Insulin resistance, which can be defined as a state of reduced responsiveness to normal circulating levels of insulin, plays a major role in the development of type 2 diabetes. This conclusion is based on the following observations: (1) cross-sectional studies demonstrating the consistent presence of insulin resistance in patients with type 2 diabetes; (2) the presence of insulin resistance in the nondiabetic offspring of patients with type 2 diabetes; (3) prospective studies demonstrating the usefulness of insulin resistance as a predictive marker of the future development of type 2 diabetes; and (4) prevention of diabetes by insulinsensitizing agents. Insulin resistance is also a key determinant of the association among obesity, diabetes, the metabolic syndrome (also commonly referred to as syndrome X), and atherosclerotic cardiovascular disease.

Although standard definitions of insulin resistance still define it in terms of the effects of insulin on glucose metabolism, the last decade has seen a shift from the traditional “glucocentric” view of diabetes to an increasingly acknowledged “lipocentric” viewpoint. This hypothesis holds that abnormalities in fatty acid metabolism may result in inappropriate accumulation of lipids in muscle, liver, and β-cells. It is further proposed that ectopic lipid accumulation is involved in the development of insulin resistance in muscle and liver as well as impairing β-cell function (so-called “lipotoxicity”). Lipid accumulation within myocytes and hepatocytes is strongly associated with insulin resistance in diabetics, nondiabetic relatives of patients with type 2 diabetes, a cohort at high risk of developing diabetes, individuals with impaired glucose tolerance, and obese subjects. In fact, nuclear magnetic resonance spectroscopy (MRS) measurements of intramyocellular lipids (IMCLs) correlate more closely with insulin resistance than any other commonly measured indices, including body mass index, waist/hip ratios, and total body fat. Nonalcoholic steatohepatitis is also recognized increasingly as a component of insulin resistance or metabolic syndrome. Somewhat counterintuitively, studies of lipodystrophic rodents and humans have provided several important pieces of information in the development of this hypothesis, the basic idea being that lipodystrophy and obesity represent states of inadequate storage capacity for surplus energy. The lipodystrophic syndromes encompass a rare group of conditions characterized by partial or complete absence of adipose tissue. The disorders may be genetic or acquired, and are further classified according to the anatomical distribution of the lipodystrophy. In obesity, the adipose depot is “overloaded” because of excess energy intake and reduced energy utilization, whereas in lipodystrophy, the limited adipose tissue storage capacity cannot cope with normal energy intake (the problem is exacerbated by the tendency of people with lipodystrophy to “over-eat” as a consequence of low plasma leptin levels). One particularly compelling piece of evidence in support of the lipotoxicity theory of diabetes was provided recently by transplanting adipose tissue into genetically engineered lipodystrophic mice. Despite only partially
restoring adipose tissue mass in recipient mice, intramuscular and intrahepatic lipids were significantly reduced, and insulin sensitivity in liver and muscle was dramatically improved. Another way in which ectopic lipid deposits can be reduced in lipodystrophic mice and humans is by replacing leptin, an anorexogenic adipocyte-derived hormone. This leads to a significant reduction in energy intake and dramatic improvements in insulin-stimulated liver and muscle carbohydrate metabolism.

In this brief review, we begin by considering the key rate-controlling steps in insulin-stimulated glucose disposal. We then review recent insights into the molecular mechanisms by which disorders of fatty acid/lipid metabolism might impair insulin-stimulated glucose disposal and delineate possible mechanisms of ectopic lipid accumulation. Finally, we examine the potential impact of inflammatory pathways on the insulin-signaling cascade and the notion that inflammation in adipose tissue may be involved in inducing systemic insulin resistance in obese states.

**Insulin Resistance and Muscle Glucose Metabolism**

$^{13}$C MRS studies of glucose disposal in normal humans suggested that skeletal muscle accounts for the majority of insulin-stimulated glucose uptake and that >80% of this glucose is then stored as glycogen. The rate of glycogen synthesis in skeletal muscle was 50% lower in diabetic subjects than in normal volunteers. The other key organ capable of storing a significant amount of glycogen is the liver, and here again, glycogen stores were reduced in diabetics. Subsequent studies focused on the rate-controlling steps in this pathway. $^{13}$C and $^{31}$P MRS were used together to monitor intracellular glucose-6-phosphate concentration and intramuscular glycogen synthesis during hyperinsulinemic–hyperglycemic clamps. Glucose-6-phosphate is an intermediate between glucose transport and glycogen synthesis (Figure 1). The increment in glucose-6-phosphate concentration was significantly reduced in type 2 diabetics, suggesting that glucose transport or phosphorylation must be the rate-controlling step in insulin-stimulated glucose disposal in skeletal muscle rather than glycogen synthase. Similar observations were also made in insulin-resistant offspring of type 2 diabetics, suggesting that this defect precedes the development of type 2 diabetes. Glucose transport in skeletal muscle is largely mediated by a specific insulin-responsive transporter known as glucose transporter 4 (GLUT4), whereas glucose phosphorylation is catalyzed by hexokinase. To determine which of these 2 steps was defective, we used a novel $^{13}$C MRS method to assess intracellular-free glucose in muscle, the idea being that if hexokinase were rate controlling in insulin-resistant type 2 diabetics, intracellular glucose concentrations should increase substantially. The fact that intracellular glucose concentrations in skeletal muscle from type 2 diabetics (during a hyperinsulinemic–hyperglycemic clamp) were 1/25 what they would have been if hexokinase were the primary rate-controlling enzyme suggested that glucose transport was rate controlling as opposed to hexokinase. Together, these data indicate that glucose transport into muscle is the rate-controlling step for insulin-stimulated muscle glycogen synthesis in patients with type 2 diabetes.

**Fatty Acid/Lipid-Induced Insulin Resistance**

Lipid infusions designed to increase plasma fatty acid concentration in humans and rodents reduce insulin-stimulated glucose disposal. Furthermore, the fall in insulin sensitivity during such clamp procedures only occurs several hours after elevations in FA concentrations, in keeping with the idea that FA accumulation in skeletal muscle and liver is responsible for this phenomenon. Randle et al originally showed that fatty acids compete with glucose for substrate oxidation in isolated rat heart muscle and rat diaphragm muscle. They speculated that an increase in fat oxidation might be responsible for insulin resistance in obese states. According to their proposal, increased fatty acid oxidation would cause an increase in the mitochondrial acetyl coenzyme A (CoA):CoA and NADH:NAD+ ratios with subsequent inactivation of pyruvate dehydrogenase. In turn, this would induce a rise in intracellular citrate levels, leading to inhibition of phosphofructokinase and glucose-6-phosphate accumulation. Because glucose-6-phosphate inhibits hexokinase activity, this would result in intracellular glucose accumulation and decreased glucose uptake. A series of studies by our group has challenged this hypothesis. Nonesterified fatty acid levels in healthy subjects were maintained at either high or low levels during hyperinsulinemic–euglycemic clamps. Maintaining high free fatty acid levels for 5 hours caused the expected reduction in insulin sensitivity, as assessed by glucose uptake, glucose oxidation, and glycogen synthesis in skeletal muscle, just as had been observed in type 2 diabetics and their insulin-resistant offspring. However, rather than increasing intracellular glucose-6-phosphate levels, as predicted by the Randle hypothesis, we found that elevating free fatty acid levels reduced intracellular glucose-6-phosphate levels. This was consistent with what had been observed in type 2 diabetics.

Fatty acid infusion could conceivably have direct effects on GLUT4 activity, or it could alter insulin-regulated GLUT4 trafficking between intracellular compartments and the cell.
membrane. To explore the latter possibility, we examined insulin-signaling intermediates in skeletal muscle biopsies from subjects exposed to high fatty acid levels for 5 hours before and during hyperinsulinemic–euglycemic clamps. Glucose oxidation and glycogen synthesis were 50% to 60% lower after the lipid infusion than with the glycerol (control) infusion and were associated with an ~90% decrease in the increment in intramuscular glucose-6-phosphate concentration, implying diminished glucose transport or phosphorylation activity. The fact that intracellular glucose concentrations were significantly lower in the lipid infusion studies compared with those during glycerol infusion implied that glucose transport was the rate-controlling step. Insulin receptor substrate-1 (IRS-1)–associated phosphoinositol 3-kinase (PI 3-kinase) activity was significantly reduced under these conditions (Figure 2). Subsequent rodent and human studies suggested that this might be a consequence of serine phosphorylation of IRS-1. An important and as yet unanswered element in this proposed mechanism for insulin resistance is the precise nature of the lipid moiety responsible for fatty acid induced–insulin resistance. Although triglyceride accumulation clearly correlates with insulin resistance, triglycerides are generally perceived to be metabolically inert associates of more favored candidates, which include long-chain acyl-CoAs and diacylglycerol. If this hypothesis is true, any perturbation that results in accumulation of fatty acyl-CoA or other fatty acid derivative within muscle and liver, either through increased delivery or decreased metabolism, ought to induce insulin resistance.

Mechanisms of Lipid Accumulation in Skeletal Muscle and Liver

Lipid accumulation in ectopic sites can occur in 3 ways: increased uptake of fatty acids, increased synthesis within the tissue involved, or reduced fatty acid oxidation/disposal. As alluded to above, it is clear that lipid infusion leads to lipid accumulation in skeletal muscle and short-term high-fat feeding elevates liver triglycerides; in both cases, insulin resistance ensues. Thiazolidinediones, now widely used as insulin sensitizers in the treatment of type 2 diabetes, act, at least in part, by lowering plasma free fatty acids and reversing the tendency to accumulate lipids in ectopic sites.

Increased fatty acid concentrations are typical of a number of insulin-resistant states, including obesity, type 2 diabetes, and insulin-resistant offspring of type 2 diabetics, suggesting that this may well contribute to ectopic lipid accumulation. We have also shown that mice overexpressing lipoprotein lipase in either skeletal muscle or liver accumulate lipid in the corresponding tissue and go on to manifest insulin resistance in a tissue-specific manner. An interesting hypothesis suggests that hyperinsulinemia may be involved in driving lipogenesis in muscle and liver by increasing sterol regulatory element-binding protein 1c (SREBP1c) expression. SREBP1c is a key transcriptional regulator of de novo lipogenesis. Finally, any defect in fatty acid oxidation would be expected to induce lipid accumulation. We demonstrated recently that insulin resistance in the elderly is and in lean healthy insulin-resistant offspring of type 2 diabetics is associated with IMCL accumulation, which in turn was linked to a reduction in mitochondrial oxidative phosphorylation activity assessed by 13C/31P MRS. In the case of the elderly, this is likely to be a consequence of acquired mitochondrial mutations, a phenomenon known to occur with aging, whereas in the insulin-resistant offspring, it is more likely that the reduction in mitochondrial oxidative phosphorylation is a primary genetic defect. Enzyme defects have also been described in isolated mitochondria derived from human skeletal muscle biopsies from type 2 diabetics. Together, these data suggest that alterations in nuclear-encoded genes that regulate mitochondrial biogenesis, such as peroxisome proliferator-activated receptor γ-coactivator 1α (PGC-1α), AMP kinase, and Ca2+/calmodulin dependent-protein kinase IV (CAMKIV), may form the genetic basis for inheritance of at least some forms of type 2 diabetes. This notion is further supported by 2 microarray studies that revealed a reduction in PGC-1α–responsive transcripts in patients with type 2 diabetes and their first-degree relatives. PGC-1α is a key regulator of mitochondrial biogenesis.

Inflammation and Insulin Resistance

Obesity is a very common cause of insulin resistance. As mentioned above, a potential mechanism for this relationship is ectopic lipid accumulation. However, obesity is also associated with a systemic chronic inflammatory response characterized by altered cytokine production and activation of inflammatory signaling pathways. Recent reports have linked this inflammatory response to the development of insulin resistance in 2 different ways. First, activation of inflammatory signaling intermediates may be directly involved in serine phosphorylation of IRS-1 within insulin-sensitive cell types such as hepatocytes and myocytes and thereby in inducing insulin resistance. Second, inflammatory cell infiltration within adipose tissue may be involved in altering adipocyte lipid metabolism (for example, tumor
necrosis factor-α (TNF-α) is reported to promote lipolysis) as well as altering cytokine production by adipose tissue, which may in turn have downstream effects in other metabolically important tissues.49

Inflammatory cytokines such as TNF-α and interleukin-6 (IL-6) have been linked to insulin resistance for some time.49 For example, TNF-α expression is increased in adipose tissue in obese rodents and humans,50 and reducing TNF-α signaling either by knocking TNF-α out or by infusing blocking antibodies can reduce insulin resistance in obese rodents.51 However, TNF-α antibody infusion in humans was shown not to alter insulin sensitivity,52 fueling lingering uncertainty about the biological relevance of this pathway in human insulin-resistant states. A key mechanism by which TNF-α was thought to induce insulin resistance involved serine phosphorylation of IRS-1.53–55 Intriguingly, Yuan et al56 proposed recently that fatty acid–induced serine phosphorylation of IRS-1 might be mediated by another inflammatory signaling intermediate, namely IkB kinase-β (IKK-β). This hypothesis has been supported by studies demonstrating that pharmacological inhibition of IKK-β activity by high-dose salicylates (high doses of salicylates are required to inhibit IKK-β activity) and heterozygous deletion of the IKK-β gene could ameliorate insulin resistance in obese rodents during high-fat feeding or lipid infusion.57 We have also shown that high-dose salicylate therapy for 2 weeks reduced fasting hyperglycemia and basal hepatic glucose production and improved peripheral glucose uptake in patients with type 2 diabetes.58 Hotamisligil et al identified another inflammatory serine kinase potentially involved in inducing serine phosphorylation of IRS-1, namely Jun kinase 1 (JNK1).59 They reported increased JNK activity in obese rodents and human adipose tissue, as well as reduced adiposity and improved insulin sensitivity in JNK1 knockout mice. A cell-permeable JNK-inhibitory peptide improved glucose tolerance and insulin sensitivity when administered to diabetic mice and mice with diet-induced insulin resistance.60 Nakatani et al61 used adenoviruses to either overexpress JNK or inhibit JNK activity (with a dominant-negative construct) in the liver. In each case, JNK activity correlated with serine phosphorylation of IRS-1 and insulin resistance. Suppressor of cytokine signaling 3 (SOCS3) is another potential contributor to the links among obesity, inflammation, and insulin resistance.62 The SOCS family of proteins is thought to participate in negative feedback loops in cytokine signaling. Their expression is usually increased by cytokine signaling through activation of signal transducers and activators of transcription— and nuclear factor κB–mediated pathways. Interestingly, 2 recent articles reported increases in SOCS3 in obese rodents.62,63 In vitro overexpression studies suggest that SOCS3 interacts directly with the insulin receptor, thereby inhibiting IRS-1 tyrosine phosphorylation and ultimately reducing insulin-stimulated glycogen synthesis in cultured myotubes. Increases in SOCS3 expression have also been observed in the hypothalamus, where SOCS3 may be involved in inducing leptin resistance.64 This notion provides a potentially unifying mechanism for insulin and leptin resistance, states that frequently coexist in obese humans.

In addition to its key role in energy storage, adipose tissue secretes a number of cytokine-like proteins (“adipokines”). Some of these appear to be secreted by adipocytes themselves, whereas others are largely produced by cells in the stromovascular fraction of adipose tissue.65 Macrophages constitute a significant proportion of the stromovascular fraction. Their numbers are significantly increased in obese states, in which they appear to make a substantial contribution to gene expression within adipose tissue.66–68 These cells are derived from bone marrow precursors and appear to infiltrate adipose tissue in obese states. Whether this inflammatory infiltrate is responsible for the development of insulin resistance is not yet clear, although Xu et al67 did suggest that the increase in inflammatory gene expression within adipose tissue preceded the dramatic increase in plasma insulin levels noted in high-fat–fed mice. They also reported downregulation of these macrophage-derived genes in response to treatment with an insulin-sensitizing agent (rosiglitazone).67

In addition to those mentioned above, deficiency or excess of a number of other adipokines may also be involved in the pathogenesis of insulin resistance. Because this topic has been the subject of a number of recent reviews, it has not been discussed in detail here.68–70 In general, the role of this cluster of proteins in modifying insulin sensitivity remains uncertain. A number of the proteins are produced by cells/tissues other than adipocytes (for example, plasminogen activator inhibitor-1 and apolipoprotein E), where some have well-defined biological roles, making it difficult to be certain of their functional significance within adipose tissue metabolism. Adiponectin is an exception to this concept because although it shares some sequence homology with type VIII and X collagen and complements component C1q, and has a similar tertiary structure to that of TNF-α, it is highly and specifically expressed in differentiated adipocytes.71 Adiponectin receptors 1 and 2 have been identified and are expressed in key insulin-sensitive tissues such as liver and skeletal muscle.72 Association studies have consistently linked plasma adiponectin levels to insulin sensitivity in rodent and human models.73 They have also suggested that adiponectin may have anti-inflammatory and antiatherogenic activity. In our opinion, the fact that we did not find significant differences in plasma levels of adiponectin, resistin, IL-6, TNF-α, or leptin in either lean insulin-resistant offspring of type 2 diabetics74 or lean insulin-resistant elderly subjects when compared with insulin-sensitive controls suggests that inflammatory changes are unlikely to be the primary abnormality in these groups of subjects. Instead, we believe that inflammatory cell recruitment and changes in cytokine production may be secondarily involved in maintaining/exacerbating the insulin-resistant state associated with obesity. However, it is likely to be a key factor in the insulin resistance associated with pathological states such as sepsis and burn trauma.75

Summary

Insulin resistance plays a major role in the pathogenesis of type 2 diabetes and the metabolic syndrome. Insulin resistance in skeletal muscle manifests primarily as a reduction in insulin-stimulated glycogen synthesis, which is in turn a consequence of reduced glucose transport. Lipid accumula-
tion within skeletal muscle and liver inhibits tyrosine phosphorylation of IRS-1 (tyrosine phosphorylation of IRS-2 is also inhibited in fatty liver). In turn, this follows serine phosphorylation of critical sites on IRS-1 and inhibits binding and activation of PI 3-kinase. A number of different serine kinases could be responsible for serine phosphorylation of IRS-1. Candidates include members of the novel protein kinase C family, which may be activated by accumulation of lipid intermediates, as well as inflammatory intermediates such as IKK-β, JNK1, and TNF-α. The latter may be activated within adipose tissue in obese states. Lipid accumulation in skeletal muscle and liver may be a result of increased delivery/synthesis of fatty acids to/in these tissues in states in which energy intake exceeds adipose tissue storage capacity (as seen in obesity and lipodystrophy) or a consequence of either acquired or inherited mitochondrial dysfunction. These novel ideas about the molecular pathogenesis of insulin resistance are beginning to provide numerous new therapeutic targets for the treatment and possible prevention of type 2 diabetes.

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