Type 2 diabetes currently affects more than a third of a billion people worldwide and is the leading cause of end-stage renal disease, non-traumatic loss of limb, and blindness in working adults, with estimated annual worldwide health care costs exceeding half a trillion dollars. Furthermore, the worldwide prevalence of type 2 diabetes is projected to increase by more than 75% during the next two decades, with the largest increases occurring in Asia and the Indian subcontinent. Although impaired beta-cell function is ultimately responsible for the progression from normoglycemia to hyperglycemia, insulin resistance predates beta-cell dysfunction and plays a major role in the pathogenesis of type 2 diabetes. After carbohydrate ingestion, glucose is deposited primarily in muscle and the liver as glycogen, and alterations in insulin responsiveness in these organs result in fasting and postprandial hyperglycemia.

In this review, I focus on recent studies using magnetic resonance spectroscopy (MRS) that have implicated ectopic lipid accumulation in the pathogenesis of insulin resistance in muscle and the liver and have clarified the role of muscle-specific insulin resistance in promoting increased hepatic lipogenesis, nonalcoholic fatty liver disease, and atherogenic dyslipidemia. I then propose a potential link between inflammation and macrophage-induced lipolysis in the progression from ectopic lipid–induced insulin resistance to impaired glucose tolerance and type 2 diabetes.
muscle cells showed that both these metabolites decreased in human muscle during induction of insulin resistance by means of a lipid infusion (Fig. 1B). The reduction in insulin-stimulated glucose-transport activity in healthy persons that is induced during a lipid infusion is similar...
to that observed in obese persons with insulin resistance,\(^\text{12}\) in patients with type 2 diabetes,\(^\text{23}\) and in lean, normoglycemic persons with insulin resistance whose parents have type 2 diabetes.\(^\text{14}\) Taken together, these data led to an alternative hypothesis that accumulation of an intracellular lipid metabolite mediates insulin resistance associated with obesity and type 2 diabetes by causing defects in insulin signaling and reduced insulin-stimulated glucose-transport activity (Fig. 1B).\(^\text{11,15}\)

### Molecular Mechanisms of Insulin Resistance in Muscle and the Liver

Insulin action in muscle and the liver requires a coordinated relay of intracellular signals involving mostly phosphorylation and dephosphorylation events. In skeletal muscle, insulin binds and activates the insulin receptor tyrosine kinase, with subsequent phosphorylation of insulin receptor substrate 1 (IRS-1) (Fig. 1B). When phosphorylated, IRS-1 binds and activates phosphatidylinositol 3-kinase (PI3K), which in turn, through signaling intermediaries, promotes translocation of glucose transporter type 4 (GLUT4) to the plasma membrane, resulting in glucose uptake into the skeletal muscle. Insulin-stimulated tyrosine phosphorylation of IRS-1 and associated PI3K activation have been shown to be impaired in muscle during lipid infusion in humans\(^\text{11}\) and rodents,\(^\text{15,16}\) indicating that the lipid-induced reduction in insulin-stimulated glucose transport could be attributable to a proximal defect in insulin signaling owing to an intracellular fatty acid–derived signal.\(^\text{11}\)

This signal was identified in studies of lipid-infused rodents and rodents fed high-fat diets, which showed transient increases in muscle diacylglycerol (DAG) content\(^\text{16}\) and sustained activation of the theta form of protein kinase C (PKC\(\theta\))\(^\text{10,13}\) leading to activation of a serine–threonine kinase cascade and inhibition of insulin signaling. Furthermore, lipid-induced PKC\(\theta\) activation in these studies could be dissociated from increases in other putative lipid signals such as muscle ceramide and triglyceride content.\(^\text{16}\) The importance of DAG–novel protein kinase C (nPKC) activation and serine phosphorylation of IRS-1 for mediating lipid-induced insulin resistance in muscle was subsequently shown in mice lacking PKC\(\theta\)\(^\text{17}\) and mice carrying Ser→Ala mutations in key residues of IRS-1 (preventing serine hyperphosphorylation of IRS-1); both types of mice were protected from lipid-induced insulin resistance in muscle.\(^\text{18}\) Additional in vitro studies have shown that IRS-1 at Ser 1101 is a target of PKC\(\theta\) that inhibits insulin signaling.\(^\text{19}\)

Similar findings have been reported in humans: DAG content has been shown to increase transiently in human skeletal muscle after infusion of lipid plus heparin\(^\text{20}\) or lipid only,\(^\text{21}\) and increased DAG content in muscle is associated with increases in PKC\(\theta\) activity and phosphorylation of IRS-1 at Ser 1101.\(^\text{21}\) In addition, increased muscle DAG content, along with increased PKC\(\theta\) activity and increased serine phosphorylation of IRS-1, has been observed in muscle of obese persons with insulin resistance\(^\text{22-23}\) and persons with type 2 diabetes.\(^\text{21,24}\)

PKC\(\theta\) activation of an nPKC has been shown to cause insulin resistance in the liver as well as in muscle. Hepatic steatosis and hepatic insulin resistance develop in rodents after just a few days of high-fat feeding, without any significant change in lipid content or insulin resistance in muscle.\(^\text{25}\)

In this model, hepatic steatosis and hepatic DAG accumulation were associated with proximal defects in insulin signaling with decreased insulin-stimulated tyrosine phosphorylation of IRS-1 and IRS-2 by the insulin receptor, ultimately interfering with insulin-induced activation of glycolgen synthesis and suppression of glucose production in the liver (Fig. 2).

The defect in insulin-stimulated hepatic glycolgen synthesis is similar to that in patients with type 2 diabetes.\(^\text{26,27}\) Though PKC\(\theta\) expression is minimal in the liver, the epsilon form of protein kinase C (PKCe), another nPKC, is expressed at high levels in the liver and is activated in rodent models of nonalcoholic fatty liver disease. The association between DAG–PKCe activation in the liver and hepatic insulin resistance has now been shown in multiple transgenic or knockout rodent models of nonalcoholic fatty liver disease.\(^\text{28-31}\) More important, increased hepatic DAG content\(^\text{33,34}\) and increased PKCe activity\(^\text{33}\) are the strongest predictors of hepatic insulin resistance in obese humans with nonalcoholic fatty liver disease.

The specific role of PKCe in the pathogenesis of hepatic insulin resistance has been genetically validated with the use of antisense oligonucleotides for knockdown of hepatic expression...
of PKCe. Antisense knockdown of hepatic PKCe expression abrogated lipid-induced defects in hepatic insulin signaling and hepatic insulin resistance in rats fed high-fat diets, despite similar increases in hepatic triacylglycerol or DAG content in control and PKCe knockdown animals. Similar protection from lipid-induced insulin resistance has also been observed in whole-body PKCe knockout mice.35

**Figure 2. Molecular Mechanisms of Lipid-Induced Hepatic Insulin Resistance.**

In the liver, a transient increase in DAG, due to an imbalance of intrahepatocellular fluxes, results in activation of the epsilon isofrom of protein kinase C (PKCe). Specifically, this transient increase in hepatocellular DAG occurs when rates of DAG synthesis, from both fatty acid re-esterification and de novo lipogenesis, exceed rates of mitochondrial long-chain CoA (fat) oxidation, rates of DAG incorporation into neutral lipid (TAG), or both. Activated PKCe binds to and inhibits the insulin receptor tyrosine kinase, leading to decreased insulin-stimulated glycogen synthesis in the liver owing to decreased phosphorylation of GSK3. This results in inhibition of glycogen synthase activity and decreased insulin suppression of hepatic gluconeogenesis through decreased phosphorylation of forkhead box subgroup O (FOXO), leading to increased FOXO translocation to the nucleus, where it promotes increased gene transcription of the gluconeogenic enzymes (e.g., phosphoenolpyruvate carboxykinase [PEP-CK] and G6P).

**Dissociation of Obesity from Insulin Resistance in Muscle and the Liver**

The most common cause of ectopic lipid deposition in skeletal muscle and the liver is a level of energy intake that exceeds the level of energy expenditure, resulting in spillover of energy storage from adipose tissue to the liver and skeletal muscle (Fig. 3). In contrast to obesity, the lipodystrophies offer a unique opportunity to assess the role of ectopic lipid deposition without any contribution from an expansion of peripheral or visceral adipose-tissue mass. The lack of subcutaneous fat leads to hypertriglyceridemia, ectopic fat deposition (including marked hepatic steatosis), and profound insulin resistance in muscle and the liver (Fig. 3).36 In lipoatrophic A-ZIP/F-1 mice, which lack adipocytes, fat accumulates in the liver and skeletal muscle, and profound insulin resistance occurs in these tissues.41 Remarkably, fat obtained from wild-type littermates and transplanted subcutaneously into these fatless mice normalizes ectopic fat content in muscle.
and the liver as well as insulin signaling and insulin action in these organs.41

Further evidence in support of the role of ectopic lipid accumulation in the pathogenesis of insulin resistance in muscle and the liver comes from studies in transgenic mice with overexpression of lipoprotein lipase.42 Transgenic mice with targeted overexpression of lipoprotein lipase in the liver have liver-specific fat accumulation and liver-specific insulin resistance. Similarly, transgenic mice with targeted overexpression of lipoprotein lipase in skeletal muscle have muscle-specific fat accumulation and muscle-specific insulin resistance.42,43 Taken together, these studies show that ectopic accumulation of intracellular lipid leads to insulin resistance in muscle and the liver even in the absence of peripheral and visceral adiposity and that DAGs are the lipid-derived metabolites responsible for triggering insulin resistance through activation of PKCe in the liver and PKCθ in muscle.

There are a few notable exceptions in which accumulation of ectopic lipid in muscle and the liver has been disassociated from insulin resistance. One exception is the Chanarin–Dorfman syndrome,44-46 which is due to a deficiency in the protein termed comparative gene identification 58 (CGI-58).45 Studies have shown that cellular compartmentalization of DAGs within lipid droplets is the likely explanation for the dissociation of ectopic lipid accumulation from insulin resistance in this syndrome.45 DAGs in lipid droplets, in contrast to DAGs located in the plasma membrane and cytosolic compartments, do not promote PKCe translocation to the plasma membrane, where PKCe binds to the insulin receptor, leading to inhibition of its tyrosine kinase activity and hepatic insulin resistance.45 Whether similar cellular compartmentalization of DAGs within lipid droplets explains the dissociation between increased ectopic lipid accumulation and insulin resistance in other situations, such as in cases of familial hypobetalipoproteinemia44 and in muscle of endurance athletes,47 remains to be determined.

**ROLE OF MITOCHONDRIAL DYSFUNCTION IN ECTOPI C LIPID ACCUMULATION**

Lipid content in muscle cells reflects a net balance between rates of fatty acid uptake by the cells and rates of mitochondrial fat oxidation. In this regard, acquired mitochondrial dysfunction has been shown to be an important predisposing factor for ectopic lipid accumulation and insulin resistance in the elderly (Fig. 3). Healthy, lean, elderly persons were shown to have markedly reduced insulin-stimulated glucose uptake by muscle as compared with that in young persons matched for lean body mass and fat mass. In elderly persons, insulin resistance in muscle was associated with increased lipid accumulation in muscle cells and a reduction of approximately 40% in both mitochondrial oxidative and phosphorylation activity, as assessed by means of in vivo 13C and 31P MRS, in comparison with mitochondrial oxidative and phosphorylation activity...
in young controls.38 These data support the hypothesis that age-associated reductions in mitochondrial function, possibly due to cumulative damage by reactive oxygen species (ROS), predispose the elderly to ectopic lipid accumulation and insulin resistance in muscle.38

Boumezbeur et al. found similar reductions in neuronal mitochondrial activity in healthy elderly persons, observations that are consistent with this hypothesis and suggest that the age-associated reductions in mitochondrial activity may be occurring in multiple organs.48 Genetic evidence that age-associated ROS-induced reductions in mitochondrial function play a critical role in the pathogenesis of age-associated insulin resistance in muscle was provided by studies of transgenic mice with an overexpression of human catalase targeted to the mitochondria.37 These mice were protected from age-associated reductions in muscle mitochondrial function and lipid (DAG–PKCθ)-induced insulin resistance in muscle. This protection from an age-induced reduction in mitochondrial function was associated with reduced mitochondrial oxidative damage, preserved ATP synthesis in muscle, and AMP-activated protein kinase–induced mitochondrial biogenesis.49,50

Taken together, these data show that acquired age-associated reductions in mitochondrial function promote ectopic lipid accumulation in skeletal muscle and insulin resistance in muscle. They also suggest that preserving mitochondrial function by reducing mitochondrial oxidative damage may be a therapeutic target for preventing age-associated reduction in muscle mitochondrial function, insulin resistance in muscle, and type 2 diabetes in the elderly.

Reductions of approximately 40% in mitochondrial oxidative and phosphorylation activity in muscle have been observed in healthy, young, lean persons with insulin resistance whose parents have type 2 diabetes.40,51 The decrease in flux in the tricarboxylic acid cycle and ATP synthesis in muscle was paralleled by a reduction of approximately 40% in mitochondrial content.52 Thus, at least in this cohort, it is likely that a reduction in mitochondrial content, owing to reduced mitochondrial biogenesis, is responsible for the reduced mitochondrial oxidative and phosphorylation activity and may be an acquired abnormality.39,52 Nevertheless, given the key role of mitochondrial activity in the regulation of fat metabolism in muscle cells,28,30,32,53,54 these data suggest that the reduced mitochondrial function may be an important predisposing factor that promotes DAG accumulation in muscle cells and insulin resistance in muscle among persons with insulin resistance whose parents have type 2 diabetes.

| GENETIC ALTERATIONS PROMOTING ECTOPIC LIPID ACCUMULATION IN THE LIVER |

Although nonalcoholic fatty liver disease is most often associated with obesity, there are important exceptions to this rule in which nonalcoholic fatty liver disease and hepatic insulin resistance are observed in lean persons.36,55,56 Healthy, young, lean, Asian Indian men have a markedly higher prevalence of hepatic steatosis associated with hepatic insulin resistance than healthy, young, lean men of other races or ethnic groups.57 Polymorphisms in the insulin-response element for the gene encoding apolipoprotein C3 (APOC3) have been shown to predispose such persons to nonalcoholic fatty liver disease and insulin resistance.58 These polymorphisms led to a 30% increase in plasma apolipoprotein C3 concentrations. The increase in apolipoprotein C3 inhibits lipoprotein lipase activity, limiting peripheral clearance of chylomicrons and causing postprandial hypertriglyceridemia. As a result, carriers of the APOC3 variant alleles have increased hepatic uptake of lipids from chylomicron remnants, predisposing them to nonalcoholic fatty liver disease and hepatic insulin resistance (Fig. 3). These results were replicated in a cohort of lean men of European descent.59

Genetic evidence in support of the role of alterations in apolipoprotein in the regulation of hepatic triglyceride synthesis comes from studies in transgenic mice that overexpress human apolipoprotein C3 in the liver. When placed on a normal chow diet, the transgenic mice manifest no metabolic phenotype. However, when placed on a high-fat diet, these mice have much greater hepatic triglyceride and DAG accumulation associated with hepatic PKCε activation and hepatic insulin resistance than their wild-type littermates.60 These studies suggest that gene–environment interactions can predispose lean per-
sons to nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes, and such interactions may also involve many potential variants in plasma apolipoproteins (e.g., variants in apolipoprotein A5 and apolipoprotein A1) that are known to affect lipoprotein lipase activity. It is also noteworthy that the APOC3 gene–environment interaction has been observed in men only, probably reflecting a protective effect of estradiol on the ability of apolipoprotein C3 to inhibit lipoprotein lipase activity and promote ectopic fat storage in premenopausal women.61 Furthermore, the APOC3 gene–environment interaction is not observed in obese persons; such persons typically have hepatic steatosis, which will mask the relatively subtle effect that these APOC3 variants have in predisposing persons to nonalcoholic fatty liver disease and hepatic insulin resistance.

Hispanics represent another large ethnic group at risk for nonalcoholic fatty liver disease, insulin resistance, and type 2 diabetes. A genome-wide association study identified a missense mutation (I148 M in PNPLA3) that is more prevalent in Hispanics than in other ethnic groups and that is strongly associated with nonalcoholic fatty liver disease.62 Though the association between this polymorphism and hepatic steatosis has been reproduced in other populations, there is, surprisingly, no association with insulin resistance. However, these studies involved obese persons who already had insulin resistance, as measured with the use of a homeostatic model assessment, which is a relatively insensitive and nonspecific method for assessing insulin resistance.

Finally, as might be expected from the associations between ectopic lipid content and insulin resistance in lipodystrophic mice and humans, genes that regulate lipogenesis (e.g., AGRP and PPARG),63 leading to lipodystrophy, and alterations in genes that regulate lipolysis (e.g., the genes encoding perilipin [PLIN1])64 also lead to ectopic lipid deposition and insulin resistance.

**Reversal of Insulin Resistance and Diabetes by Reduction of Ectopic Fat**

Further evidence that ectopic lipid accumulation in muscle and the liver plays a causal role in the pathogenesis of insulin resistance and type 2 diabetes in humans is provided by studies showing that reduction of ectopic lipid content is associated with reversal of insulin resistance in these organs. One study showed that restoring plasma leptin to physiologic levels in patients with diabetes and lipodystrophy normalized fasting plasma glucose and plasma lipid concentrations.65 These improvements in insulin-stimulated glucose metabolism, which may be attributable to reversal of insulin resistance in muscle and the liver, were associated with large reductions in hepatic triglyceride content and muscle-cell fat content.66

Similarly, modest weight loss (approximately 10% of body weight) with a hypocaloric diet resulted in a marked reduction in hepatic triglyceride concentrations and normalization of hepatic insulin sensitivity, rates of hepatic glucose production, and fasting plasma glucose concentrations in patients with type 2 diabetes.67 Similarly, Lim et al. found marked reductions in liver fat and hepatic insulin resistance and reversal of type 2 diabetes in patients following a hypocaloric diet.68 Reductions in muscle-cell fat and the reversal of insulin resistance in muscle have also been observed after weight reduction of approximately 10% in young, lean persons with insulin resistance whose parents had type 2 diabetes.69

Thiazolidinediones also reduce hepatic steatosis and improve insulin sensitivity in muscle and the liver67,68 by enhancing adipocyte insulin sensitivity and shifting ectopic lipid from muscle and the liver to subcutaneous adipose tissue.67
Figure 4. Mechanism by which Selective Insulin Resistance in Skeletal Muscle Leads to Atherogenic Dyslipidemia and Nonalcoholic Fatty Liver Disease.

In healthy, young, lean persons, selective insulin resistance in skeletal muscle leads to diversion of ingested carbohydrate from muscle glycogen synthesis to the liver. This process, in combination with the compensatory hyperinsulinemia, leads to increased hepatic de novo lipogenesis, resulting in increased plasma triglyceride levels, reduced plasma high-density lipoprotein (HDL) levels, and increased hepatic triglyceride synthesis. This abnormal pattern of energy storage after carbohydrate ingestion can be reversed after a single 45-minute bout of moderate-intensity exercise with the use of an elliptical trainer, which promotes increased glucose uptake and glycogen synthesis in muscle through adenosine 5′-monophosphate–activated protein kinase (AMPK) activation of glucose-transport activity.

Mal pattern of energy storage was completely abrogated after a single bout of moderate-intensity exercise with the use of an elliptical trainer, which promoted muscle glycogen synthesis after carbohydrate ingestion through increased glucose-transport activity. These data show that insulin resistance in muscle is an early therapeutic target for the treatment and prevention of atherogenic dyslipidemia and nonalcoholic fatty liver disease in young persons with insulin resistance, who are prone to the metabolic syndrome and type 2 diabetes.

MACROPHAGE-INDUCED LIPOLYSIS, INFLAMMATION, AND FASTING HYPERGLYCEMIA

Although lipid-induced insulin resistance occurs early in the pathogenesis of type 2 diabetes and can be dissociated from inflammation at this stage, a key question concerns identification of the factors that promote the progression from insulin resistance associated with ectopic lipid accumulation to impaired glucose tolerance and fasting hyperglycemia. The canonical view of this process attributes impaired pancreatic beta-cell and alpha-cell function, along with inflammation, to this transition, in which beta-cell and alpha-cell defects lead to increased hepatic gluconeogenic gene transcription and inflammation inhibits insulin action through the release of cytokines and adipocytokines. Increased cytokine levels in turn lead to inhibition of insulin signaling and increased hepatic gluconeogenic protein transcription through activation of the nuclear factor κB, Jun N-terminal kinase, and ceramide biosynthetic pathways.

An alternative hypothesis linking inflammation to the progression to fasting hyperglycemia is the potential effect of macrophage-induced lipolysis on the regulation of hepatic gluconeogenesis (Fig. 5). In this regard, increased lipolysis in rat models of poorly controlled type 1 diabetes and type 2 diabetes results in increased hepatic gluconeogenesis in vivo by two nontranscriptionally mediated mechanisms. First, increased lipolysis leads to increased fatty acid delivery to the liver, resulting in increased hepatic acetyl CoA concentrations and increased hepatic gluconeogenesis through allosteric activation of pyruvate carboxylase. Second, increased lipolysis leads to increased glycerol delivery to the liver, resulting in increased conversion of glycerol to glucose through a substrate-driven mechanism. Subsequent long-term increases in hepatic gluconeogenesis could lead to impaired insulin secretion by beta cells and inappropriate glucagon secretion by alpha cells as a result of glucose toxicity, exacerbating fasting and postprandial hyperglycemia.

Although speculative, this hypothesis proposes that macrophage-induced lipolysis, as opposed to alterations in circulating cytokines and hepatic gluconeogenic protein transcription, is the major culprit in the transition from insulin resistance to impaired glucose tolerance and type 2 diabetes. This hypothesis is also consistent with a study that showed no relationship between hepatic gluconeogenic protein expression and fasting hyperglycemia in obese persons.
Ectopic lipid–induced insulin resistance represents a surfeit of intracellular energy in the form of DAGs, leading to activation of PKCθ in muscle and PKCε in the liver and subsequent inhibition of insulin signaling in these tissues. This hypothesis can explain the insulin resistance associated with obesity, aging, lipodystrophy, prediabetes, and type 2 diabetes and the reversal of insulin resistance and diabetes after weight loss and thiazolidinedione therapy. Teleologically, insulin resistance in muscle and the liver that is induced by DAGs and nPKCs may represent a cell-autonomous mechanism for turning off energy storage in liver and muscle cells when intracellular lipids are in excess and routing this excess energy to adipose tissue for storage.

Although reduction of ectopic lipid content and insulin resistance by means of weight-loss interventions (ideally combined with exercise) is clearly the preferred medical therapy for these disorders, recidivism after weight loss is extremely common. Bariatric surgery is more successful at achieving long-term weight loss, but this procedure is invasive, expensive, and not without risks. Consequently, there is a need for a drug that reduces ectopic liver fat and insulin resistance. In this regard, fibroblast growth factor 21 has been shown to be effective in reducing liver DAG–PKCε activity as well as hepatic insulin resistance in animals and is now under investigation in clinical trials.74

Another potential approach to decreasing ectopic lipid content has been the application of a liver-targeted mitochondrial protonophore to promote subtle increases in hepatic mitochondrial uncoupling. This approach has been shown to reverse hypertriglyceridemia, hepatic steatosis, insulin resistance, and hyperglycemia in rat models of nonalcoholic fatty liver disease and type 2 diabetes, with a relatively wide therapeutic index.75 In addition to decreasing hepatic triglyceride and DAG content, PKCε activity, and hepatic insulin resistance, this approach reduces hepatic acetyl CoA content, leading to decreased rates of hepatic gluconeogenesis and marked reductions in both fasting and postprandial hyperglycemia.75 Furthermore, by increasing liver-fat oxidation by 60%, this approach decreases hepatic production of very-low-density lipoprotein, resulting in decreased export of triglyceride to muscle and protection from lipid-induced insulin resistance in muscle.

In summary, these studies show the critical role of ectopic lipid accumulation in the pathogenesis of insulin resistance in muscle and the liver. This model also explains the improvements in insulin action with exercise, weight loss, and thiazolidinediones. Furthermore, increasing hepatic energy expenditure by promoting mitochondrial uncoupling could be a novel approach for treating the related epidemics of nonalcoholic fatty liver disease, the metabolic syndrome, and type 2 diabetes.
REFERENCES


