. This could imply that a possible mechanism of defects in GLUT4 translocation in human muscle involves the deficit in actinin-2 abundance observed (23). Changes in mitochondrial, extracellular matrix, and cytoskeletal/structural genes and proteins in human skeletal muscle insulin sensitivity are summarized in Table 1.

**Proposed Model of the Relationships Among Changes in Expression of Extracellular Matrix and Cytoskeletal Proteins and Mitochondrial Changes in Insulin-Resistant Muscle**

Our proposed model of the relationship between inflammation and insulin resistance in skeletal muscle is shown in Fig. 5. In this model, an inflammatory response leads to changes in the extracellular matrix that are reminiscent of fibrosis. The extracellular matrix remodeling that is observed in insulin resistance may then alter mechanosignal transduction mediated by cytoskeletal elements such as intermediate (desmin) filaments or the actin cytoskeleton, resulting in altered sensing of contractile activity and ensuing gene expression changes that lead to decreased muscle fiber type I remodeling and regeneration and reduced mitochondrial number and function (perhaps mediated by changes in PGC-1α expression). Changes in sensing of contractile activity could then result in alterations in expression of cytoskeletal and structural proteins by a feedback mechanism, reinforcing this vicious cycle. Changes in these genes and proteins contribute to the mitochondrial abnormalities observed in insulin-resistant muscle and may in the end lead to decreased fat oxidation, accumulation of ectopic lipid, insulin-signaling abnormalities, and ultimately insulin resistance. We point out that this mechanism is compatible with and comple-
mentary to other current hypotheses regarding the vicious cycle connecting inflammation, mitochondrial changes, lipid accumulation, and insulin-signaling defects. The novel aspect of this mechanism is that it connects inflammatory processes with changes in insulin sensitivity by means of altered mechanosignal transduction due to fibrotic changes.

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DISCLOSURES

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