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

Fig. 1. The distribution of the M values (mg·kg⁻¹·min⁻¹), measured by insulin-stimulated glucose disposal during the euglycemic clamp in 45 normal, glucose-tolerant, healthy subjects. Data were obtained during an 80 mU·m⁻²·min⁻¹ insulin infusion and were taken from unpublished (Mandarino LJ) and published (14, 23, 32) sources.

Fig. 2. Sources of inflammation associated with insulin resistance in skeletal muscle. IRS-1, insulin receptor substrate-1.
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 factor 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 metalloproteinases, and others (Fig. 3). These changes were confirmed at the protein level using immunoblot analysis. The pattern of gene and protein changes was strikingly similar to typical fibrotic responses seen in response to inflammation in a number of other circumstances, including (perhaps not coincidentally) nonalcoholic steatohepatitis (4). In some diseases, such as scleroderma or pulmonary fibrosis, connective tissue growth factor (CTGF) expression is increased in response to inflammatory stimuli such as TGFβ (49). After lipid infusion, CTGF expression levels were increased, although there was no evidence of increases in expression of TGFβ or other inflammatory cytokines directly in muscle (49), suggesting that circulating cytokines could be responsible for these effects.

Collagen Content Is Increased in Naturally Occurring Insulin Resistance

On the basis of our findings that experimental insulin resistance induced by a lipid infusion also profoundly increases expression of a wide array of genes encoding extracellular matrix proteins, we set out to determine whether similar changes could be found in the setting of "naturally" occurring insulin resistance in muscle of obese nondiabetic individuals and patients with type 2 diabetes mellitus. To answer this, we used percutaneous biopsies of the vastus lateralis muscle taken from lean, healthy volunteers and insulin-resistant obese nondiabetic and diabetic participants (4). As expected, the obese and diabetic individuals were insulin resistant compared with lean controls. Collagen content of muscle was assessed by two means. First, hydroxyproline content of acid hydrolysates of muscle was used as a marker for collagen (4), and second, immunofluorescence histochemistry was employed to localize the site or sites of collagen deposition. Hydroxyproline content of tissue from both obese and diabetic volunteers showed a profound increase, providing evidence for increased collagen content. Immunofluorescence staining also showed increased collagen types I and III deposition and revealed that, similar to data from the lipid infusion experiments (Fig. 4A), the major increases in collagen deposition occurred in the endomysium, the matrix layer surrounding individual muscle fibers. Excess deposition of collagen appeared to be similar in obese nondiabetic and type 2 diabetic individuals (Fig. 4B), suggesting that this was associated with insulin resistance rather than hyperglycemia.
Fig. 4. A: hydroxyproline content of acid hydrolysates of biopsies of vastus lateralis muscle from lean (n = 10), obese (n = 10), and diabetic (n = 10) subjects. Data are shown as means ± SE. *P < 0.05 vs. lean control values. Data are redrawn from Berria et al. (4). B: immunofluorescence staining of 5-μm sections of biopsies of vastus lateralis muscle from lean, obese, and diabetic subjects for types I (top) and III (bottom) collagen. Data are redrawn from Berria et al. (4).
through dystroglycan and sarcoglycan complexes in the sarclemma. 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-

Table 1. Changes in mitochondrial, extracellular matrix, and cytoskeletal/structural genes and proteins in human skeletal muscle insulin sensitivity

<table>
<thead>
<tr>
<th>Naturally Occurring Insulin Resistance</th>
<th>Lipid-Induced Insulin Resistance</th>
<th>Reduction in Lipid Supply by Acipimox Treatment</th>
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</thead>
<tbody>
<tr>
<td>Decreased PGC-1α and oxidative phosphorylation</td>
<td>Hwang et al. (23), Lefort et al. (23), Patti et al. (42), Mootoo et al. (37)</td>
<td>Richardson et al. (49)</td>
</tr>
<tr>
<td>Increased collagens and other extracellular matrix genes, such as CTGF</td>
<td>Berria et al. (4)</td>
<td>Richardson et al. (49)</td>
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<tr>
<td>Increased proteasomes and chaperones</td>
<td>Hwang et al. (23)</td>
<td>No change</td>
</tr>
<tr>
<td>Decreased structural proteins</td>
<td>Hwang et al. (23)</td>
<td>No change</td>
</tr>
</tbody>
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PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; CTGF, connective tissue growth factor.
DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


