



Published in final edited form as:

Nature. 2014 June 5; 510(7503): 84–91. doi:10.1038/nature13478.

The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes

Rachel J. Perry¹, Varman T. Samuel^{1,2}, Kitt F. Petersen^{1,3}, and Gerald I. Shulman^{1,3,4,5}

¹Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA

²VA Connecticut Healthcare System West Haven, Connecticut 06516, USA

³Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen DK-2200, Denmark

⁴Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut 06520, USA

⁵Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06535-8012, USA

Abstract

Non-alcoholic fatty liver disease and its downstream sequelae, hepatic insulin resistance and type 2 diabetes, are rapidly growing epidemics, which lead to increased morbidity and mortality rates, and soaring health-care costs. Developing interventions requires a comprehensive understanding of the mechanisms by which excess hepatic lipid develops and causes hepatic insulin resistance and type 2 diabetes. Proposed mechanisms implicate various lipid species, inflammatory signalling and other cellular modifications. Studies in mice and humans have elucidated a key role for hepatic diacylglycerol activation of protein kinase C ϵ in triggering hepatic insulin resistance. Therapeutic approaches based on this mechanism could alleviate the related epidemics of non-alcoholic fatty liver disease and type 2 diabetes.

Modern global health care faces challenges that are drastically different from past generations, largely owing to the increasing worldwide prevalence of obesity. This is exemplified by a change in focus to centre on obesity-related liver disease. Although viral hepatitis continues to be an important health concern, non-alcoholic fatty liver disease (NAFLD) is the now most common liver disorder in the Western world, where the rates of adult and paediatric obesity have soared to an estimated 20–30% of the US population^{1,2}. In east and south Asian communities, NAFLD is also on the rise, with estimates that its prevalence reaches as high as 60% in urban areas^{3,4}. Startlingly, NAFLD has been found to be highly prevalent among young lean south Asian Indians^{5,6}.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence should be addressed to: G.I.S. (gerald.shulman@yale.edu).

The authors declare no competing financial interests.

Readers are welcome to comment on the online version of this paper at go.nature.com/cghfru.

A strong association between NAFLD and type 2 diabetes has been demonstrated: more than 90% of obese patients with type 2 diabetes have NAFLD⁷. Insulin resistance is common in both conditions⁵. Patients with NAFLD almost universally have hepatic insulin resistance, which increases the risk of impaired fasting glucose and type 2 diabetes^{5,8-11}. In addition, a subset of patients with NAFLD will develop non-alcoholic steatohepatitis (NASH) with histological changes such as steatosis, lobular inflammation and/or hepatocellular ballooning¹². Around 20% of patients with NASH will progress to liver cirrhosis and liver failure^{13,14}. NASH-associated cirrhosis is now the third most common indication for liver transplantation in the United States¹⁵. Health policies that can prevent NAFLD and new treatments that can reverse the disease will offer tremendous benefits, in terms of both lives saved and health-care costs.

Thus, in this Perspective we will discuss the link between hepatic lipid accumulation and hepatic insulin resistance and focus on the role of diacylglycerol, a lipid metabolite that activates novel protein kinase C iso-forms (PKCs) and thereby impairs insulin signalling, in the pathogenesis of lipid-induced hepatic insulin resistance. Although several other mechanisms have been proposed to explain this association, these alternatives have been reviewed elsewhere¹⁶. As we will discuss here, diacylglycerol-induced novel PKC activation has emerged as a common mechanism to explain the development of insulin resistance in liver and skeletal muscle in a variety of experimental and clinical models.

Molecular mechanism of lipid-induced insulin resistance

Insulin action requires a coordinated, intricate relay of intracellular signals, involving mostly phosphorylation and dephosphorylation events. In the canonical view of hepatic insulin signalling, insulin binds and activates the insulin receptor tyrosine kinase (IRTK), which in turn promotes tyrosine kinase phosphorylation of insulin receptor substrates (IRS), most importantly IRS2 in the liver (Fig. 1)¹⁷. Phosphorylation of IRS2 generates binding sites for Src homology 2 domain proteins, including phosphatidylinositol-3-OH kinase (PI(3)K)¹⁸. The binding of PI(3)K to IRS2 recruits phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃), which in turn recruits Akt¹⁹. Under insulin-stimulated conditions, 3-phosphoinositide-dependent kinase-1 phosphorylates and activates Akt, which is thought to suppress hepatic glucose production through two key mechanisms: first, decreased expression of gluconeogenic enzymes by phosphorylation and nuclear exclusion of the fork-head box protein FOXO1 and its pro-gluconeogenic targets, and second, activation of glycogen synthase by phosphorylation and inactivation of glycogen synthase kinase-3 β . Although this relatively linear construct is useful for interrogating insulin signalling in experimental models, it fails to capture the interwoven mechanisms that have evolved to regulate hepatic glucose and lipid metabolism. For example, although acute insulin signalling following a meal can decrease messenger RNA expression of gluconeogenic enzymes, it probably does not acutely alter the protein levels of these enzymes. Gluconeogenic enzymes are also conventionally thought to be subject to allosteric activation: acetyl coenzyme A (acetyl-CoA) activates pyruvate carboxylase^{20,21}, and fructose-2,6-bisphosphate inhibits fructose-1,6-bisphosphatase²². And, although insulin might activate glycogen synthesis, glucose is necessary to inhibit glycogen phosphorylase and effectively promote net hepatic glycogen synthesis^{23,24}.

The development of NAFLD is strongly associated with hepatic insulin resistance. This relationship is most apparent when NAFLD is induced in rats after just 3 days of being fed a high-fat diet. The ability of insulin to suppress hepatic glucose production is diminished in this model even without changes in body weight, adiposity or muscle insulin resistance²⁵. Hepatic insulin resistance in this model was associated with increased hepatic diacylglycerol content and increased translocation of the primary novel PKC isoform in liver, protein kinase-C ϵ (PKC ϵ)^{26,27}, to the plasma membrane at which it was found to bind and inhibit the activity of the intracellular kinase domain of the insulin receptor. This was associated with reduced insulin-stimulated phosphorylation of IRS2 and IRS2-associated PI(3)K activity and phosphorylation of Akt2. Consequently, the ability of insulin to activate glycogen synthesis and inhibit gluconeogenesis was impaired (Fig. 1)²⁵. The crucial role of PKC ϵ in mediating lipid-induced hepatic insulin resistance has been convincingly demonstrated by knocking down expression of PKC ϵ in the liver of rodents. Rats treated with an antisense oligonucleotide (ASO) to decrease hepatic expression of PKC ϵ were protected from lipid-induced hepatic insulin resistance, although hepatic diacylglycerol and triglyceride content were unchanged²⁶. Furthermore, these animals were found to have preserved IRTK activity with intact signalling through downstream proteins. Similarly, *Prkce* whole-body knockout mice are also protected from lipid-induced insulin resistance²⁸.

This model for lipid-induced hepatic insulin resistance has been translated to humans. Potential mechanisms for hepatic insulin resistance were assessed in a group of patients undergoing bariatric surgery. Although the participants were all obese, there was a significant variation in insulin resistance. Notably, individuals with very similar body mass index could manifest markedly different degrees of insulin resistance. By contrast, hepatic diacylglycerol content and PKC ϵ activation were the strongest predictors of hepatic insulin resistance in liver biopsies obtained from these individuals²⁹. There was no association between insulin sensitivity and other factors implicated in causing hepatic insulin resistance, including ceramide content, endoplasmic reticulum (ER) stress markers or inflammatory cytokine concentrations. These results were replicated in another study showing that hepatic diacylglycerol content was the best predictor of hepatic insulin resistance in obese humans, whereas there was no association with hepatic ceramide content or markers of inflammation³⁰. Indeed, hepatic inflammation has been suggested to be a consequence, not a cause, of insulin resistance³¹. Thus, although excess calorie intake certainly leads to obesity, only those who develop hepatic steatosis will develop insulin resistance. These data argue that the key step in the pathogenesis of hepatic insulin resistance is the accumulation of hepatic diacylglycerol leading to activation of PKC ϵ . Experimental models of altered hepatic lipid content allow us to further test this mechanism and could also inform the development of potential therapeutic interventions.

The model outlined focuses on the inability of insulin to alter hepatic glucose metabolism. Despite this, the ability of insulin to activate lipogenesis seems to be intact in most models of NAFLD. Much has been written about the paradox of selective insulin resistance, with investigators proposing the existence of branch points in the insulin signalling pathway^{32,33}, but space limitations preclude discussion of selective insulin resistance in this Perspective.

Instead, we will discuss the mechanisms that govern hepatic lipid accumulation and its relationship to insulin's ability to control hepatic glucose metabolism.

Regulation of fat delivery to liver

Hepatic lipid content is regulated by the balance between hepatic lipid uptake, synthesis, oxidation and export (Fig. 2). Hepatic lipid uptake is a function of substrate delivery and transport into the hepatocyte, and several genetic models exemplify this aspect of hepatic lipid metabolism. Transgenic mice with liver-specific overexpression of lipoprotein lipase (LpL) develop liver-specific lipid accumulation and liver-specific insulin resistance, whereas transgenic mice with muscle-specific overexpression of LpL develop muscle-specific lipid accumulation and muscle-specific insulin resistance³⁴. In these models, tissue-specific insulin resistance followed ectopic lipid accumulation. In a similar example, mice that lack the primary fatty-acid transporter in hepatocytes, FATP5, are protected from diet-induced NAFLD, indicating that excess fatty-acid transport into the hepatocyte is required for NAFLD and hepatic insulin resistance³⁵.

Studies in mice and humans have implicated adipose tissue lipolysis as an important source of fatty acids that promote NAFLD and hepatic insulin resistance. Whole-body lipolysis increases with total fat mass in humans^{36,37}; however, the relationship between lipolysis and insulin sensitivity seems to be largely independent of body mass. For example, insulin-resistant obese adolescents have higher visceral fat content than their weight-matched, insulin-sensitive counterparts³⁸. The relationship between adipose lipolysis and hepatic lipid content is exemplified by manipulation of the genes that regulate adipose lipolysis. As in humans, the effect of lipolysis on insulin sensitivity in rodents seems to be independent of body weight. Obese mice lacking the fatty-acid-binding protein FABP in adipocytes are more insulin sensitive than their obese littermates with normal FABP³⁹. Conversely, leptin-deficient obesity-prone mice with increased rates of adipocyte lipolysis due to knockout of the gene encoding adipocyte phospholipase A2 show increases in ectopic lipid storage and insulin resistance despite reduced body weight compared with littermates with normal lipolytic rates⁴⁰. These data suggest a facilitative role for the increases in adipose tissue lipolysis in providing substrates for ectopic lipid deposition and insulin resistance.

These genetic rodent models inform our understanding of human disease. Patients with conditions resulting from mutations in LpL (for example, hyperlipoproteinaemia type 1) are prone to developing insulin resistance⁴¹. In humans, the single nucleotide polymorphism (SNP) rs56225452, putatively representing a gain-of-function mutation in the FATP5 promoter, was associated with insulin resistance and NAFLD⁴². In Asian Indian individuals, as well as those of other ethnic groups, variants (C-482T, T-455C or both) in apolipoprotein C3 (APOC3), which can inhibit LpL and hepatic lipase, are associated with hypertriglyceridaemia and NAFLD⁴³. These polymorphisms led to around 30% higher plasma APOC3 concentrations and post-prandial hypertriglyceridaemia through the inhibitory effect of APOC3 on LpL activity. As a result, the livers of individuals with APOC3 variants take up a greater amount of lipid from chylomicrons, remnants of lipoprotein particles, predisposing these lean subjects to NAFLD and hepatic insulin resistance. These results were replicated in another cohort of lean males of European

descent⁴⁴. Of note, this *APOC3*-gene–environment interaction has only been observed in lean males, probably reflecting a protective effect of oestradiol on the ability of APOC3 to inhibit LpL and promote ectopic fat storage⁴⁵, and the ability of obesity-associated NAFLD⁴⁴ to mask the relatively subtle affect of this gene–environment interaction on the development of hepatic insulin resistance^{46,47}.

The effect of increased plasma Apoc3 concentrations on the development of NAFLD and hepatic insulin resistance has been genetically validated in transgenic mice that have increased hepatic overexpression of Apoc3. These mice are more prone to diet-induced NAFLD and diacylglycerol–PKC ϵ -induced hepatic insulin resistance than their wild-type littermates⁴⁸. Interestingly, although hypertriglyceridaemia was present in transgenic mice fed both a normal and high-fat diet, severe hepatic steatosis and hepatic insulin resistance only developed in Apoc3 transgenic mice fed a high-fat diet, reflecting an important gene–environment interaction. Moreover, the phenotype was due to both inhibition of peripheral lipase activity and diminished hepatic triacylglycerol export as very-low-density lipoprotein (VLDL)⁴⁸.

Lessons learned from lipodystrophy

The importance of adipose tissue lipid storage is exemplified when adipose tissue is altogether absent. In ‘fatless’ mice expressing the dominant-negative protein A-ZIP/F-1 in adipocytes, the absence of visceral and peripheral fat leads to ectopic lipid accumulation and severe hepatic and muscle insulin resistance. Insulin resistance can be corrected by transplantation of white adipose tissue from normal mice, further illustrating the importance of adipose tissue as a ‘safe’ storage depot⁴⁹. Lipoatrophic mice with the gene encoding peroxisome proliferator-activated receptor- γ (Ppar- γ) knocked out in white adipose tissue or with the gene encoding hepatic 1-acylglycerol-3-phosphate O-acyltransferase 2 (Agpat2) knocked out globally display a similar phenotype: the loss of visceral and subcutaneous fat is associated with hepatic steatosis and hepatic insulin resistance^{50,51}.

Similar associations are evident in humans with lipodystrophies. Patients with these disorders represent a rare example of severe hepatic insulin associated with extreme hepatic steatosis in the absence of visceral or peripheral fat accumulation^{52–54}. Leptin treatment can decrease calorie intake and effectively normalize hepatic lipid content and hepatic insulin action^{53,54}. Similarly, patients with partial lipodystrophy owing to mutations in the scaffolding protein perilipin-1, which inhibits adipose triglyceride lipase, have reduced peripheral fat mass but develop NAFLD because of increased adipose tissue lipolysis resulting from inhibition of adipose tissue triglyceride lipase⁵⁵. These patients can also develop profound insulin resistance. These data again point to ectopic lipid accumulation in the liver, which might occur as a result of the diversion of substrates from other storage depots, as the crucial mediator of hepatic insulin resistance.

Regulation of hepatic lipid synthesis

Hepatic triacylglycerol synthesis is the sum of two main processes: the synthesis of fatty acids (*de novo* lipogenesis, DNL) and esterification of fatty acids into fatty-acid glyceride species (for example, mono-, di- and triacylglyceride).

Contributions of *de novo* lipogenesis to triacylglycerol synthesis

Although DNL is thought to make a relatively small contribution to hepatic triacylglycerol accumulation relative to esterification^{56,57}, rates of postprandial DNL do increase significantly in both young and elderly patients with NAFLD^{58–60}. Diet-induced NAFLD might stimulate a feed-forward loop exacerbating DNL and ectopic lipid deposition. For example, fructose-fed hamsters have hypertriglyceridaemia, NAFLD and insulin resistance associated with increased DNL⁶¹, and because fructose inhibits fatty-acid oxidation both directly and indirectly, excess fructose intake is likely to stimulate DNL and hepatic insulin resistance^{62,63}. However, reducing lipogenic gene expression by knockdown of the upstream gene encoding peroxisome proliferator-activated receptor γ coactivator-1 β (PGC-1 β)⁶⁴ protects against fructose-induced hepatic insulin resistance⁶⁵. Similarly knockdown of the genes encoding the acetyl CoA carboxylases ACC1 and ACC2, which are crucial in the regulation of DNL and lipid oxidation, respectively, reduced liver triacylglycerol and diacylglycerol content, decreased PKC ϵ activation and protected mice from lipid-induced hepatic insulin resistance⁶⁶.

Skeletal muscle insulin resistance promotes hepatic lipogenesis

Skeletal muscle insulin resistance typically accompanies insulin resistance at other sites, possibly because of the diversion of substrates from insulin-resistant muscle to the liver (Fig. 3). The independent effect of muscle insulin resistance to exacerbate NAFLD has also been demonstrated in rodents. Mice that lack insulin-responsive glucose transporter 4 (Glut4) in muscle have NAFLD⁶⁷. Similarly, mice lacking the Akt substrate As160 have decreased glucose uptake in adipose tissue and slow-twitch muscles, and develop insulin resistance in the liver as well as adipose tissue and skeletal muscle⁶⁸. Muscle insulin resistance might also be independently associated with NAFLD. For example, the severity of NAFLD in mice fed a high-fat, high-cholesterol diet was demonstrated to correlate with peripheral insulin resistance, and not with hepatic insulin resistance⁶⁹.

These results have been translated to humans, in which selective muscle insulin resistance in healthy young lean individuals has been shown to predispose them to increased hepatic DNL, hepatic triacylglycerol accumulation and atherogenic dyslipidaemia after eating high-carbohydrate meals. This is because ingested glucose is diverted away from muscle glycogen storage to the liver, in which it is converted to triacylglycerol driven by the compensatory hyperinsulinaemia that is secondary to muscle insulin resistance⁵⁷. Further evidence to support this hypothesis stems from a study demonstrating that a single 45 minute bout of exercise on an elliptical trainer increased postprandial muscle glycogen synthesis following carbohydrate ingestion, resulting in a 40% reduction in hepatic DNL and a 30% reduction in hepatic triglyceride synthesis⁷⁰.

Fatty acid esterification contributes to triacylglycerol synthesis

Most liver triglyceride is formed through esterification of fatty acids^{56,57}. Diacylglycerol is an intermediate in the esterification pathway and, thus, genetic models that manipulate hepatic lipid esterification can be used to further examine the diacylglycerol–novel-PKCs hypothesis of insulin resistance. Rats overexpressing the rate-controlling enzyme in triglyceride esterification, mitochondrial glycerol-3-phosphate acyltransferase 1 (GPAT1),

have hepatic insulin resistance associated with increased PKC ϵ activity⁷¹. These data are in contrast to mice in which the gene encoding mitochondrial GPAT was knocked down. These animals exhibit suppression of PKC ϵ activity and improved hepatic insulin sensitivity⁷², offering further evidence in support of the diacylglycerol–PKC ϵ hypothesis of hepatic insulin resistance.

Diacylglycerol acyltransferase 2 (DGAT2) catalyses the final step in triglyceride synthesis from diacylglycerol. Although inhibition of DGAT2 may be expected to acutely increase cellular diacylglycerol content, chronic reduction in hepatic DGAT2 expression due to ASO treatment results in decreased hepatic diacylglycerol content due to downregulation of the lipogenic pathway. Consistent with the diacylglycerol–PKC ϵ hypothesis this reduction in hepatic diacylglycerol content was associated with reduced PKC ϵ activation and protection from lipid-mediated hepatic insulin resistance^{73,74}.

The effects of hepatic overexpression of DGAT2 are less clear. Monetti *et al.* reported that although transgenic mice had increased hepatic diacylglycerol content, there was no impact on hepatic insulin sensitivity⁷⁵. Jornayvaz *et al.* also demonstrated an increase in hepatic diacylglycerol content in the same mice, but reported increased PKC ϵ activation, decreased hepatic insulin signalling and hepatic insulin resistance⁷⁶. Technical differences in study execution could explain this difference. In hyperinsulinaemic–euglycaemic clamp studies to test insulin resistance, both groups found that DGAT2 transgenic mice fed a normal diet failed to normally suppress hepatic glucose production. However, the teams reported differing results for wild-type mice fed a normal diet. Jornayvaz *et al.* showed that these control mice had normal insulin suppression of hepatic glucose production, whereas Monetti *et al.* reported that control mice did not suppress hepatic glucose production. Thus, the key to interpreting the phenotype attributed to increased hepatic DGAT2 expression and diacylglycerol accumulation in this model is whether or not the control animals fed a normal diet had complete suppression of hepatic glucose production in response to insulin. In a different study in humans, SNPs in DGAT2 predicted a smaller decrease in liver fat content after very modest (3 kg) weight loss compared with individuals without SNPs in DGAT2. However, insulin signalling was not measured in these studies, and the participants' very modest weight loss may prevent us from uncovering meaningful information about the role of DGAT2 (ref. 77). Further studies are needed to understand the role of DGAT2 in hepatic insulin sensitivity and its potential link to whole-body adiposity.

Lipid export

Export of triglyceride as VLDL is the only means of reducing hepatic lipid content other than through fat oxidation. NAFLD-associated insulin resistance is seen in genetic models of impaired VLDL export⁷⁸, whereas, in models of increased VLDL export, NAFLD is ameliorated, independent of body weight^{79,80}. As previously discussed, impaired hepatic lipid export also contributes to the development of NAFLD and hepatic insulin resistance in mice overexpressing ApoC3 (ref. 48).

Regulation of hepatic lipid oxidation

Rodent models with altered fat oxidation can be used to both test relationships between diacylglycerol, PKC ϵ activation and insulin resistance, and to validate potential therapeutic targets. Mice lacking Ppar- α , a key regulator of hepatic lipid oxidation, are prone to NAFLD and fail to benefit from the insulin sensitizing effects of omega-3 fatty acids⁸¹. Mice that are genetically deficient in the dehydrogenase LCAD have diminished mitochondrial fatty-acid oxidation and increased *de novo* diacylglycerol synthesis, increased liver PKC ϵ activity and hepatic insulin resistance⁸². Interestingly, humans and mice with loss-of-function mutations in LCAD exhibit fasting hypoglycaemia, which has been ascribed to defects in amino-acid metabolism and may not be related to alterations in hepatic insulin sensitivity⁸³.

Hypoglycaemia has also been dissociated from insulin resistance in mice fed a ketogenic diet. These mice develop profound hepatic insulin resistance associated with increased diacylglycerol concentrations and PKC ϵ activation, despite hypoglycaemia stemming from reductions in fasting hepatic gluconeogenesis⁸⁴.

Changes in whole-body energy metabolism also affect hepatic lipid balance. Thyroid hormone receptor- α knockout mice have increased energy expenditure (as well as decreased expression of lipogenic enzymes) and are protected from diet-induced NAFLD, PKC ϵ activation and hepatic insulin resistance⁸⁵. Similarly, infusion of fibroblast growth factor 21 (Fgf21) in mice resulted in increased energy expenditure in the liver and white adipose tissue, lowered liver diacylglycerol concentrations, decreased hepatic PKC ϵ translocation and protection from lipid-induced hepatic insulin resistance⁸⁶. By contrast, mice deficient for fatty acid amide hydrolase (FAAH) have reduced whole-body energy expenditure due to diminished hypothalamic–pituitary axis activity and impaired thyroid function. The absence of FAAH resulted in increased liver diacylglycerol, increased PKC ϵ translocation and hepatic insulin resistance⁸⁷. These data again indicate that the link between NAFLD and insulin resistance is an increase in diacylglycerol concentration leading to activation of PKC ϵ activity.

As already mentioned, inhibition of ACC1 and ACC2 leads to both a decrease in lipid synthesis and an increase in lipid oxidation. The latter effect is due to the disinhibition of carnitine palmitoyltransferase-1 (CPT1) and more long-chain fatty acyl CoAs entering the mitochondria. Mutation of key serine residues in Acc1 and Acc2 in mice prevents inactivation of these enzymes by AMP-activated protein kinase (AMPK), leading to increased lipid synthesis and decreased lipid oxidation⁸⁸. These mice have increased hepatic diacylglycerol content, activation of PKC ϵ and develop hepatic insulin resistance. By contrast, AMPK is activated in mice with a deletion of the mitochondrial gene encoding the sodium-dicarboxylate cotransporter Slc13a5, the mammalian homologue of the INDY protein in *Drosophila*. The increased AMPK activity leads to reductions in Acc1 and Acc2 activity, resulting in decreased liver diacylglycerol content and reduced PKC ϵ translocation. Together, these changes protected *Slc13a5* knockout mice from both diet- and age-associated hepatic insulin resistance⁸⁹.

Many groups have studied whether people with NAFLD have alterations in hepatic oxidative flux either as a cause or consequence of ectopic lipid accumulation. Results in

humans have been mixed. When an indirect tracer method was used to assess hepatic tricarboxylic acid (TCA) cycle flux and anaplerosis, both were found to be markedly increased in individuals with NAFLD^{90,91}. But when a ³¹P magnetic resonance spectroscopy technique was used to assess hepatic ATP production, a reduction in hepatic energy metabolism was found in individuals with type 2 diabetes^{92,93}. Because the role of hepatic oxidative flux is key to both the understanding of pathogenesis and potential treatment of NAFLD and type 2 diabetes, the development of methods to directly measure rates hepatic oxidative metabolism is of crucial importance⁹⁴.

Dissociation of NAFLD and hepatic insulin resistance

The requirement of NAFLD for hepatic insulin resistance has been questioned by several groups and has been recently reviewed^{95–97}. It is well established that it is possible to experimentally induce insulin resistance without NAFLD, or induce NAFLD without insulin resistance under certain conditions. For instance, blocking hepatic VLDL secretion with a choline-deficient diet or by genetic modification of the export machinery increases hepatic triglyceride concentrations but does not cause insulin resistance^{98,99}. Similarly, mice with liver-specific knockout of the gene encoding the protein phosphatase Shp1 develop NAFLD, but are protected from insulin resistance¹⁰⁰. Hepatic carbohydrate responsive element-binding protein and sterol regulatory element-binding protein-1 have recently been independently implicated in dissociating NAFLD and insulin resistance in mice and humans^{101,102}. In addition, mice with the gene encoding the lipase activator CGI-58 knocked down have profound hepatic steatosis due to suppression of hepatic triglyceride lipolysis. However, these mice are protected against high-fat-diet-induced hepatic insulin resistance¹⁰³. To explain this paradox, the authors of one study examined the subcellular localization of diacylglycerols in liver cells and found that knockdown of the gene encoding CGI-58 promoted diacylglycerol accumulation in lipid droplets, while protecting against diacylglycerol accumulation in the cell membrane and preventing PKC ϵ translocation to the cell membrane¹⁰⁴. These results are consistent with earlier studies in humans, also showing that compartmentation of diacylglycerols in the cytosolic²⁹ and membrane¹⁰⁵ compartments could be an important factor in the pathogenesis of liver and muscle insulin resistance. The different results between diacylglycerol accumulation in the membrane and the cytosolic compartments might reflect differences in the measurement and fractionating or lipid-extraction techniques used in the different studies and/or in the length of fasting. Nevertheless, the CGI-58 ASO data clearly indicate that lipids sequestered in lipid droplets do not promote PKC ϵ activation and hepatic insulin resistance, and that this compartmentation of diacylglycerols and triacylglycerols in lipid droplets may explain other models of NAFLD that are not associated with hepatic insulin resistance, as discussed later. Future work will better discern the importance of specific lipid compartments in the pathogenesis of insulin resistance.

Similarly, liver-specific *Hdac3* knockout mice are prone to developing hepatic steatosis when fed both normal and high-fat diets and have been shown to be protected from lipid-induced hepatic resistance¹⁰⁶. This apparent disconnect is probably also due to altered partitioning of diacylglycerols to the lipid droplet. Loss of *Hdac3* in mice was associated with an increase in the lipid droplet protein Plin2 and decreased activation of PKC ϵ . When

Plin2 expression was normalized with a specific ASO, the improvements in glucose tolerance were no longer evident. Taken together these data strongly suggest that diacylglycerols need access to a particular subcellular compartment to inhibit IRTK activity, whereas diacylglycerol accumulation in the lipid droplet is protective and does not lead to inhibition of IRTK activity and insulin resistance.

Genetic variants in the triglyceride hydrolase PNPLA3 (also known as adiponutrin) have been proposed to disassociate NAFLD from insulin resistance in normal weight and obese humans⁴⁶. However, this conclusion was based on fasting plasma glucose and insulin measurements, and hepatic insulin responsiveness was not directly assessed in this study. Furthermore, because many of the subjects in this study were obese and relatively insulin resistant it is difficult to determine if PNPLA3 could exacerbate hepatic insulin resistance in these individuals. Additional insights into the role of PNPLA3 have been gained using an ASO to decrease expression of PNPLA3 in rats¹⁰⁷. Reduced expression of PNPLA3 decreased hepatic lipid content owing to decreased lipid esterification, pointing to the potential duality of PNPLA3's functions in both triglyceride synthesis and hydrolysis¹⁰⁸. Similarly, in obese Taiwanese children, variants (which had previously been identified¹⁰⁹ to strongly correlate with NAFLD in adults of European ancestry who were not all obese) did not correlate with NAFLD, but again this may be due to the obfuscating effects of obesity in this cohort; however, there was a significant association between glucokinase regulatory protein (GCKR) and NAFLD¹¹⁰.

Reversal of NAFLD ameliorates hepatic insulin resistance

The most effective intervention to reverse NAFLD and hepatic insulin resistance in humans is weight loss. Hepatic steatosis quickly resolves in both obese patients with type 2 diabetes and non-diabetic normal weight individuals with NAFLD after a hypocaloric diet and a modest weight loss of less than 10% of total body weight¹¹¹. This is accompanied by resolution of hepatic steatosis and normalization of fasting plasma glucose concentrations, hepatic glucose production and hepatic insulin sensitivity^{111,112}. However, recidivism following weight loss is extremely common: less than 50% of those who have lost more than 10% of their body weight are able to maintain the weight loss after 1 year¹¹³, and after 5 years, less than 25% have maintained their weight¹¹⁴. Nevertheless, improvements in insulin sensitivity and NAFLD can persist for 2 years after weight loss in overweight and obese individuals even after the weight is regained, arguing that there is a dissociation between obesity and insulin sensitivity or NAFLD¹¹⁵.

In regards to potential medical therapies to treat NAFLD, thiazolidinediones, which are potent PPAR- γ activators, have been shown to lead to significant reductions in liver-fat content and improvements in hepatic insulin sensitivity^{116–120}. This effect probably occurs by thiazolidinedione activation of PPAR- γ in subcutaneous fat tissue leading to increased insulin sensitivity and suppression of lipolysis, resulting in a redistribution of liver fat to the subcutaneous fat cells¹¹⁶.

Increasing hepatic mitochondrial fat oxidation by promoting subtle increases in mitochondrial uncoupling could be another therapeutic target for NAFLD, hepatic insulin

resistance and type 2 diabetes. Promoting mitochondrial uncoupling by treating rats fed a high-fat diet with low doses of the mitochondrial protonophore 2,4-dinitrophenol (DNP) was shown to reduce hepatic triglyceride content, increase insulin-stimulated IRS2 tyrosine phosphorylation and PI(3)K activity, and improve insulin-mediated suppression of hepatic glucose production²⁵. Consistent with these findings, simply reversing hepatic steatosis using a liver-targeted DNP to increase hepatic mitochondrial fat oxidation by about 60% was found to reverse hypertriglyceridaemia, hepatic and peripheral insulin resistance, and hyperglycaemia in rat models of NAFLD and type 2 diabetes. Furthermore, correction of liver and muscle insulin resistance was associated with marked reductions in diacylglycerol-PKC ϵ and diacylglycerol-PKC θ activity in liver and muscle, respectively; and occurred independently of any changes in body weight, inflammatory mediators, FGF21, adiponectin or liver ceramide content¹²¹. Moreover, liver-targeting DNP resulted in a 50-fold increase in the ratio of toxic to effective doses compared with DNP. Taken together these data support the key role of diacylglycerol activation of novel PKC in mediating liver insulin resistance and demonstrate the potential feasibility of dissociating the toxic effects of DNP from its beneficial effects to promote subtle increases in hepatic mitochondrial uncoupling and hepatic fat oxidation to treat the related epidemics of NAFLD and type 2 diabetes.

Outlook

Hepatic insulin resistance is a complex phenomenon. Although many questions regarding the nature of the insulin-signalling defect remain unanswered, hepatic insulin resistance is almost universally associated with increases in hepatic triacylglycerol and diacylglycerol concentrations, with the latter leading to PKC ϵ activation and subsequent inhibition of IRTK activity. The diacylglycerol-PKC ϵ hypothesis of hepatic insulin resistance has recently been validated in humans with NAFLD. Exceptions to this rule can be attributed to compartmentation of diacylglycerols to the lipid droplet, in which it does not lead to inhibition of IRTK activity. NAFLD occurs when lipid supply to the liver exceeds rates of lipid oxidation and lipid export. Thus therapies targeted to reduce fatty-acid delivery to the liver, suppress diacylglycerol production, or raise mitochondrial fat oxidation by promoting subtle increases in hepatic mitochondrial uncoupling are of great interest to ameliorate NAFLD and type 2 diabetes.

Acknowledgments

Space limitations preclude this from being a comprehensive review, and this unfortunately limits appropriate recognition of many of our colleagues worldwide who have contributed immeasurably to the development of this field. This work was supported by grants from the National Institutes of Health: R01 DK-40936, R01 DK-49230, R24 DK-085836, R01 AG-23686, U24 DK-059635, UL1 RR-024139, P30 DK-45735, T32-DK101019 and I01-BX000901 (V.T.S.), and the Novo Nordisk Foundation for Basic Metabolic Research, University of Copenhagen.

References

1. Browning JD, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology*. 2004; 40:1387–1395. [PubMed: 15565570]
2. Smits MM, Ioannou GN, Boyko EJ, Utzschneider KM. Non-alcoholic fatty liver disease as an independent manifestation of the metabolic syndrome: results of a US national survey in three ethnic groups. *J Gastroenterol Hepatol*. 2013; 28:664–670. [PubMed: 23286209]

3. Fan JG, et al. Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. *J Hepatol.* 2005; 43:508–514. [PubMed: 16006003]
4. Amarapurkar DN, et al. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? *J Gastroenterol Hepatol.* 2007; 22:788–793. [PubMed: 17565631]
5. Petersen KF, et al. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci USA.* 2006; 103:18273–18277. This study reported ethnic differences in the prevalence of NAFLD and insulin resistance. [PubMed: 17114290]
6. Das K, et al. Nonobese population in a developing country has a high prevalence of nonalcoholic fatty liver and significant liver disease. *Hepatology.* 2010; 51:1593–1602. [PubMed: 20222092]
7. Tolman KG, Fonseca V, Dalpiaz A, Tan MH. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. *Diabetes Care.* 2007; 30:734–743. [PubMed: 17327353]
8. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology.* 1990; 12:1106–1110. This study reported that steatohepatitis was sevenfold more common in severely obese compared with patients of normal weight contributing to type 2 diabetes risk. [PubMed: 2227807]
9. Silverman JF, et al. Liver pathology in morbidly obese patients with and without diabetes. *Am J Gastroenterol.* 1990; 85:1349–1355. [PubMed: 2220728]
10. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology.* 2010; 51:679–689. This review summarizes the link between obesity, NAFLD and insulin resistance and the possible role of inflammation in these processes. [PubMed: 20041406]
11. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest.* 2000; 106:171–176. This review describes the cellular and molecular mechanisms of liver and muscle insulin resistance and proposes the diacylglycerol and novel PKC hypothesis of lipid-induced insulin resistance. [PubMed: 10903330]
12. Kleiner DE, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* 2005; 41:1313–1321. [PubMed: 15915461]
13. Hui JM, et al. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology.* 2003; 38:420–427. [PubMed: 12883486]
14. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol.* 2010; 53:372–384. [PubMed: 20494470]
15. Charlton MR, et al. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology.* 2011; 141:1249–1253. [PubMed: 21726509]
16. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell.* 2012; 148:852–871. This review provides a balanced and detailed discussion of the potential roles of inflammation, ER stress, ceramides and other factors in the pathogenesis of liver and muscle insulin resistance. [PubMed: 22385956]
17. Cheng Z, Tseng Y, White MF. Insulin signaling meets mitochondria in metabolism. *Trends Endocrinol Metab.* 2010; 21:589–598. [PubMed: 20638297]
18. Hanke S, Mann M. The phosphotyrosine interactome of the insulin receptor family and its substrates IRS-1 and IRS-2. *Mol Cell Proteomics.* 2009; 8:519–534. [PubMed: 19001411]
19. Franke TF, Kaplan DR, Cantley LC, Toker A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. *Science.* 1997; 275:665–668. [PubMed: 9005852]
20. Adina-Zada A, et al. Allosteric regulation of the biotin-dependent enzyme pyruvate carboxylase by acetyl-CoA. *Biochem Soc Trans.* 2012; 40:567–572. [PubMed: 22616868]
21. Adina-Zada A, Zeczycki TN, Attwood PV. Regulation of the structure and activity of pyruvate carboxylase by acetyl CoA. *Arch Biochem Biophys.* 2012; 519:118–130. [PubMed: 22120519]
22. Pilkis SJ, el-Maghrabi MR, Claus TH. Fructose-2,6-bisphosphate in control of hepatic gluconeogenesis. From metabolites to molecular genetics. *Diabetes Care.* 1990; 13:582–599. [PubMed: 2162755]

23. Petersen KF, Laurent D, Rothman DL, Cline GW, Shulman GI. Mechanism by which glucose and insulin inhibit net hepatic glycogenolysis in humans. *J Clin Invest.* 1998; 101:1203–1209. [PubMed: 9502760]
24. Roden M, et al. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest.* 1996; 97:2859–2865. [PubMed: 8675698]
25. Samuel VT, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem.* 2004; 279:32345–32353. The authors of this paper established a model of selective hepatic insulin resistance and demonstrated that this resistance was associated with increased hepatic diacylglycerol content and increased PKC ϵ activation for the first time. [PubMed: 15166226]
26. Samuel VT, et al. Inhibition of protein kinase C ϵ prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *J Clin Invest.* 2007; 117:739–745. This paper conclusively demonstrated the key role of PKC ϵ activation in mediating lipid-induced hepatic insulin resistance. [PubMed: 17318260]
27. Dries DR, Gallegos LL, Newton AC. A single residue in the C1 domain sensitizes novel protein kinase C isoforms to cellular diacylglycerol production. *J Biol Chem.* 2007; 282:826–830. [PubMed: 17071619]
28. Raddatz K, et al. Time-dependent effects of Prkce deletion on glucose homeostasis and hepatic lipid metabolism on dietary lipid oversupply in mice. *Diabetologia.* 2011; 54:1447–1456. [PubMed: 21347625]
29. Kumashiro N, et al. Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease. *Proc Natl Acad Sci USA.* 2011; 108:16381–16385. This paper reports that intracellular diacylglycerol, associated with PKC ϵ activation, is the strongest predictor of insulin resistance in obese patients. [PubMed: 21930939]
30. Magkos F, et al. Intrahepatic diacylglycerol content is associated with hepatic insulin resistance in obese subjects. *Gastroenterology.* 2012; 142:1444–1446.e2. [PubMed: 22425588]
31. Funke A, et al. Cholesterol-induced hepatic inflammation does not contribute to the development of insulin resistance in male LDL receptor knockout mice. *Atherosclerosis.* 2014; 232:390–396. [PubMed: 24468153]
32. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab.* 2008; 7:95–96. [PubMed: 18249166]
33. Chavez JA, Summers SA. Lipid oversupply, selective insulin resistance, and lipotoxicity: molecular mechanisms. *Biochim Biophys Acta.* 2010; 1801:252–265. [PubMed: 19796706]
34. Kim JK, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci USA.* 2001; 98:7522–7527. This paper reports that overexpression of LpL in liver resulted in liver-specific triglyceride accumulation and liver-specific insulin resistance, whereas muscle-specific overexpression of LpL resulted in muscle-specific triglyceride accumulation and muscle-specific insulin resistance. [PubMed: 11390966]
35. Doege H, et al. Silencing of hepatic fatty acid transporter protein 5 *in vivo* reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. *J Biol Chem.* 2008; 283:22186–22192. [PubMed: 18524776]
36. Mittendorfer B, Magkos F, Fabbrini E, Mohammed BS, Klein S. Relationship between body fat mass and free fatty acid kinetics in men and women. *Obesity (Silver Spring).* 2009; 17:1872–1877. [PubMed: 19629053]
37. Pardina E, et al. Increased expression and activity of hepatic lipase in the liver of morbidly obese adult patients in relation to lipid content. *Obes Surg.* 2009; 19:894–904. [PubMed: 18972174]
38. Weiss R, et al. The ‘obese insulin-sensitive’ adolescent: importance of adiponectin and lipid partitioning. *J Clin Endocrinol Metab.* 2005; 90:3731–3737. [PubMed: 15797955]
39. Cao H, et al. Regulation of metabolic responses by adipocyte/macrophage Fatty Acid-binding proteins in leptin-deficient mice. *Diabetes.* 2006; 55:1915–1922. [PubMed: 16804058]
40. Jaworski K, et al. AdPLA ablation increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. *Nature Med.* 2009; 15:159–168. [PubMed: 19136964]
41. Mingrone G, et al. Triglyceride-induced diabetes associated with familial lipoprotein lipase deficiency. *Diabetes.* 1999; 48:1258–1263. [PubMed: 10342813]

42. Auinger A, et al. A promoter polymorphism in the liver-specific fatty acid transport protein 5 is associated with features of the metabolic syndrome and steatosis. *Horm Metab Res.* 2010; 42:854–859. [PubMed: 20945272]
43. Petersen KF, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med.* 2010; 362:1082–1089. The authors of this paper show that variants in APOC3 were associated with a high prevalence of NAFLD and insulin resistance in lean Asian-Indian men. [PubMed: 20335584]
44. Peter A, Kantartzis K, Machicao F, Machann J. Visceral obesity modulates the impact of apolipoprotein C3 gene variants on liver fat content. *J Obes.* 2012; 36:774–782. This paper, which follows up on the findings of ref. 43 shows that the association between APOC3 is only observable in lean, not obese individuals, demonstrating that obesity may mask the predisposing effects of APOC3 genetic variants on NAFLD and insulin resistance.
45. Camporez JPG, et al. Cellular mechanism by which estradiol protects female ovariectomized mice from high-fat diet-induced hepatic and muscle insulin resistance. *Endocrinology.* 2013; 154:1021–1028. [PubMed: 23364948]
46. Verrijken A, Beckers S, Francque S, Hilden H. A gene variant of PNPLA3, but not of APOC3, is associated with histological parameters of NAFLD in an obese population. *Obesity (Silver Spring).* 2013; 21:2138–2145. [PubMed: 23512881]
47. Kozlitina J, Boerwinkle E, Cohen JC, Hobbs HH. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. *Hepatology.* 2011; 53:467–474. [PubMed: 21274868]
48. Lee H-Y, et al. Apolipoprotein CIII overexpressing mice are predisposed to diet-induced hepatic steatosis and hepatic insulin resistance. *Hepatology.* 2011; 54:1650–1660. This study demonstrates that transgenic mice with hepatic overexpression of human APOC3 predisposes them to severe hepatic steatosis and hepatic insulin resistance when fed a high-fat diet, whereas there is no metabolic phenotype when they are fed a normal diet. [PubMed: 21793029]
49. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J Biol Chem.* 2000; 275:8456–8460. This study clearly illustrated the mechanism by which lipodystrophy syndromes lead to insulin resistance. [PubMed: 10722680]
50. Wang F, Mullican SE, DiSpirito JR, Peed LC, Lazar MA. Lipodystrophy and severe metabolic disturbance in mice with fat-specific deletion of PPAR γ . *Proc Natl Acad Sci USA.* 2013; 110:18656–18661. [PubMed: 24167256]
51. Cortés VA, et al. Leptin ameliorates insulin resistance and hepatic steatosis in Agpat2^{-/-} lipodystrophic mice independent of hepatocyte leptin receptors. *J Lipid Res.* 2014; 55:276–288. [PubMed: 24293639]
52. Savage DB, Murgatroyd PR, Chatterjee VK, O'Rahilly S. Energy expenditure and adaptive responses to an acute hypercaloric fat load in humans with lipodystrophy. *J Clin Endocrinol Metab.* 2005; 90:1446–1452. [PubMed: 15613417]
53. Petersen KF, et al. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest.* 2002; 109:1345–1350. This study established the mechanism by which leptin replacement therapy reverses liver and muscle insulin resistance in patients with lipodystrophy. [PubMed: 12021250]
54. Simha V, Szczepaniak LS, Wagner AJ, DePaoli AM, Garg A. Effect of leptin replacement on intrahepatic and intramyocellular lipid content in patients with generalized lipodystrophy. *Diabetes Care.* 2003; 26:30–35. [PubMed: 12502655]
55. Gandotra S, et al. Perilipin deficiency and autosomal dominant partial lipodystrophy. *N Engl J Med.* 2011; 364:740–748. [PubMed: 21345103]
56. Diraison F, Moulin P, Beylot M. Contribution of hepatic *de novo* lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab.* 2003; 29:478–485. [PubMed: 14631324]
57. Petersen KF, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci USA.* 2007; 104:12587–12594. In this paper the authors demonstrate that selective insulin resistance in skeletal muscle promotes the development of atherogenic dyslipidaemia and NAFLD. [PubMed: 17640906]

58. Donnelly KL, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest*. 2005; 115:1343–1351. [PubMed: 15864352]
59. Petersen KF, et al. Reversal of muscle insulin resistance by weight reduction in young, lean, insulin-resistant offspring of parents with type 2 diabetes. *Proc Natl Acad Sci USA*. 2012; 109:8236–8240. This study provides evidence that skeletal muscle insulin resistance in young lean insulin-resistant offspring of parents with type 2 diabetes can be attributed to increased intramyocellular lipid content. [PubMed: 22547801]
60. Flannery C, Dufour S, Rabøl R, Shulman GI, Petersen KF. Skeletal muscle insulin resistance promotes increased hepatic *de novo* lipogenesis, hyperlipidemia, and hepatic steatosis in the elderly. *Diabetes*. 2012; 61:2711–2717. [PubMed: 22829450]
61. Bilz S, et al. Activation of the farnesoid X receptor improves lipid metabolism in combined hyperlipidemic hamsters. *Am J Physiol Endocrinol Metab*. 2006; 290:E716–E722. [PubMed: 16291572]
62. Delarue J, Normand S, Couet C, Pachiaudi C. Effects of free fatty acids on the metabolic response to oral fructose in lean healthy humans. *Int J Obes Relat Metab Disord*. 1996; 20:130–136. [PubMed: 8646249]
63. Zhang C, et al. Endoplasmic reticulum stress is involved in hepatic SREBP-1c activation and lipid accumulation in fructose-fed mice. *Toxicol Lett*. 2012; 212:229–240. [PubMed: 22698815]
64. Lin J, et al. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1 β coactivation of SREBP. *Cell*. 2005; 120:261–273. [PubMed: 15680331]
65. Nagai Y, et al. The role of peroxisome proliferator-activated receptor γ coactivator-1 β in the pathogenesis of fructose-induced insulin resistance. *Cell Metab*. 2009; 9:252–264. [PubMed: 19254570]
66. Savage DB, et al. Reversal of diet-induced hepatic steatosis and hepatic insulin resistance by antisense oligonucleotide inhibitors of acetyl-CoA carboxylases 1 and 2. *J Clin Invest*. 2006; 116:817–824. The authors of this study reported that decreasing hepatic expression of hepatic ACC1 and ACC2 in rats by ASO decreased hepatic lipogenesis and increased liver fat oxidation, resulting in protection from lipid-induced hepatic steatosis and hepatic insulin resistance. [PubMed: 16485039]
67. Kim JK, et al. Glucose toxicity and the development of diabetes in mice with muscle-specific inactivation of GLUT4. *J Clin Invest*. 2001; 108:153–160. [PubMed: 11435467]
68. Wang HY, et al. AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues. *Biochem J*. 2013; 449:479–489. [PubMed: 23078342]
69. Asai, A., et al. Dissociation of hepatic insulin resistance from susceptibility of non-alcoholic fatty liver disease induced by a high fat and high carbohydrate diet in mice. *Am J Physiol Gastrointest Liver Physiol*. 2014. <http://dx.doi.org/10.1152/ajpgi.00291.2013>
70. Rabøl R, Petersen KF, Dufour S, Flannery C, Shulman GI. Reversal of muscle insulin resistance with exercise reduces postprandial hepatic *de novo* lipogenesis in insulin resistant individuals. *Proc Natl Acad Sci USA*. 2011; 108:13705–13709. The authors of this paper demonstrate that reversal of muscle insulin resistance in healthy young lean insulin-resistant individuals with a single 45 minute bout of elliptical exercise reversed the abnormal pattern of energy distribution of energy storage following carbohydrate ingestion, thus offering strong evidence in support of a key role for selective muscle insulin resistance in promoting NAFLD and atherogenic dyslipidaemia as proposed in ref. 57. [PubMed: 21808028]
71. Nagle CA, et al. Hepatic overexpression of glycerol-sn-3-phosphate acyltransferase 1 in rats causes insulin resistance. *J Biol Chem*. 2007; 282:14807–14815. [PubMed: 17389595]
72. Neschen S, Morino K, Hammond LE, Zhang D, Liu ZX. Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA: glycerol-sn-3-phosphate acyltransferase 1 knockout mice. *Cell Metab*. 2005; 2:55–65. [PubMed: 16054099]
73. Yu XX, et al. Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. *Hepatology*. 2005; 42:362–371. [PubMed: 16001399]
74. Choi CS, et al. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. *J Biol Chem*. 2007; 282:22678–22688. [PubMed: 17526931]

75. Monetti M, et al. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metab.* 2007; 6:69–78. [PubMed: 17618857]
76. Jornayvaz FR, et al. Hepatic insulin resistance in mice with hepatic overexpression of diacylglycerol acyltransferase 2. *Proc Natl Acad Sci USA.* 2011; 108:5748–5752. [PubMed: 21436037]
77. Kantartzis K, et al. The *DGAT2* gene is a candidate for the dissociation between fatty liver and insulin resistance in humans. *Clin Sci.* 2009; 116:531–537. [PubMed: 18980578]
78. Shindo N, et al. Involvement of microsomal triglyceride transfer protein in nonalcoholic steatohepatitis in novel spontaneous mouse model. *J Hepatol.* 2010; 52:903–912. [PubMed: 20392512]
79. Morán-Ramos S, et al. *Opuntia ficus indica* (nopal) attenuates hepatic steatosis and oxidative stress in obese Zucker (fa/fa) rats. *J Nutr.* 2012; 142:1956–1963. [PubMed: 23014486]
80. Singhal NS, Patel RT, Qi Y, Lee YS, Ahima RS. Loss of resistin ameliorates hyperlipidemia and hepatic steatosis in leptin-deficient mice. *Am J Physiol Endocrinol Metab.* 2008; 295:E331–E338. [PubMed: 18505833]
81. Neschen S, et al. n-3 Fatty acids preserve insulin sensitivity *in vivo* in a peroxisome proliferator-activated receptor- α -dependent manner. *Diabetes.* 2007; 56:1034–1041. [PubMed: 17251275]
82. Zhang D, et al. Mitochondrial dysfunction due to long-chain Acyl-CoA dehydrogenase deficiency causes hepatic steatosis and hepatic insulin resistance. *Proc Natl Acad Sci USA.* 2007; 104:17075–17080. [PubMed: 17940018]
83. Houten SM, et al. Impaired amino acid metabolism contributes to fasting-induced hypoglycemia in fatty acid oxidation defects. *Hum Mol Genet.* 2013; 22:5249–5261. [PubMed: 23933733]
84. Jornayvaz FR, et al. A high fat, ketogenic diet causes hepatic insulin resistance in mice, despite increasing energy expenditure and preventing weight gain. *Am J Physiol Endocrinol Metab.* 2010; 299:E808–E815. [PubMed: 20807839]
85. Jornayvaz FR, et al. Thyroid hormone receptor- α gene knockout mice are protected from diet-induced hepatic insulin resistance. *Endocrinology.* 2012; 153:583–591. [PubMed: 22147010]
86. Camporez JPG, et al. Cellular mechanisms by which FGF21 improves insulin sensitivity in male mice. *Endocrinology.* 2013; 154:3099–3109. [PubMed: 23766126]
87. Brown WH, et al. Fatty acid amide hydrolase ablation promotes ectopic lipid storage and insulin resistance due to centrally mediated hypothyroidism. *Proc Natl Acad Sci USA.* 2012; 109:14966–14971. [PubMed: 22912404]
88. Fullerton MD, et al. Single phosphorylation sites in *Acc1* and *Acc2* regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nature Med.* 2013; 19:1649–1654. [PubMed: 24185692]
89. Birkenfeld AL, et al. Deletion of the mammalian *INDY* homolog mimics aspects of dietary restriction and protects against adiposity and insulin resistance in mice. *Cell Metab.* 2011; 14:184–195. [PubMed: 21803289]
90. Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab.* 2011; 14:804–810. [PubMed: 22152305]
91. Satapati S, et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. *J Lipid Res.* 2012; 53:1080–1092. [PubMed: 22493093]
92. Szendroedi J, et al. Abnormal hepatic energy homeostasis in type 2 diabetes. *Hepatology.* 2009; 50:1079–1086. [PubMed: 19637187]
93. Schmid AI, et al. Liver ATP synthesis is lower and relates to insulin sensitivity in patients with type 2 diabetes. *Diabetes Care.* 2011; 34:448–453. [PubMed: 21216854]
94. Befroy DE, et al. Direct assessment of hepatic mitochondrial oxidative and anaplerotic fluxes in humans using dynamic ^{13}C magnetic resonance spectroscopy. *Nature Med.* 2014; 20:98–102. [PubMed: 24317120]
95. Farese RV Jr, Zechner R, Newgard CB, Walther TC. The problem of establishing relationships between hepatic steatosis and hepatic insulin resistance. *Cell Metab.* 2012; 15:570–573. [PubMed: 22560209]

96. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science*. 2011; 332:1519–1523. [PubMed: 21700865]
97. Sun Z, Lazar MA. Dissociating fatty liver and diabetes. *Trends Endocrinol Metab*. 2013; 24:4–12. [PubMed: 23043895]
98. Niebergall LJ, Jacobs RL, Chaba T, Vance DE. Phosphatidylcholine protects against steatosis in mice but not non-alcoholic steatohepatitis. *Biochim Biophys Acta*. 2011; 1811:1177–1185. [PubMed: 21745592]
99. Jacobs RL, et al. Impaired *de novo* choline synthesis explains why phosphatidylethanolamine *N*-methyltransferase-deficient mice are protected from diet-induced obesity. *J Biol Chem*. 2010; 285:22403–22413. [PubMed: 20452975]
100. Xu E, et al. Hepatocyte-specific Ptpn6 deletion promotes hepatic lipid accretion, but reduces NAFLD in diet-induced obesity: potential role of PPAR γ . *Hepatology*. 2013; 59:1803–1815. [PubMed: 24327268]
101. Ruiz R, et al. Sterol regulatory element binding protein-1 (SREBP-1) is required to regulate glycogen synthesis and gluconeogenic gene expression in mouse liver. *J Biol Chem*. 2014; 289:5510–5517. [PubMed: 24398675]
102. Benhamed F, et al. The lipogenic transcription factor ChREBP dissociates hepatic steatosis from insulin resistance in mice and humans. *J Clin Invest*. 2012; 122:2176–2194. [PubMed: 22546860]
103. Brown JM, et al. CGI-58 knockdown in mice causes hepatic steatosis but prevents diet-induced obesity and glucose intolerance. *J Lipid Res*. 2010; 51:3306–3315. [PubMed: 20802159]
104. Cantley JL, et al. CGI-58 knockdown sequesters diacylglycerols in lipid droplets/ER-preventing diacylglycerol-mediated hepatic insulin resistance. *Proc Natl Acad Sci USA*. 2013; 110:1869–1874. The authors of this study demonstrate the importance of compartmentation of DAG in modulating hepatic insulin resistance. [PubMed: 23302688]
105. Bergman BC, Hunerdosse DM, Kerege A, Playdon MC, Perreault L. Localisation and composition of skeletal muscle diacylglycerol predicts insulin resistance in humans. *Diabetologia*. 2012; 55:1140–1150. [PubMed: 22252470]
106. Sun Z, et al. Hepatic Hdac3 promotes gluconeogenesis by repressing lipid synthesis and sequestration. *Nature Med*. 2012; 18:934–942. [PubMed: 22561686]
107. Kumashiro N, et al. Role of patatin-like phospholipase domain-containing 3 on lipid-induced hepatic steatosis and insulin resistance in rats. *Hepatology*. 2013; 57:1763–1772. [PubMed: 23175050]
108. Kumari M, et al. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. *Cell Metab*. 2012; 15:691–702. [PubMed: 22560221]
109. Speliotes EK, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet*. 2011; 7:e1001324. [PubMed: 21423719]
110. Lin YC, Chang PF, Chang MH, Ni YH. Genetic variants in GCKR and PNPLA3 confer susceptibility to nonalcoholic fatty liver disease in obese individuals. *Am J Clin Nutr*. 2014; 99:869–874. [PubMed: 24477042]
111. Petersen KF, et al. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes*. 2005; 54:603–608. This study demonstrates that moderate (<10%) body weight reduction in obese patients with type 2 diabetes eating a 1,200 calorie hypocaloric diet corrected fasting plasma glucose concentrations, normalized rates of hepatic glucose reversed NAFLD and reversed hepatic insulin resistance independent of changes in circulating adipocytokines. [PubMed: 15734833]
112. Lim EL, et al. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia*. 2011; 54:2506–2514. [PubMed: 21656330]
113. Weiss EC, Galuska DA, Kettel Khan L, Gillespie C, Serdula MK. Weight regain in U.S. adults who experienced substantial weight loss, 1999–2002. *Am J Prev Med*. 2007; 33:34–40. [PubMed: 17572309]

114. McGuire MT, Wing RR, Hill JO. The prevalence of weight loss maintenance among American adults. *Int J Obes Relat Metab Disord*. 1999; 23:1314–1319. [PubMed: 10643690]
115. Haufe S, et al. Long-lasting improvements in liver fat and metabolism despite body weight regain after dietary weight loss. *Diabetes Care*. 2013; 36:3786–3792. [PubMed: 23963894]
116. Mayerson AB, Hundal RS, Dufour S, Lebon V. The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes*. 2002; 51:797–802. This study demonstrated that thiazolidinediones improve insulin sensitivity in patients with type 2 diabetes by decreasing hepatic steatosis and promoting a redistribution of fat to the subcutaneous fat compartment. [PubMed: 11872682]
117. Kim JK, et al. Differential effects of rosiglitazone on skeletal muscle and liver insulin resistance in A-ZIP/F-1 fatless mice. *Diabetes*. 2003; 52:1311–1318. [PubMed: 12765938]
118. Prieur X, et al. Thiazolidinediones partially reverse the metabolic disturbances observed in Bcl2/seipin-deficient mice. *Diabetologia*. 2013; 56:1813–1825. [PubMed: 23680914]
119. Dutchak PA, et al. Fibroblast growth factor-21 regulates PPAR γ activity and the antidiabetic actions of thiazolidinediones. *Cell*. 2012; 148:556–567. [PubMed: 22304921]
120. Miyazaki Y, et al. Rosiglitazone improves downstream insulin receptor signaling in type 2 diabetic patients. *Diabetes*. 2003; 52:1943–1950. [PubMed: 12882909]
121. Perry RJ, et al. Reversal of hypertriglyceridemia, fatty liver disease, and insulin resistance by a liver-targeted mitochondrial uncoupler. *Cell Metab*. 2013; 18:740–748. The authors of this article demonstrate that a liver-targeted mitochondrial uncoupling agent (DNP) resulted in around a 60% increase in hepatic fat oxidation, reductions in liver and muscle triglyceride and diacylglycerol content and reversal of liver and muscle insulin resistance in rat models of NAFLD and type 2 diabetes. [PubMed: 24206666]

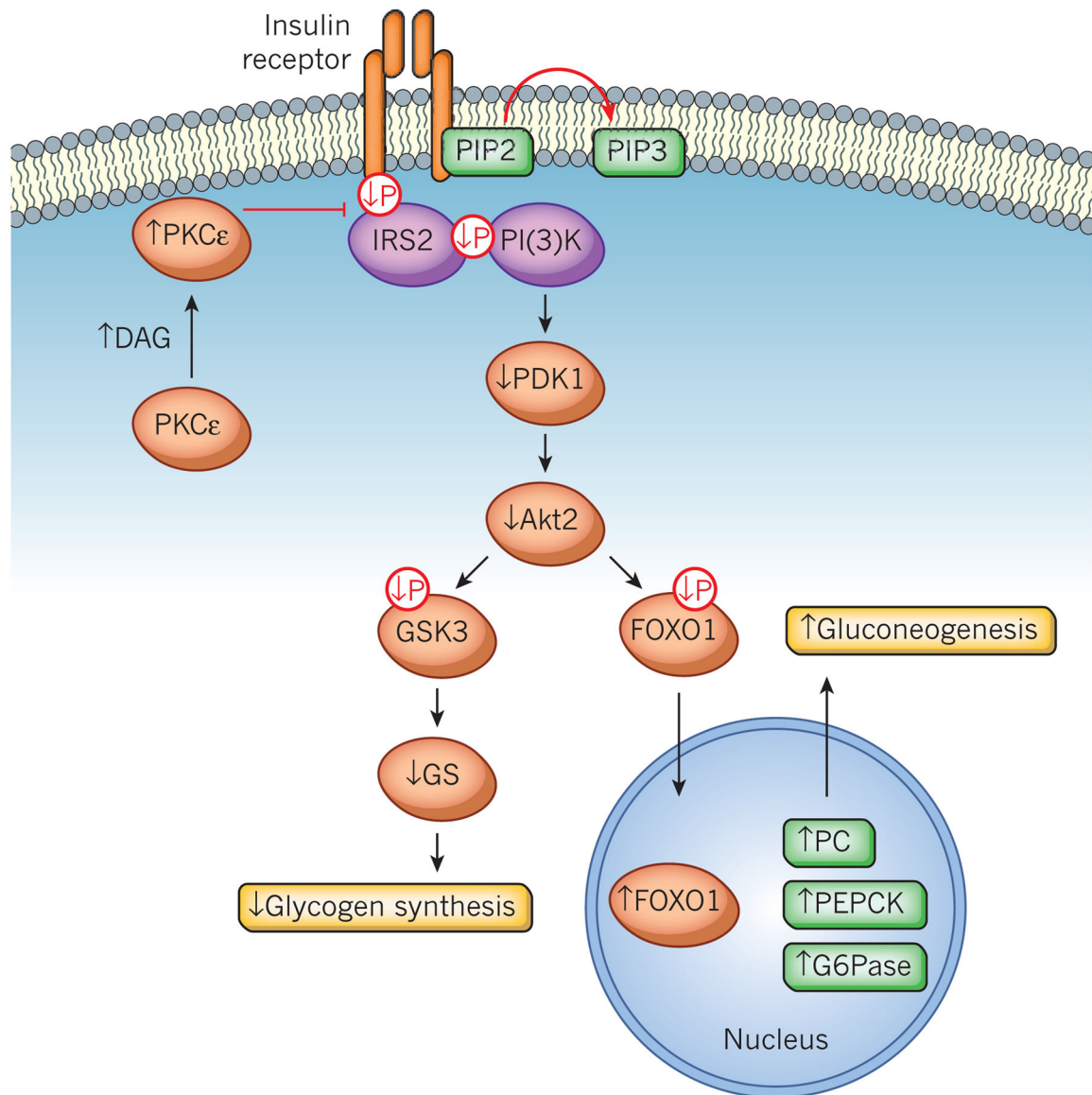


Figure 1. Molecular mechanism by which excess diacylglycerol leads to hepatic insulin resistance and hyperglycaemia

Increases in liver diacylglycerol (DAG) cause protein kinase C ϵ (PKC ϵ) activation and translocation to the cell membrane, which results in inhibition of insulin signalling. Reduced phosphorylation of insulin receptor substrate-2 (IRS2) and PI(3)K impairs Akt2 activity by reductions in 3-phosphoinositide-dependent protein kinase 1 (PDK1) activity, suppressing glycogen synthase kinase-3 (GSK3) phosphorylation and reducing insulin-stimulated liver glycogen synthesis through reduced glycogen synthase (GS) activity. Impaired Akt2 activity also reduces insulin suppression of hepatic gluconeogenesis by promoting Forkhead box protein O1 (FOXO1) translocation to the nucleus due to reduced phosphorylation and increasing expression of the gluconeogenic proteins pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase). PIP3, phosphatidylinositol (3,4,5)-triphosphate.

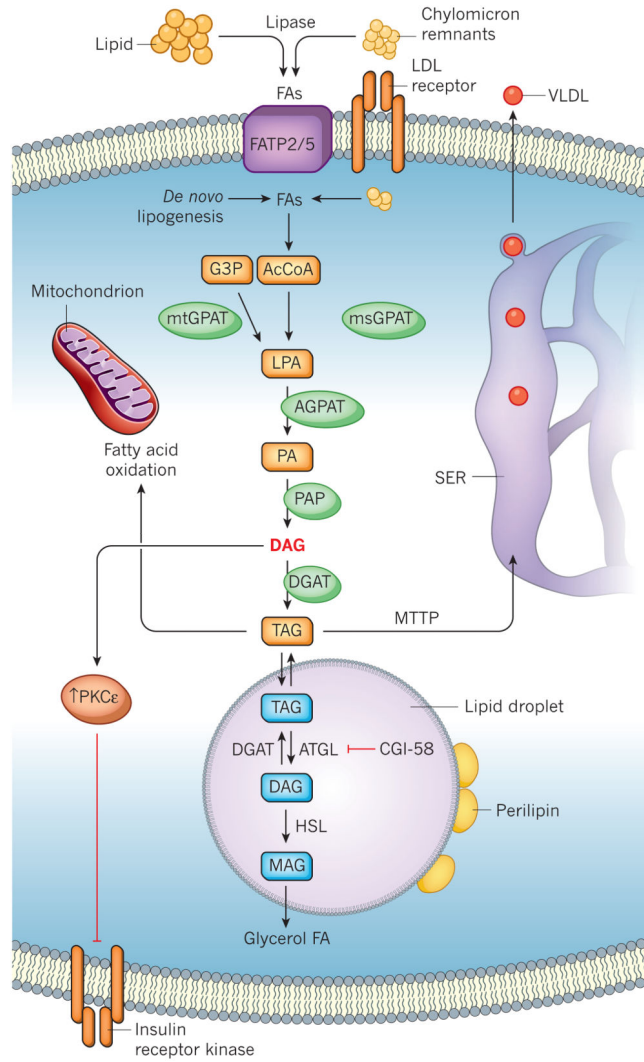


Figure 2. NAFLD develops due to an imbalance between lipid supply and demand
 Fatty acids (FAs) derived from lipolysis and from chylomicron remnants are taken up through fatty-acid transport proteins (FATPs), mainly FATP2 and FATP5 in the liver; chylomicron remnants are also taken up through the low-density lipoprotein (LDL) receptor. A small fraction of intracellular fatty acid supply in the liver also comes from *de novo* lipogenesis in the cytosol. Fatty acids can also be re-esterified to lysophosphatidic acid (LPA) by acyl-coenzyme A (AcCoA) and the conversion of glycerol 3-phosphate (G3P) by either mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT) or microsomal GPAT (msGPAT). Fatty-acyl CoAs (shown here as phosphatidic acid, PA) formed by 1-acylglycerol-3-phosphate O-acyltransferase-2 (AGPAT2) are then added to the glycerol backbone by phosphatidic acid phosphatase (PAP) to generate diacylglycerol (DAG), and by diacylglycerol acyltransferases (DGAT) to generate triacylglycerol (TAG). Increased DAG causes protein kinase Cε (PKCε) translocation to the cell membrane, which inhibits insulin signalling. Lipids may also be sequestered in lipid droplets as monoacylglycerol (MAG), DAG and TAG, but these are not thought to be responsible for hepatic insulin resistance. By inhibition of adipose triglyceride lipase (ATGL), comparative gene identification-58

(CGI-58) bound to perilipin is mainly responsible for lipid sequestration in the droplet. By contrast, intracellular hepatic lipid content is reduced by two mechanisms: mitochondrial fatty acid oxidation and export from the smooth endoplasmic reticulum (SER) as very-low-density lipoprotein (VLDL). HSL, hormone-sensitive lipase; MTTP, microsomal triglyceride transfer protein.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

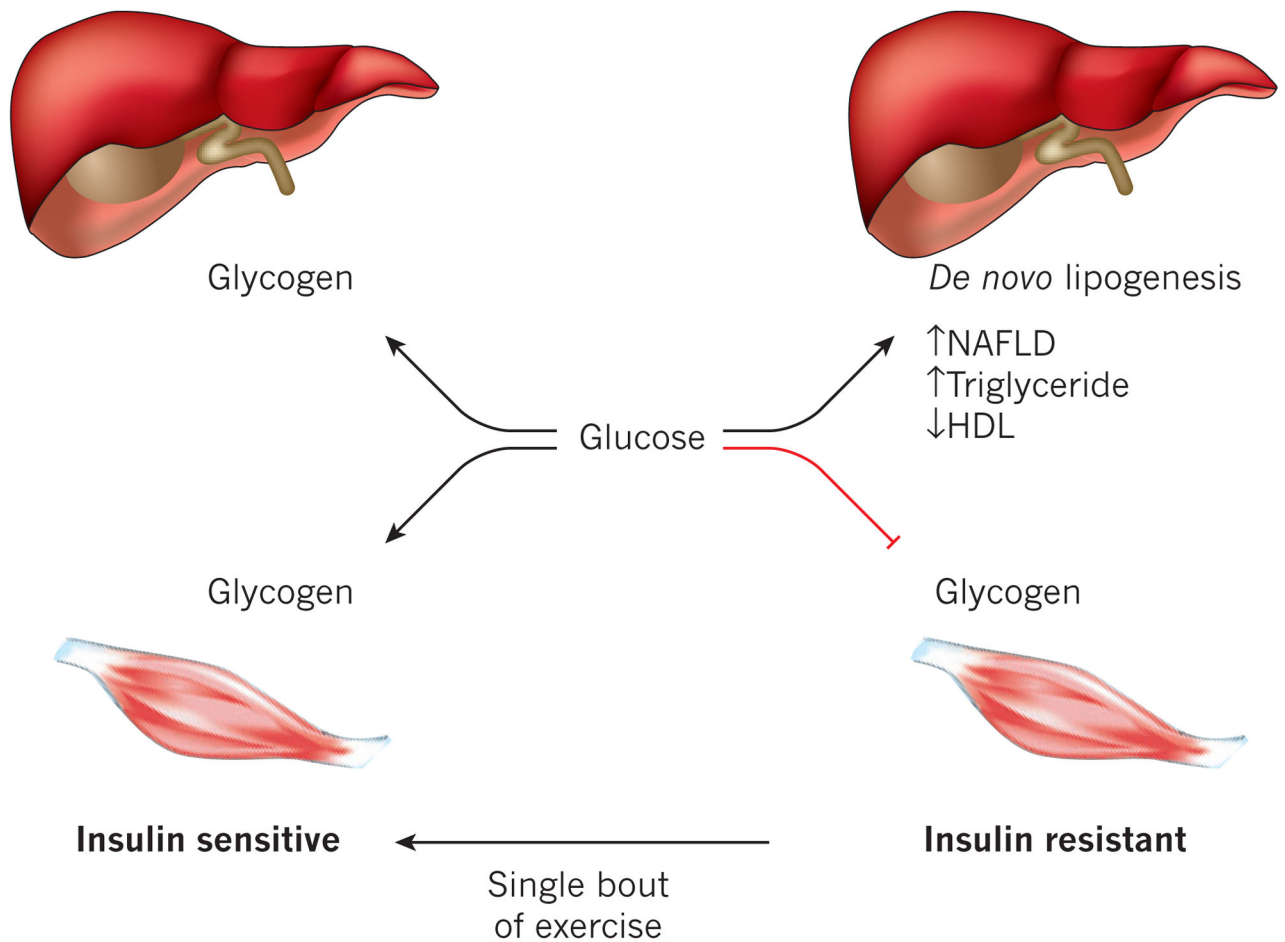


Figure 3. Mechanism by which selective skeletal muscle insulin resistance contributes to hepatic insulin resistance

In insulin-sensitive subjects, insulin stimulates glycogen synthesis in both liver and muscle; however, in those with skeletal muscle insulin resistance, insulin fails to promote glycogen synthesis, diverting substrate to *de novo* lipogenesis. Increased lipid synthesis in patients with muscle insulin resistance thus produces non-alcoholic fatty liver disease (NAFLD), with increased triglyceride and reduced high-density lipoprotein (HDL) export from the liver. However, these defects in muscle insulin signalling can be reversed by a single 45 minute bout of exercise.