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Review

# Nonalcoholic fatty liver disease: A main driver of insulin resistance or a dangerous liaison?☆

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ABSTRACT

Insulin resistance is one of the key components of the metabolic syndrome and it eventually leads to the development of type 2 diabetes, making it one of the biggest medical problems of modern society. Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are tightly associated with insulin resistance. While it is fairly clear that insulin resistance causes hepatic steatosis, it is not known if NAFLD causes insulin resistance. Hepatic inflammation and lipid accumulation are believed to be the main drivers of hepatic insulin resistance in NAFLD. Here we give an overview of the evidence linking hepatic lipid accumulation to the development of insulin resistance, including the accumulation of triacylglycerol and lipid metabolites, such as diacylglycerol and ceramides. In particular, we discuss the role of obesity in this relation by reviewing the current evidence in terms of the reported changes in body weight and/or adipose tissue mass. We further discuss whether the activation or inhibition of inflammatory pathways, Kupffer cells and other immune cells influences the development of insulin resistance. We show that, in contrast to what is commonly believed, neither hepatic steatosis nor hepatic inflammation is sufficient to cause insulin resistance. Many studies show that obesity cannot be ignored as an underlying factor in this relationship and NAFLD is therefore less likely to be one of the main drivers of insulin resistance.



## 1. Introduction

The easy availability of appealing food high in calories is driving excess nutrient intake and the development of obesity and the metabolic syndrome. One of the key components of the metabolic syndrome is insulin resistance, which precedes the development of type 2 diabetes (T2D) [1]. Insulin resistance is reversible, but once T2D has established and beta-cells are damaged, the disease progresses [2]. As the number of people with T2D and healthcare costs continue to rise, it is important to prevent the development of T2D by improving insulin sensitivity. However, much is still unclear about the mechanisms leading to insulin resistance, which makes it difficult to develop new and effective therapies.

Nonalcoholic fatty liver disease (NAFLD) is a feature of the metabolic syndrome and is strongly associated with insulin resistance. In NAFLD, triacylglycerols (TAGs) accumulate in the liver (hepatic steatosis) due to an imbalance between lipid storage and lipid removal [3]. This is caused by a higher dietary fat intake, increased de novo lipogenesis, and increased lipolysis in adipose tissue [4]. In addition, macrophages and other immune cells are recruited to the liver and secrete pro-inflammatory cytokines [5,6]. This state of hepatic inflammation is known as nonalcoholic steatohepatitis (NASH) and it can progress toward cirrhosis and hepatocellular carcinoma [4]. Because NAFLD has become a major disease burden in Western society [7], it is important to determine the exact role of NAFLD in the development of insulin resistance. Low-grade chronic inflammation and lipid accumulation in the liver and other organs (ectopic

*Abbreviations:* AKT (or PKB), protein kinase B; ATGL, adipose triacylglycerol lipase; CGI-58, comparative gene identification 58; ChREBP, carbohydrate responsive element-binding protein; CPT1a, carnitine palmitoyl transferase 1a; CREB, cAMP response element-binding; DAG, diacylglycerol; DGAT2, acyl-CoA:diacylglycerol acyltransferase 2; ER, endoplasmic reticulum; FATP2, fatty acid transporter protein 2; FATP5, fatty acid transporter protein 5; GNMT, glycine N-methyltransferase; HOMA-IR, homeostasis model assessment of insulin resistance; IκBα, inhibitor of NF-κB α; IKK2, inhibitor of κB-kinase-β; IL-1R, interleukin 1 receptor; IL-6Rα, interleukin-6 receptor α; IR, insulin receptor; IRS1/2, insulin receptor substrate 1/2; JNK, c-Jun NH2-terminal kinase; LXR, liver X receptor; mTorc1, mammalian target of rapamycin complex 1; MTP, mitochondrial trifunctional protein; MTTP, microsomal triglyceride transfer protein; MUFA, monounsaturated fatty acid; MyD88, myeloid differentiation primary response gene 88; NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; NF-κB, nuclear factor κB; NIK, NF-κB inducing kinase; NKT-cells, natural killer T-cells; NOX4, NAD(P)H oxidase homologue 4; OSM, oncostatin M; PC, phosphatidylcholine; PKC, protein kinase C; PI3K, phosphatidylinositol 3-kinase; PP2A, protein phosphatase 2A; RANK, receptor activator of NF-κB; RANKL, receptor activator of NF-κB ligand; SFA, saturated fatty acid; shRNA, short hairpin RNA; SOCS3, suppressor of cytokine signaling 3; SREBP-1c, sterol regulatory element-binding protein 1c; T2D, type 2 diabetes; TAG, triacylglycerol; TLR, Toll-like receptor; TNFα, tumor necrosis factor α; TNFR, tumor necrosis factor receptor; TRAF2, TNF-receptor associated factor; VLDL, very low density lipoprotein

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lipid accumulation) have both been implicated in causing insulin resistance [8–10]. Therefore, hepatic steatosis and hepatic inflammation in NAFLD are believed to contribute to insulin resistance as well [11]. However, since NAFLD is often accompanied by obesity in mouse models, it is difficult to differentiate the effects of obesity and NAFLD on insulin resistance. Thus, it remains unclear whether NAFLD is causally involved in the development of insulin resistance.

In this review, we discuss the evidence linking NAFLD to hepatic and systemic insulin resistance and explore the evidence for NAFLD having a causal role in the development of insulin resistance. We specifically discuss the role of hepatic lipid accumulation and hepatic inflammation. We focus on the role of TAGs and lipid metabolites, including diacylglycerols (DAGs) and ceramides, and on the main inflammatory pathways, including nuclear factor κB (NF-κB) and c-Jun NH2-terminal kinase (JNK). We also discuss the role of Kupffer cells, since these are of particular importance in regulating hepatic inflammation. We highlight data derived from studies using liver-specific mouse models and investigate which effects are independent of body weight changes. Although mouse studies are crucial in understanding disease mechanisms, no mouse model fully captures human NAFLD. Thus, some of the mechanisms we propose still need to be validated in man.

## 2. Hepatic lipid accumulation and insulin resistance

### 2.1. Evidence that insulin resistance causes hepatic lipid accumulation

There is a strong association between hepatic lipid accumulation and insulin resistance. This association is at least partly caused by the dual effect of insulin on hepatic glucose production (gluconeogenesis) and de novo lipogenesis in the liver. The liver controls blood glucose homeostasis by gluconeogenesis and this is inhibited by insulin. In hepatic insulin resistance, the inhibition is no longer effective [12] and the pancreas compensates for this by increasing the production of insulin to maintain normal glucose levels. However, there is strong evidence that insulin resistance is selective and only occurs for the pathway involved in glucose metabolism, but not for de novo lipogenesis. The increased insulin levels therefore overstimulate de novo lipogenesis, leading to increased production of lipids (reviewed by [13]). In the liver, the molecular basis for this mechanism was shown to involve sterol regulatory element-binding protein (*Srebp*)-1c, an important lipogenic transcription factor. While genes involved in glucose metabolism were downregulated in models for insulin resistance, *Srebp-1c* remained activated [14]. The inhibition of the gluconeogenic pathway and activation of the lipogenic pathway both require activation of the insulin receptor (IR) [15,16]. There must therefore be a point downstream of the IR where the glucose pathway and the lipogenesis pathway diverge. Evidence for this branch point comes from a study in which the inhibition of the mammalian target of rapamycin complex 1 (mTorc1), which is required for lipid synthesis, selectively blocked lipogenesis, but not the suppression of gluconeogenesis [17]. More recently, another downstream target of the IR, NAD(P)H oxidase homologue 4 (*Nox4*) has been implicated. Inactivation of this pathway by knockdown of *Nox4* in hepatocytes reduced the insulin-stimulated glucose uptake, while lipogenesis was maintained [18]. However, a recent review discussed the possibility that alterations in nutrient handling in peripheral tissues due to insulin resistance contribute more to the lipid phenotype in the liver than selective hepatic insulin resistance [19].

### 2.2. Evidence that hepatic lipid accumulation causes insulin resistance

Although the above studies indicate that insulin resistance causes hepatic lipid accumulation, this does not exclude steatosis from also being a cause of insulin resistance. Indeed, numerous studies, including dietary intervention studies, have been reported in which hepatic steatosis is accompanied by insulin resistance in mice (reviewed by [20]). The possibility therefore arises that the relationship between steatosis and insulin resistance is a vicious cycle, in which systemic insulin resistance leads to hepatic steatosis, and hepatic steatosis then leads to an exacerbation of hepatic insulin resistance. In order to determine whether hepatic steatosis is a consequence or a cause of insulin resistance, mouse models with liver-specific defects in genes involved in hepatic lipid metabolism are of crucial importance. Below we describe the impact of alterations in fatty acid uptake, de novo lipogenesis, beta-oxidation and VLDL-export on hepatic steatosis and insulin resistance. See Fig. 1 and Box 1 for details of how these models fit into the pathways involved in hepatic lipid metabolism.

In support of a causal role for hepatic steatosis in the development of insulin resistance, an improvement in hepatic steatosis and NAFLD was associated with increased systemic insulin sensitivity in mice with liver-specific knockdown of fatty acid transporter protein 5 (*Fatp5*) [21]. *Fatp5* is involved in the uptake of fatty acids from the blood. Moreover, studies investigating important transcription factors for lipogenic genes (*Srebp-1c*, and carbohydrate responsive element-binding protein (*Chrebp*)) found a relationship between steatosis and insulin resistance in liver-specific models [22,23]. Overexpression of *Srebp-1c* in mice increased the homeostasis model assessment of insulin resistance (HOMA-IR) 3-fold in parallel with an increase in steatosis [22], whereas knockdown of *Chrebp* in ob/ob mice improved hepatic steatosis and systemic insulin resistance [23]. In addition, mice fed with a high-fat diet and with increased fatty acid oxidation driven by constitutively active carnitine palmitoyl transferase 1a (*Cpt1a*) in the liver are protected against the development of steatosis and hepatic and systemic insulin resistance [24].

However, each of the above studies reported alterations in body weight between the mice (Table 1). Due to the co-existing nature of steatosis and insulin resistance with obesity, it is therefore likely that the level of adiposity drives the etiology of insulin resistance in these studies. Indeed, the importance of the effect of increased adiposity on insulin resistance is well illustrated by a study in which mice lacking tumor necrosis factor receptors (TNFRs) were more obese and more insulin resistant than their wild type controls. After matching both groups for body weight, the differences in insulin resistance between them almost completely disappeared [25]. In addition, severe weight loss and loss of white adipose tissue are associated with improved insulin sensitivity in mice fed a methionine- and choline-deficient diet, a well-characterized model for NAFLD [26].

In line with this, several studies in which body weight is not affected do not show a relationship between steatosis and insulin resistance (Table 1, Fig. 1). Increased uptake of fatty acids by hepatic *Cd36* overexpression in mice resulted in increased hepatic TAG accumulation, but did not affect glucose tolerance [27]. Similarly, treatment with an agonist for the liver X receptor (*Lxr*), which activates *Srebp-1c* and *Chrebp*, induced hepatic steatosis in lean and ob/ob mice, but this did not affect their hepatic insulin resistance [28]. In addition, increases in fatty acid oxidation in the liver by overexpression of *Cpt1a*, resulted in a reduction in hepatic TAG content in rats. This was not associated with changes in the HOMA-IR [29]. Moreover, liver-specific deletion of adipose triacylglycerol lipase (*Atgl*), an enzyme involved in TAG processing, increased steatosis in mice. However, hepatic insulin resistance and systemic insulin resistance were not affected in this model [30]. Another study also found a dissociation between hepatic steatosis and insulin resistance. Enhancing hepatic fatty acid oxidation by expression of constitutively active *Cpt1a* improved hepatic insulin signaling and glucose and insulin tolerance, without reducing the level of hepatic steatosis [31].

Nevertheless, there are several studies that, in the absence of obesity as a confounding factor, support a causal role for hepatic steatosis in the development of insulin resistance. For instance, hepatic steatosis as a result of a 50% reduction in mitochondrial fatty acid oxidation is associated with the development of systemic insulin resistance in aged mice [32]. In this study, insulin resistance occurred at the same time point that steatosis developed [32]. In addition, fatty liver Shionogi mice have

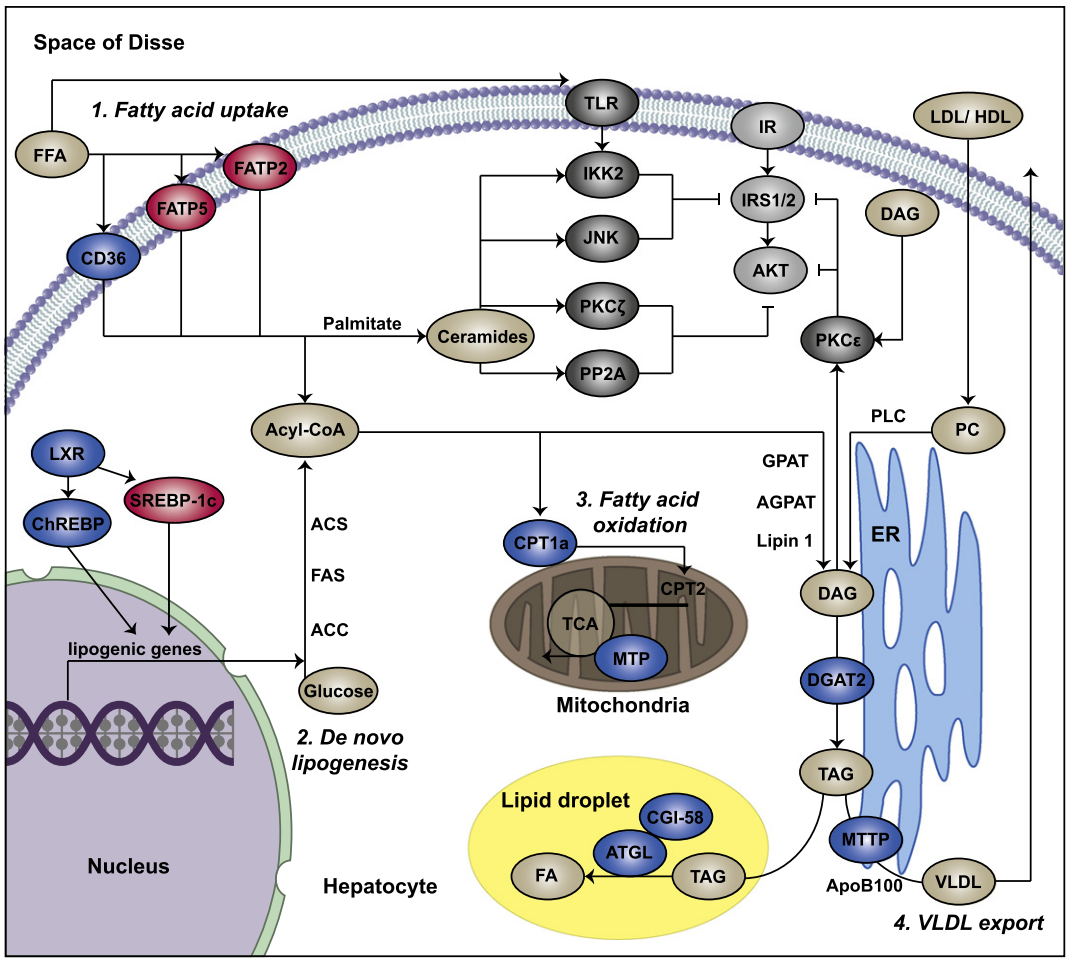


**Fig. 1.** Hepatic steatosis in the etiology of insulin resistance. Simplified overview of the pathways involved in lipid accumulation in the liver and their proposed roles in the development of insulin resistance. Lipid accumulation occurs due to increased uptake of free fatty acids (1), increased de novo lipogenesis (2), reduced fatty acid oxidation (3) and/or reduced VLDL export (4) (see Box 1 for details of hepatic lipid metabolism). The metabolites of these pathways are shown in brown. Proteins involved in hepatic lipid accumulation are shown in red and blue. The red proteins have been implicated in the development of insulin resistance, whereas the blue proteins have been shown not to affect insulin resistance in at least one study. The molecules that directly interfere with the insulin signaling pathway (gray) are shown in black. DAGs, ceramides and free fatty acids have been implicated in the etiology of insulin resistance by inhibiting IRS1/2 and AKT signal transduction. DAGs (especially membrane associated) are believed to interfere with insulin signaling through activation of PKCε. Ceramides have been shown to activate IKK2, JNK, PP2A and PKCζ, which all interfere with insulin signaling. Free fatty acids activate TLRs, which in turn activate IKK2, and IKK2 then inhibits IRS1. Abbreviations: ACC, acetyl-CoA carboxylase; ACS, fatty acyl-CoA synthetase; AGPAT, 1-acylglycerol-3-phosphate acyltransferase; AKT (or PKB), protein kinase B; ATGL, adipose triacylglycerol lipase; CGI-58, comparative gene identification 58; ChREBP, carbohydrate responsive element-binding protein; CPT1a, carnitine palmitoyl transferase 1a; CPT2, carnitine palmitoyl transferase 2; DAG, diacylglycerol; DGAT2, acyl-CoA:diacylglycerol acyltransferase 2; ER, endoplasmic reticulum; FA, fatty acid; FAS, fatty acid synthase; FATP2, fatty acid transporter protein 2; FATP5, fatty acid transporter protein 5; FFA, free fatty acid; GPAT, glycerol-3-phosphate acyltransferase; HDL, high-density lipoprotein; IKK2, inhibitor of κB-kinase-β; IR, insulin receptor; IRS1/2, insulin receptor substrate 1/2; JNK, c-Jun NH2-terminal kinase; LDL, low-density lipoprotein; LXR, liver X receptor; MTP, mitochondrial trifunctional protein; MTTP, microsomal triglyceride transfer protein; PC, phosphatidylcholine; PKC, protein kinase C; PLC, phospholipase C; PP2A, protein phosphatase 2A; SREBP-1c, sterol regulatory element-binding protein 1c; TAG, triacylglycerol; TCA, tricarboxylic acid cycle; TLR, Toll-like receptor; VLDL, very low density lipoprotein.

reduced very low density lipoprotein (VLDL) export due to reduced expression of microsomal triglyceride transfer protein (*Mttp*). This has been shown to induce hepatic steatosis accompanied by systemic insulin resistance in these mice [33]. Likewise, reductions in hepatic steatosis are associated with a protection against systemic insulin resistance in mice lacking hepatic fatty acid transporter protein 2 (*Fatp2*) [34] as well as in db/db mice with constitutively active *Cpt1a* in the liver [24]. However, in mice lacking *Fatp2*, a reduction in fat pad weight was observed, even though body weight was similar [34], which may explain the protection against insulin resistance found in this study.

Together, these studies show that the relationship between steatosis and insulin resistance is still controversial. For each of the mechanisms that can contribute to steatosis, many studies find conflicting evidence for a relationship with insulin resistance (Fig. 1). In only a few studies, insulin resistance was increased in line with enhanced steatosis, independent of body weight gain (Table 1). Overall, hepatic steatosis in itself does not seem to be sufficient or necessary to induce hepatic insulin resistance [35], suggesting that it is not a main driver of insulin resistance.

### 2.3. Lipid species and insulin resistance

A possible explanation for the contradictory findings underlying the relationship between hepatic steatosis and insulin resistance is that hepatic steatosis is a general measure, referring to the buildup of neutral lipids, such as TAGs and cholesterol esters, in lipid droplets in hepatocytes. The accumulation of lipid species and metabolites, such as DAGs, ceramides and saturated fatty acids (SFAs), is more closely linked to the development of insulin resistance [9,10,35,36]. Indeed, a correlation between the accumulation of harmful lipid metabolites in the liver and hepatic insulin resistance has been observed in several animal models (Table 2). DAGs are used for the formation of TAGs and this process is catalyzed by the enzyme acyl-CoA:diacylglycerol acyltransferase 2 (*Dgat2*) [37]. Manipulations in this enzyme therefore affect DAG and

Box 1
Hepatic lipid metabolism.

FFA are transported across the plasma membrane by FATP2, FATP5 and CD36 and are converted to ceramides or to acyl-CoA. Acyl-CoAs are also formed during de novo lipogenesis. SREBP-1c and ChREBP are transcription factors for de novo lipogenic genes. The activity of these transcription factors is stimulated by LXR. Acyl-CoAs are transported to the mitochondria for fatty acid oxidation or are incorporated in TAG. During fatty acid oxidation, CPT1a and CPT2 facilitate the transport of acyl-CoA across the outer and inner mitochondrial membranes. The last three steps of the oxidation of fatty acids in the tricarboxylic acid cycle are catalyzed by MTP. TAGs are formed from Acyl-CoA in multiple steps, with DAG as one of the intermediate metabolites. Once DAG is formed, DGAT2 catalyzes the final step toward TAG synthesis. In addition, PC, derived from HDL and LDL, is converted to DAG by PLC and then further converted to TAG. VLDL particles are assembled from TAGs and ApoB100 with the help of MTTP in the ER. In addition, TAGs are stored in lipid droplets, from which they can be mobilized by ATGL and its activator CGI-58.

TAG levels. Downregulation of *Dgat2* in the liver of rats leads to a reduction in liver TAG and DAG content and protects against the development of hepatic and systemic insulin resistance [38]. In support of the detrimental role of DAGs in insulin resistance, Chan et al. showed that a reduction in hepatic DAG by fenofibrate treatment improved glucose intolerance and restored hepatic insulin signaling in mice [39].

However, in these studies, the protection against insulin resistance occurred in parallel with a decrease in body weight, suggesting that the effects on hepatic and systemic insulin resistance are caused by alterations in adiposity and not by alterations in lipid species. Consistent with this, several other studies in which body weight is not a confounding factor do not show a relationship between lipid species and insulin resistance (Table 2, Fig. 2). Increased levels of TAGs, DAGs and ceramides by hepatic *Dgat2* overexpression in mice did not affect hepatic or systemic insulin resistance [40]. In addition, Minehira et al. showed that blocking VLDL secretion in mice by hepatic deletion of *Mttp* and the resulting increase in TAGs, DAGs and ceramides did not induce hepatic or systemic insulin resistance [41]. Similarly, knockdown of comparative gene identification 58 (CGI-58) in the liver of mice on a chow diet, led to the accumulation of lipid species, without worsening systemic insulin resistance [42]. Phosphatidylcholine (PC), half of which is derived from lipoproteins, is an important source of TAG in the liver [43]. Interference in PC metabolism, by knocking down glycine N-methyltransferase (*Gnmt*), also did not result in systemic insulin resistance, even though hepatic TAG and DAG levels were elevated [44]. Additional evidence against a role for lipid species in the development of insulin resistance comes from mice heterozygote for mitochondrial trifunctional protein (*Mtp*). These mice have a 50% reduction in fatty acid oxidation, that leads to a reduction in ceramides, but an increase in hepatic insulin resistance [45]. Moreover, mice with increased TAGs and DAGs due to liver-specific overexpression of *Chrepb* were protected against the development of hepatic and systemic insulin resistance [46]. Despite the fact that their body weight was not altered, their fat mass was decreased [46], which may partly explain the protection against insulin resistance observed in this study. Another explanation may be the enrichment of beneficial lipid species (mono-unsaturated fatty acids, MUFAs) and the reduction of detrimental lipid species (SFAs) found in mice overexpressing *Chrebp* in the liver [46].

Of note, there are only a few studies that report that lipid species induce insulin resistance, independent of body weight (Table 2). Strikingly, a group, using the same mouse model of *Dgat2* overexpression in the liver as previously described [40], found that these mice have increased hepatic TAGs, ceramides and DAGs, and exacerbated hepatic, but not systemic, insulin resistance [47]. Similarly, a modification in another enzyme involved in TAG processing shows a relationship between lipid metabolites and insulin resistance. Mice in which *Atgl* is overexpressed in the liver have reduced steatosis and a decreased amount of DAGs and ceramides in the liver, associated with a mild improvement of hepatic insulin signaling [48].

Despite this controversy, there is much evidence for the mechanisms by which these lipid metabolites may interfere with the insulin signaling cascade (Fig. 1). Following insulin binding to the IR, the IR induces tyrosine phosphorylation of the insulin receptor substrates (IRS1 and IRS2). At these phosphorylation sites, other signaling molecules, such as phosphatidylinositol 3-kinase (PI3K), bind and transmit the signal further to protein kinase B (PKB or AKT) [9]. Ceramides induce protein kinase C (PKC)ζ and protein phosphatase 2A (PP2A) activation (reviewed by [36]). PKCs and PP2A have been shown to interfere with insulin signaling [49–52]. In addition, ceramides were shown to upregulate kinases that are believed to inhibit insulin signaling, including JNK and inhibitor of κB-kinase-β (IKK2) [53–55]. In adipocytes, ceramides and glucosylceramides were found to antagonize insulin action, whereas in myotubes only ceramides seemed to play a role [56]. Glucosylceramides also do not seem to be important in the liver, as liver-specific inhibition of glucosylceramide synthesis did not improve insulin resistance in mice [57]. If another class of ceramides does interfere with insulin signaling in the liver, it is still unknown. Specific DAG species, e.g. sn-1,2 DAGs, are also believed to activate PKC [58]. In the liver, particularly PKCε has been implicated [59]. SFAs were shown to induce insulin resistance through DAG-induced PKCε activation, independent of ceramides, and PKCε impaired IRS2 signaling [60]. That PKCε is responsible for the observed effect on insulin resistance is supported by the fact that antisense oligonucleotide-mediated inhibition of PKCε in the liver improved insulin resistance, independent of DAG levels [61]. In addition, DAGs were shown to be necessary for endoplasmic reticulum (ER)-stress to induce hepatic insulin resistance [39]. Finally, lipid species may induce inflammation through the activation of Toll-like receptors (TLRs) and could thereby cause insulin resistance indirectly [62].

### 2.4. Hepatic lipid accumulation and insulin resistance: unresolved questions

The relationship between steatosis and insulin resistance is still controversial (Fig. 1). In many studies the relationship between steatosis and systemic as well as hepatic insulin resistance is explained by adiposity. However, there is also evidence that hepatic lipids induce insulin resistance under certain circumstances. Unfortunately, the complex and multifactorial etiology of insulin resistance makes it difficult to elucidate what these specific circumstances are. Since C57BL/6 mice were used in the majority of studies (Tables 1 and 2), environmental factors, such as gut microbiota or breeding circumstances, are likely to be involved. The fact that two groups using the same mouse model, at the same age and on the same diet, found contradictory results [40,47] emphasizes this point. Determining the factors that explain the differences between these two studies would help us to understand which environmental factors are involved in the development of insulin resistance and/or which experimental procedures are the most reliable. In addition, it may depend on the fatty acid composition in the liver, as an increase in the hepatic MUFA/SFA ratio was associated with protection against metabolic disease, despite high DAG levels [46]. On the other hand, other authors reported that increased MUFAs in the liver are associated with worsened insulin resistance [22]. Thus, the role of the fatty acid composition in insulin resistance warrants further investigation. In addition, it would be of interest to determine if the fatty acid composition of TAGs and DAGs is relevant in the development of insulin resistance, as alterations in their fatty acid composition have been shown to occur in patients with NAFLD [63].

**Table 1**
Animal models investigating hepatic steatosis and insulin resistance.

| Mechanism | Model[a] | Diet[b] | Steatosis | Body weight | Insulin resistance | Assessed by | Ref. |
|---|---|---|---|---|---|---|---|
| Fatty acid uptake | FATP5 knockdown liver using AAV | HFD (60% fat) | Decreased | Decreased | Systemic IR decreased | Glucose levels, GTT, ITT | [21] |
| | CD36 overexpression liver using AV | LFD (10% fat) | Increased | Not affected | Systemic IR not affected | Glucose levels, GTT | [27] |
| | FATP2 knockdown liver using AAV | HFD (60% fat) | Decreased | Not affected[c] | Systemic IR decreased | Glucose levels, GTT, HOMA-IR | [34] |
| De novo lipogenesis | SREBP-1c overexpression liver using transgenic approach | Standard chow | Increased | Increased | Systemic IR increased | HOMA-IR, QUICKI | [22] |
| | ChREBP knockdown liver using AV in ob/ob mice | Standard chow | Decreased | Decreased | Systemic IR decreased | Glucose and insulin levels, GTT, ITT, WAT and muscle insulin signaling | [23] |
| | LXR agonist in diet | Standard chow | Increased | Not affected | Systemic and hepatic IR not affected | Glucose and insulin levels, HIEC, hepatic gene expression | [28] |
| | | Standard chow (ob/ob) | Increased | Not affected | Systemic IR decreased, hepatic IR not affected | Glucose and insulin levels, HIEC, hepatic gene expression | |
| Beta-oxidation | Constitutively active CPT1a liver using AAV in DIO or db/db mice | HFD (60% fat) | Decreased | Decreased | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, PTT, hepatic, muscle and WAT insulin signaling, hepatic gene expression | [24] |
| | | Chow (db/db mice) | Decreased | Not affected | Decreased systemic IR | Glucose and insulin levels | |
| | CPT1a overexpression liver using AV in Sprague–Dawley rats | Standard chow | Decreased | Not affected | Systemic IR not affected | Glucose and insulin levels, HOMA-IR | [29] |
| | | HFD (41% fat) | Decreased | Not affected | Systemic IR not affected | Glucose and insulin levels, HOMA-IR | |
| | Constitutively active CPT1a liver using AV in DIO or ob/ob mice | Chow (ob/ob mice) | Not affected | Not affected | Systemic IR decreased | Insulin levels, GTT, ITT | [31] |
| | | HF/HSD (35% CHO, 45% fat) | Not affected | Not affected | Hepatic and systemic IR decreased | Insulin levels, GTT, ITT, hepatic insulin signaling | |
| | MTP heterozygote mice | Standard chow young | Not affected | Not affected | Systemic IR not affected | Glucose and insulin levels, GTT, ITT | [32] |
| | | Standard chow aged | Increased | Not affected | Systemic IR increased | Glucose and insulin levels, GTT, ITT | |
| VLDL export | Fatty liver Shionogi mice | Standard chow | Increased | Not affected | Systemic IR increased | HOMA-IR | [33] |
| Lipolysis | ATGL knockdown liver | Standard chow | Increased | Not affected | Hepatic and systemic IR not affected | Glucose levels, GTT, ITT, PTT, hepatic gene expression | [30] |
| | | HFD (59% fat) | Increased | Not affected | Systemic IR not affected | GTT, ITT | |

Abbreviations: AAV, adeno-associated virus; ATGL, adipose triacylglycerol lipase; AV, adenovirus; CHO, carbohydrate; ChREBP, carbohydrate responsive element-binding protein; DIO, diet-induced obese; FATP2, fatty acid transporter protein 2; FATP5, fatty acid transporter protein 5; GTT, glucose tolerance test; HFD, high fat diet; HF/HSD, high fat/high sucrose diet; HIEC, hyperinsulinemic–euglycemic clamp; HOMA-IR, homeostasis model assessment of insulin resistance; ITT, insulin tolerance test; IR, insulin resistance; LFD, low-fat diet; LXR, liver X receptor; MTP, mitochondrial trifunctional protein; PTT, pyruvate tolerance test; ref, reference; SREBP-1c, sterol regulatory element-binding protein 1c; VLDL, very low density lipoprotein; WAT, white adipose tissue.

[a] All models were mice on a C57BL/6 background, unless otherwise stated.

[b] All percentages are calories from fat, unless otherwise stated.

[c] Body weight not affected, but decreased adipose tissue mass.

**Table 2**
Animal models investigating hepatic lipid species and metabolites in the development of insulin resistance.

| Model[a] | Diet[b] | Hepatic inflammation | Lipid accumulation | Body weight | Insulin resistance | Assessed by | Ref. |
|---|---|---|---|---|---|---|---|
| DGAT2 hepatic knockdown using ASOs in Sprague–Dawley rats | HFD (59% fat) | n.d. | Decreased: TAGs and DAGs; not affected: long-chain acyl CoAs | Decreased | Hepatic and systemic IR decreased | Glucose and insulin levels, HIEC, hepatic insulin signaling | [38] |
| PPARα activation using fenofibrate | HFruD (35% fructose) | Not affected | Decreased: TAGs and DAGs; increased: ceramides | Decreased | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, HOMA-IR, hepatic and muscle insulin signaling | [39] |
| DGAT2 hepatic overexpression using transgenic approach | Standard chow | Not affected | Increased: TAGs, DAGs, ceramides and long chain acyl CoA | Not affected | Hepatic and systemic IR not affected | Glucose and insulin levels, GTT, ITT, HIEC, hepatic insulin signaling | [40] |
| MTTP knockdown liver (C57BL/6 and 129/SvJae mix) | Standard chow | n.d. | Increased: TAGs, DAGs and ceramides | Not affected | Hepatic and systemic IR not affected | Glucose and insulin levels, GTT, HIEC, hepatic insulin signaling and gene expression | [41] |
| CGI-58 knockdown using ASOs liver and WAT | Standard chow | Not affected | Increased: TAGs, DAGs and ceramides; not affected: long chain acyl CoAs | Not affected[c] | Systemic IR not affected | Glucose levels, GTT, ITT, hepatic gene expression | [42] |
| | HFD (45% fat) | Not affected | Increased TAGs, DAGs, ceramides; decreased: long chain acyl CoAs | Decreased[c] | Systemic IR decreased | Glucose and insulin levels, GTT, ITT, hepatic gene expression | |
| GNMT knock-out mice | Standard chow | n.d. | Increased: TAGs and DAGs | Not affected[c] | Systemic IR not affected | GTT, ITT | [44] |
| MTP heterozygote mice | Standard chow | Not affected | Increased: TAGs; not affected: DAGs; decreased ceramides | Decreased | Hepatic IR increased | Glucose and insulin levels, HIEC, hepatic insulin signaling and gene expression | [45] |
| ChREBP overexpression liver using AV | Standard chow | Decreased | Increased: TAGs, DAGs and MUFAs; not affected: ceramides, SFAs and PUFAs | Not affected[c] | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, ITT, PTT, hepatic insulin signaling and gene expression | [46] |
| | HFD (60% fat, modified) | Not affected | Increased: TAGs, DAGs and MUFAs; decreased: SFAs | Not affected[c] | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, hepatic insulin signaling and gene expression | |
| DGAT2 hepatic overexpression using transgenic approach | Standard chow | Not affected | Increased TAGs, DAGs and ceramides | Not affected | Hepatic IR increased, systemic IR not affected | Insulin levels, HIEC, hepatic insulin signaling | [47] |
| ATGL overexpression using AV | HFD (59% fat) | Not affected | Decreased: TAGs, DAGs and ceramides | Not affected | Hepatic IR decreased | Glucose and insulin levels, ITT, PTT, hepatic insulin signaling and gene expression | [48] |

Abbreviations: ASO, antisense oligonucleotide; ATGL, adipose triacylglycerol lipase; AV, adenovirus; CGI-58, comparative gene identification 58; ChREBP, carbohydrate responsive element-binding protein; DAGs, diacylglycerols; DGAT2, acyl-CoA: diacylglycerol acyltransferase 2; GNMT, glycine N-methyltransferase; HFD, high fat diet; HFruD, high fructose diet; HIEC, hyperinsulinemic-euglycemic clamp; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; ITT, insulin tolerance test; GTT, glucose tolerance test; MTP, mitochondrial trifunctional protein; MTTP, microsomal triglyceride transfer protein; MUFA, mono-unsaturated fatty acid; n.d., not determined; PPARα, peroxisome proliferator-activated receptor α; PTT, pyruvate tolerance test; ref, reference; PUFA, poly-unsaturated fatty acid; SFA, saturated fatty acid; TAGs, triacylglycerols; VLDL, very low density lipoprotein; WAT, white adipose tissue.

[a] All models were mice on a C57BL/6 background, unless otherwise stated.

[b] All percentages are calories from fat, unless otherwise stated.

[c] No differences in body weight, but decreased adipose tissue mass.

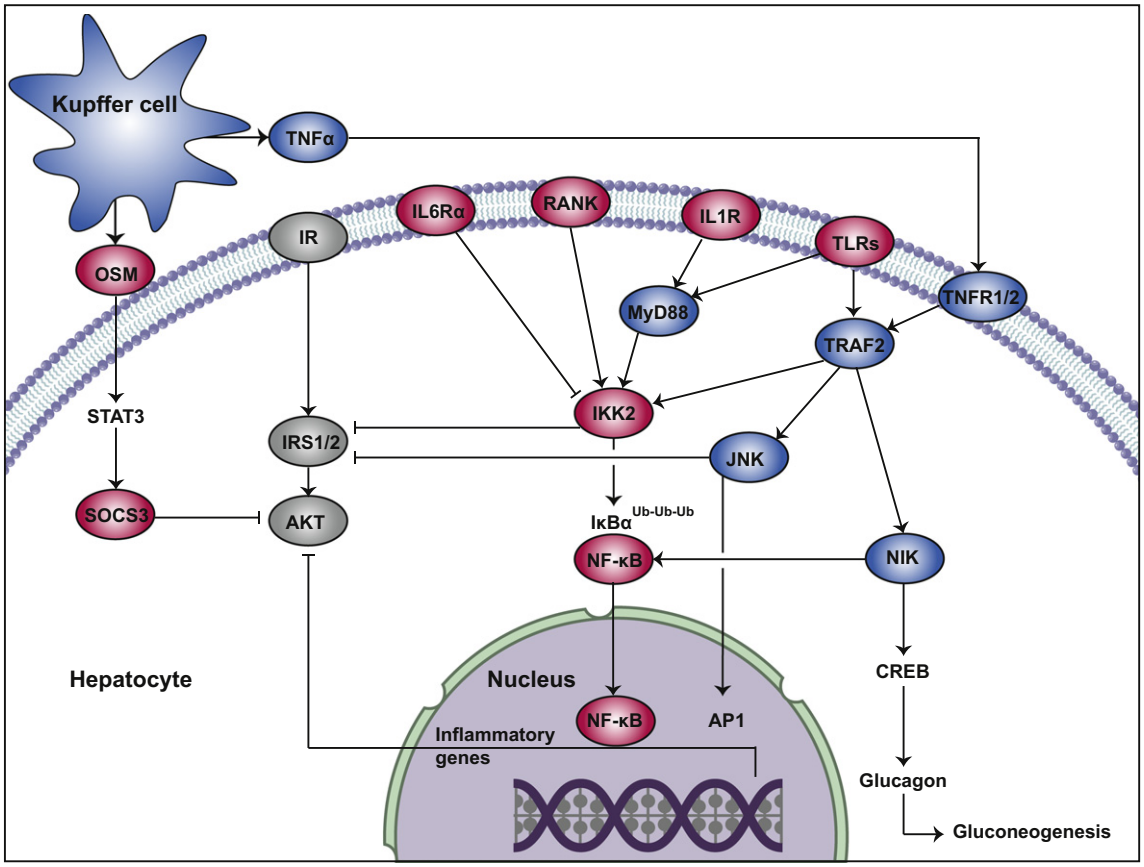


**Fig. 2.** Hepatic inflammation in the etiology of insulin resistance. Simplified overview of the pathways involved in the development of inflammation and insulin resistance in hepatocytes. The proteins involved in hepatic inflammation are shown in red and blue. The red proteins were shown to affect the development of insulin resistance, whereas the blue proteins were shown not to affect insulin resistance in at least one study. The proteins involved in the insulin signaling pathway are shown in gray. Kupffer cells secrete OSM, which interferes with AKT phosphorylation via activation of SOCS3 and STAT3 pathway. In addition, activated Kupffer cells secrete TNFα, which binds to TNFR1/2. Activation of TLR, IL-1R or the TNFR1 and/or 2 leads to the activation of adaptors proteins, such as MyD88 and TRAF2. These adaptor proteins transmit the signal IKK2, JNK or NIK. IKK2 is also activated by RANK signaling, but inhibited by IL-6Rα signaling. IKK2 activation results in the ubiquitination and subsequent degradation of IκBα. This releases NF-κB, which then translocates to the nucleus and starts the transcription of pro-inflammatory genes. JNK activates the transcription factor AP-1, resulting in the transcription of pro-inflammatory genes. IKK2, JNK and the pro-inflammatory genes are believed to interfere with insulin signaling at the level of IRS1/2 and AKT. NIK activates NF-κB and improves CREB stability. CREB promotes glucagon-induced gluconeogenesis. Abbreviations: AKT (or PKB), protein kinase B; AP1, activator protein 1; CREB, cAMP response element-binding; IκBα, inhibitor of NF-κB α; IKK2, inhibitor of κB-kinase-β; IL-1R, interleukin 1 receptor; IL-6Rα, interleukin-6 receptor α; IR, insulin receptor; IRS1/2, insulin receptor substrate 1/2; JNK, c-Jun NH2-terminal kinase; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor κB; NIK, NF-κB inducing kinase; OSM, oncostatin M; RANK, receptor activator of NF-κB; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; TLR, Toll-like receptor; TNFα, tumor necrosis factor α; TNFR1/2, tumor necrosis factor receptor 1/2; TRAF2, TNF-receptor associated factor.

Finally, an explanation for the dissociations between DAG accumulation and insulin resistance found in several studies could lie in differences in the intracellular localization of lipid species [35]. It seems that only membrane-associated DAGs are responsible for PKC inhibition. The dissociation between DAGs and insulin resistance in mice treated with antisense oligonucleotides to inhibit CGI-58 [42] was later explained by the fact that DAG levels were increased only in lipid droplets and not in the cell membrane [64]. Since the localization of lipid species has not been published in most studies, it is unclear whether this explains the discrepancies between the other studies. Future studies need to elucidate the other circumstances under which lipids induce insulin resistance.

## 3. Hepatic inflammation

### *3.1. Does hepatic inflammation cause hepatic insulin resistance?*

Many animal models have been developed to gain insight into the role of hepatic inflammation in the etiology of insulin resistance (Tables 3 and 4, Fig. 2). In the sections below, we will describe the data on the role of the main inflammatory pathways in the development of insulin resistance.

#### *3.1.1. IKK2–NF-κB and insulin resistance*

NF-κB is one of the most important transcription factors of inflammatory cytokines and is activated in NASH [65]. In addition, NF-κB activation has been implicated in the etiology of insulin resistance [66], suggesting that its activation in NASH could also be important for insulin resistance. The canonical pathway involved in NF-κB activation is regulated by IKK2 (Fig. 2). When IKK2 becomes activated, it phosphorylates the inhibitor of NF-κB, IκBα, which then becomes ubiquitinated and subsequently degraded. This releases NF-κB for translocation into the nucleus and initiates the transcription of pro-inflammatory genes [67]. In a mouse model, Cai et al. found that a constitutively active form of IKK2 led to hepatic inflammation and hepatic as well as systemic insulin resistance [68]. In line with this, knockdown of IKK2 in hepatocytes protected mice from hepatic insulin resistance [69], indicating that hepatic inflammation induced by IKK2 plays an important role in the development of insulin resistance. Upstream signals that activate the IKK2/NF-κB pathway are mediated by the TLR and interleukin 1 receptor (IL-1R). Both TLR and IL-1R activate IKK2 via myeloid differentiation primary response gene 88 (MyD88), making it a key molecule in the activation of this pathway [70]. These proteins have also been intensively studied in relation to insulin resistance. IL-1R-deficient mice were protected from diet-induced systemic insulin resistance, without a

**Table 3**
Animal models investigating the role of hepatic inflammation in insulin resistance.

| Pathway | Model[a] | Diet[b] | Steatosis | Hepatic inflammation | Adipose tissue inflammation | Body weight | Insulin resistance | Assessed by | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| NF-κB | Constitutively active human IKK2 in hepatocytes | Standard chow | Decreased | Increased | n.d. | Not affected | Hepatic and systemic IR increased | Glucose and insulin levels, GTT, HIEC, HOMA-IR, hepatic and muscle insulin signaling and hepatic gene expression | [68] |
| | Hepatocyte-specific knockdown of IKK2 in DIO or ob/ob mice | Standard chow | n.d. | Not affected | Not affected | n.d. | Not affected | Glucose and insulin levels, GTT, HIEC, hepatic insulin signaling and gene expression | [69] |
| | | HFD (60% fat) | n.d. | Decreased | Not affected | n.d. | Hepatic IR decreased, systemic IR not affected | Glucose and insulin levels, GTT, HIEC, hepatic insulin signaling and gene expression | |
| | | Standard chow (ob/ob) | n.d. | n.d. | Not affected | n.d. | Hepatic IR decreased, systemic IR not affected | Glucose and insulin levels, GTT, HIEC, hepatic insulin signaling | |
| | TLR9 knockout mice | CDAA diet (14% fat) | Decreased | Decreased | n.d. | Decreased | Systemic IR decreased | HOMA-IR | [71] |
| | IL-1R knockout mice | CDAA diet (14% fat) | Decreased | Not affected | n.d. | Not affected[c] | Systemic IR decreased | HOMA-IR | |
| | MyD88 knockout mice | CDAA diet (14% fat) | Decreased | Decreased | n.d. | Not affected[c] | Systemic IR decreased | HOMA-IR | |
| | TLR2 knockout mice | Standard chow | Decreased | Not affected | Not affected | Not affected | Systemic and hepatic IR decreased | Glucose and insulin levels, GTT, ITT, PTT, HOMA-IR, hepatic, WAT and muscle insulin signaling | [72] |
| | | HFD (60% fat) | n.d. | Decreased | Decreased | Decreased | Systemic and hepatic IR decreased | Glucose and insulin levels, GTT, ITT, HOMA-IR, hepatic, WAT and muscle insulin signaling | |
| | MyD88 knockout mice | Standard chow | n.d. | Not affected | n.d. | Not affected | Systemic IR increased | Glucose and insulin levels, GTT | [73] |
| | | HFD (60% fat) | n.d. | Inconsistent | n.d. | Not affected | Systemic IR increased | Glucose and insulin levels, GTT | |
| | IκBα super-repressor expression in liver db/db mice using AV | Standard chow | Increased | Not affected | n.d. | Not affected | Hepatic IR decreased | Glucose and insulin levels, GTT, PTT, HOMA-IR, hepatic insulin signaling and gene expression | [78] |
| TNFR1/2 | TNFR1 knockout mice | Standard chow | n.d. | n.d. | n.d. | Decreased | Systemic IR not affected | Insulin levels, GTT, ITT | [80] |
| | | HFD (55% fat) | n.d. | n.d. | n.d. | Decreased | Systemic IR decreased | Insulin levels, GTT, ITT | |
| | TNFα knockout mice (C57BL/6 and 129 mix) | Standard chow | n.d. | n.d. | n.d. | Not affected | Systemic IR not affected, hepatic IR not affected | Glucose and insulin levels, GTT, ITT | [81] |
| | | HFD (60% fat) | n.d. | n.d. | n.d. | Not affected[c] | Systemic IR decreased, hepatic IR not affected | Glucose and insulin levels, GTT, ITT, WAT, muscle and liver insulin signaling | |
| | Ob/ob TNFR1/2 double knockout mice | Standard chow | n.d. | n.d. | n.d. | Not affected | Systemic IR decreased, hepatic IR not affected | Glucose and insulin levels, GTT, ITT | |
| | TNFR1/2 double knockout mice | HF/HSD (35.5% w/w fat, 36.6% w/w CHO) | n.d. | n.d. | Decreased | Increased | Systemic IR increased | Glucose and insulin levels, GTT, ITT | [25] |
| | TNFR1 knockout mice | HF/HSD (35.5% w/w fat, 36.6% w/w CHO) | n.d. | n.d. | n.d. | Not affected | Systemic IR not affected | Glucose and insulin levels, GTT, ITT | [82] |
| | TNFR2 knockout mice | HF/HSD (35.5% w/w fat, | n.d. | n.d | n.d. | Decreased | Systemic IR not affected | Glucose and insulin levels, GTT, ITT | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 36.6% w/w CHO) | | | | | | | |
| | TNFR1/2 double knockout mice | HF/HSD (35.5% w/w fat, 36.6% w/w CHO) | n.d. | n.d. | n.d. | Not affected | Systemic IR increased | Glucose and insulin levels, GTT, ITT | |
| | Db/db TNFR1 knockout mice | Standard chow | n.d. | n.d. | n.d. | Not affected | Systemic IR not affected | Glucose and insulin levels, GTT, ITT | |
| | TNFR1 deletion in aromatase knockout mice | Phytoestrogen-low chow | Increased | n.d. | n.d. | Not affected | Systemic IR increased | Glucose and insulin levels, GTT, ITT | [83] |
| | TNFR1 gain of function mutation | Standard chow | Not affected | Increased | Not affected | Not affected | Systemic and hepatic IR not affected | Insulin levels, GTT, hepatic insulin signaling and gene expression | [84] |
| | | HFD (36% w/w fat) | Not affected | Increased | Not affected | Not affected | Hepatic and systemic IR not affected | Insulin levels, GTT, hepatic insulin signaling and gene expression | |
| | Hepatocyte-specific knockdown of TRAF2 | Standard chow | n.d. | n.d. | n.d. | Not affected | Hepatic and systemic IR not affected[d] | Glucose and insulin levels, GTT, ITT, hepatic insulin signaling | [85] |
| | | HFD (60% fat) | Not affected | Not affected | n.d. | Not affected | Hepatic and systemic IR not affected[d] | Glucose and insulin levels, GTT, ITT, PTT, hepatic insulin signaling and gene expression | |
| Other | Inhibition of NIK in the liver using AV | HFD (45% fat) | n.d. | n.d. | n.d. | Not affected | Hepatic and systemic IR not affected[d] | Glucose and insulin levels, GTT, ITT, PTT | [87] |
| | Hepatocyte-specific overexpression NIK | Standard chow | n.d. | n.d. | n.d. | n.d. | Hepatic and systemic IR not affected[d] | GTT, PTT, hepatic gene expression | |
| | Hepatocyte-specific knockdown of RANK in DIO | Standard chow | Not affected | n.d. | n.d. | Not affected | Hepatic and systemic IR not affected | Glucose and insulin levels, GTT, ITT, HOMA-IR, HIEC, hepatic insulin signaling | [89] |
| | | HFD (58% fat) | Decreased | n.d. | n.d. | Not affected | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, ITT, HOMA-IR, HIEC, hepatic insulin signaling | |
| | Inhibition of RANK in the liver using lentivirus | HFD (58% fat) | n.d. | Decreased | n.d. | n.d. | Hepatic and systemic IR decreased | Glucose and insulin levels, HOMA-IR, HIEC | |
| JNK | Overexpression of WT-JNK in liver using AV | Standard chow | n.d. | n.d. | n.d. | Not affected | Hepatic IR increased, systemic IR not affected | Glucose and insulin levels, GTT, HIEC, hepatic insulin signaling and gene expression | [91] |
| | Overexpression DN-JNK in liver using AV in db/db mice | Standard chow | n.d. | n.d. | n.d. | Not affected | Hepatic IR decreased, systemic IR not affected | Glucose and insulin levels, ITT, HIEC, hepatic insulin signaling and gene expression | |
| | Overexpression DN-JNK in liver using AV in DIO | HF/HSD | n.d. | n.d. | n.d. | Not affected | Systemic IR decreased | Glucose and insulin levels, GTT | |
| | JNK knockdown in liver using AV | HFD (60% fat) | Not affected | n.d. | n.d. | Not affected | Hepatic and systemic IR decreased | Glucose and insulin levels, ITT, hepatic insulin signaling and gene expression | [92] |
| | Hepatocyte-specific knockdown of JNK | Standard chow | Increased | Increased | n.d. | Not affected | Hepatic IR increased, systemic IR not affected | Glucose and insulin levels, GTT, HIEC, hepatic insulin signaling and gene expression | [94] |
| | | HFD (60% fat) | Not affected | n.d. | n.d. | n.d. | Hepatic and systemic IR not affected | Glucose and insulin levels GTT, ITT, HIEC | |

Abbreviations: AV, adenovirus; CDAA, choline-deficient L-amino acid defined diet; CHO, carbohydrate; DIO, diet-induced obese; DN-JNK, dominant-negative type JNK; GK, Goto-Kakizaki; HFD, high fat diet; HF/HSD, high fat/high sucrose diet; IκBα, inhibitor of NF-κB α; IKK2, inhibitor of κB-kinase-β; IL-1R, interleukin 1 receptor; IL-1Ra, interleukin 1 receptor antagonist; JNK, c-Jun NH2-terminal Kinase; MyD88, myeloid differentiation primary response gene 88; n.d., not determined; NIK, NF-κB inducing kinase; TNFα, tumor necrosis factor α; NF-κB, nuclear factor κB; RANK, receptor activator of NF-κB; ref, reference; TLR, toll-like receptor; TNFR1, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; TRAF2, TNF-receptor associated factor 2; WT-JNK, wild type JNK.

[a] All models were mice on a C57BL/6 background, unless otherwise stated.

[b] All percentages are calories from fat, unless otherwise stated.

[c] No changes in body weight, but decreased adipose tissue mass.

[d] The effects on glucose metabolism were mediated by glucagon.

**Table 4**
Animal models investigating the role of Kupffer cells and other immune cells in insulin resistance.

| Model[a] | Diet[b] | Steatosis | Hepatic inflammation | Adipose tissue inflammation | Body weight | Insulin resistance | Assessed by | Ref. |
|---|---|---|---|---|---|---|---|---|
| GdCl3 KC depletion in Wistar rats | HFD (45% fat) | Decreased | Decreased | Not affected | Not affected | Hepatic IR decreased, systemic IR not affected | Glucose and insulin levels, HIEC | [96] |
| | HSD (68% sucrose) | Decreased | Decreased | Not affected | Not affected | Hepatic IR decreased, systemic IR not affected | Glucose and insulin levels, HIEC | |
| GdCl3 KC depletion | Standard chow | Not affected | Decreased | Not affected | Not affected[c] | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, HOMA-IR, hepatic insulin signaling and gene expression | [98] |
| | HFD (72% fat) | Decreased | n.d. | n.d. | Decreased | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, HOMA-IR, hepatic insulin signaling and gene expression | |
| GdCl3 KC depletion in Wistar rats | Standard chow | Not affected | Decreased | n.d. | Not affected | Hepatic and systemic IR not affected | Glucose and insulin levels, GTT, hepatic insulin signaling | [100] |
| | HFD (60%, enriched in MUFAs) | Decreased | Increased | n.d. | Not affected | Hepatic and systemic IR increased | Glucose and insulin levels, GTT, hepatic insulin signaling | |
| Clo liposome depletion KC | HFD (60% fat) | Not affected | Decreased | Not affected | Not affected[c] | Hepatic IR decreased | Glucose and insulin levels, HIEC, hepatic insulin signaling | [97] |
| Clo liposome depletion KC | HFD (59.3% fat) | Increased | Increased | Not affected | n.d. | Hepatic and systemic IR increased | Glucose and insulin levels, HOMA-IR, GTT, ITT, PTT, hepatic insulin signaling | [101] |
| Clo liposome depletion KC after 10 weeks HFD | HFD (60% fat) | Not affected | Decreased | Not affected | Not affected | Hepatic and systemic IR not affected | Glucose and insulin levels, HIEC, hepatic and muscle insulin signaling | [102] |
| Clo liposome depletion before 4 weeks HFD | HFD (60% fat) | Not affected | Decreased | Decreased | Decreased | Hepatic and systemic IR decreased | Glucose and insulin levels, GTT, hepatic and muscle insulin signaling | |
| Clo liposome depletion KC in IL-6Rα$^{LKO}$ mice | Standard chow | Decreased | n.d. | n.d. | n.d. | Systemic IR decreased | Insulin levels, GTT | [104] |
| Hematopoietic PPARδ deletion with BMT (129/SvJ mice) | HFD (60% fat) | Increased | Increased | Increased | Not affected[d] | Hepatic and systemic IR increased | Insulin levels, GTT, ITT, hepatic, WAT and muscle insulin signaling | [103] |
| HFD-induced KC activation | 4 week HFD (60% fat) | n.d. | Not affected | n.d. | n.d. | Hepatic IR not affected | Hepatic insulin signaling | [105] |
| | 8 week HFD (60% fat) | n.d. | Increased | n.d. | n.d. | Hepatic IR increased | Hepatic insulin signaling | |
| HFCD-induced KC activation LDLR$^{-/-}$ mice | HFCD (21% w/w fat, 0.2% w/w cholesterol) | Increased | Increased | n.d. | Increased | Hepatic and systemic IR not affected | Insulin levels, HOMA-IR, GTT, muscle and liver insulin signaling | [106] |
| Neutrophil elastase knockout mice | HFD (60% fat) | n.d. | Decreased | Decreased | Decreased | Hepatic and systemic IR decreased | GTT, ITT, HIEC, hepatic and WAT insulin signaling | [110] |
| Dietary fatty acid-induced NKT-cell deficiency | SFA enriched diet (35.9% fat) | Increased | Increased | n.d. | Increased | Systemic IR increased | GTT | [111] |
| | MUFA enriched diet (35.9% fat) | Increased | Increased | n.d. | Increased | Systemic IR increased | GTT | |
| NKT-cell depletion by Cd1d knockdown in mice on BALB/c background | HFD (58% fat) | Increased | Increased | Decreased | Increased | Systemic IR increased | Glucose levels, GTT, ITT, WAT insulin signaling | [112] |

Abbreviations: BMT, bone marrow transplantation; Clo, clodronate; $GdCl_3$, gadolinium chloride; HFCD, high fat/high cholesterol diet; HFD, high fat diet; HSD, high sucrose diet; IL-6Rα$^{LKO}$, interleukin-6 receptor α liver-specific knockout; KC, Kupffer cells; LDLR$^{-/-}$, low-density lipoprotein receptor knockout; MUFA, mono-unsaturated fatty acid; n.d., not determined; NKT-cell, natural killer T-cell; ref, reference; SFA, saturated fatty acid; WAT, white adipose tissue.

[a] All models were mice on a C57BL/6 background, unless otherwise stated.

[b] All percentages are calories from fat, unless otherwise stated.

[c] Body weight not affected, but decreased adipose tissue mass.

[d] Body weight not affected, but increased adipose tissue mass.

reduction in inflammatory cell infiltration in the liver [71]. In addition, mice deficient for TLR9 or MyD88 had reduced hepatic infiltration of inflammatory cells and were protected against systemic insulin resistance [71]. In TLR2 knockout mice, reduced hepatic inflammatory gene expression and signaling were also associated with protection against systemic and hepatic insulin resistance [72]. However, these four mouse models also showed a reduction in body weight and/or fat mass [71,72], which may explain the protection against insulin resistance found in these studies. In line with this, in another study investigating MyD88 (in which no differences in body weight were reported), mice deficient for this protein displayed an increased susceptibility to the development of systemic insulin resistance [73]. Unfortunately, to our knowledge, no liver-specific models regarding these proteins and their effects on glucose metabolism have been described.

The mechanisms by which IKK2 induces insulin resistance have been partly elucidated with in vitro data showing that IKK2 induces serine phosphorylation, instead of tyrosine phosphorylation, of IRS1 [74,75]. When IRS1 is phosphorylated in this way, the insulin signaling pathway is inhibited [76,77]. A similar mechanism is likely to play a role in the liver. In mice with constitutively active IKK2 in the liver, Cai et al. found that the insulin-stimulated tyrosine phosphorylation of the IR and IRS2 was reduced in the liver [68]. Conversely, hepatic IKK2 deficiency resulted in improved binding of PI3K to IRS1 and IRS2 in the liver following a high fat diet. In agreement with this, the activation of hepatic AKT was improved in these mice [69].

Taken together, these findings show that IKK2–NF-κB activation is involved in the development of insulin resistance. Since the in vitro data indicate that IKK2 inhibits insulin signaling directly, the effects of IKK2 on insulin resistance are not necessarily mediated through changes in inflammatory endpoints. Indeed, in a study by Tamura et al. in which NF-κB was inhibited in the liver by injecting db/db mice with an adeno-associated virus carrying the IκBα super-repressor, mice were protected against the development of hepatic insulin resistance without a significant reduction in the expression of the most important pro-inflammatory cytokines in the liver [78]. Of note, in this study, the effects on insulin resistance were independent of alterations in AKT, IRS1 and IRS2 phosphorylation [78], indicating that other, unidentified signaling pathways may be involved in the development of insulin resistance in this model.

### 3.1.2. Other pathways that activate NF-κB and insulin resistance

Another major inflammatory pathway implicated in the development of insulin resistance is the TNFR signaling cascade, which also activates NF-κB [79]. In several studies, mice lacking *Tnfr* 1 and/or 2 or tumor necrosis factor α (*Tnfα*) were protected against the development of systemic insulin resistance [80,81]. As TNFα signaling can activate IKK2 and JNK, TNFα may thereby induce insulin resistance [8]. However, several papers showed that *Tnfr1*- or *Tnfr2*-deficiency did not protect against systemic insulin resistance in mice [25,82]. Moreover, *Tnfr1* deletion in estrogen-deficient mice increased systemic insulin resistance [83]. Unfortunately, some of these studies were complicated by differences in body weight or fat mass and differences in liver inflammation were not always investigated (Table 3). We recently reported that a gain-of-function mutation in *Tnfr1* did not affect body weight gain or adipose tissue inflammation in mice, but did induce liver inflammation and promote the progression of NAFLD to NASH [84]. Since this did not affect hepatic or systemic insulin resistance, the inflammation induced in the liver by TNFR1 activation is probably not involved in the development of hepatic and systemic insulin resistance. Unfortunately, to our knowledge, there is no data available on liver-specific transgenic mouse models of *Tnfα* or its receptors to corroborate these findings.

There is a hepatocyte-specific model for a protein related to TNFR signaling, TNF-receptor associated factor 2 (TRAF2) [85]. This protein mediates the signaling transduction of TNFR and TLR to the canonical and non-canonical NF-κB pathways and to JNK. Hepatocyte-specific deletion of *Traf2* did not affect liver inflammation, stressing the importance of investigating the effect of modulations in inflammatory genes on inflammatory endpoints. In addition, hepatic *Traf2* deletion did not affect hepatic and systemic insulin resistance. However, it did reduce gluconeogenesis and hyperglycemia by reducing glucagon action. This indicates that the effects of modulations in inflammatory genes on glucose metabolism can be mediated by other pathways than insulin resistance.

Recent findings have implicated a non-canonical NF-κB pathway in mediating glucose metabolism. In this pathway, NF-κB inducing kinase (NIK) promotes NF-κB activity, but only via specific receptors [86]. NIK seems to be particularly important in the liver, because in several models of insulin resistance, Sheng et al. found that NIK levels were upregulated in the liver but not in muscle [87]. Liver-specific inhibition of this protein improved glucose metabolism, whereas hepatocyte-specific overexpression induced glucose intolerance [87]. Sheng et al. showed that TNFα induced NIK activity and that NIK could directly affect glucose metabolism independent of insulin resistance by mediating the glucagon action via increased cAMP response element-binding (CREB) stability.

The receptor activator of NF-κB ligand (RANKL) is another activator of NF-κB which is expressed in the liver [88]. Mice with a hepatocyte-specific deletion of its receptor, *Rank*, and mice in which *Rank* was downregulated in the liver by lentiviral administration of a short hairpin RNA (shRNA) against *Rank*, were shown to be protected against high fat diet-induced hepatic and systemic insulin resistance [89]. No changes in body weight or fat mass were found between the groups. In mice in which *Rank* was downregulated by an shRNA, NF-κB activity was inhibited and in vitro data showed that RANKL stimulated the expression of inflammatory genes in hepatocytes and Kupffer cells dependent on RANK [89], suggesting that the effects on insulin resistance in this model are mediated through changes in inflammation.

### 3.1.3. JNK and insulin resistance

JNK is activated in response to cytokines, pathogens and cellular stress and activates the transcription factor activator protein 1 [90]. The role of JNK in the development of hepatic insulin resistance has been investigated in many tissue-specific mouse models. Suppression of hepatic JNK, by administering an adenovirus carrying the dominant-negative form of *Jnk* or an shRNA against *Jnk*, improved hepatic insulin sensitivity without affecting body weight gain [91,92]. In line with this, overexpression of the wild type form of *Jnk* in the liver produced the opposite result, without alterations in body weight gain [91]. Unfortunately, this study did not examine the effects of modifications in *Jnk* on inflammation and steatosis. The mechanisms by which JNK is believed to induce insulin resistance are similar to those found to play a role with IKK2. In cell culture studies, JNK was shown to induce serine phosphorylation of IRS1 [75,93]. This has also been shown to occur in the liver of mice overexpressing JNK [91].

However alterations in JNK-induced NF-κB activation do not consistently affect inflammation and insulin resistance as one would expect. For example, mice in which *Jnk* was deleted in hepatocytes paradoxically have increased inflammation, steatosis and are more insulin resistant in the liver compared to wild type mice, without any changes in fat or lean mass [94]. The paradoxical increase in inflammation in this model underscores the importance of including inflammatory endpoints, such as cytokines, chemokines and markers for macrophages and Kupffer cells in future studies. In addition, in this study, hepatocyte-specific deletion of *Jnk* also increased insulin clearance, providing another example of how alterations in inflammatory genes affect the glucose metabolism independent of insulin resistance.

### 3.1.4. Kupffer cells and insulin resistance

Kupffer cells are the resident macrophages of the liver and they are thought to play an important role in the regulation of hepatic inflammation. They amount to approximately 10% of the total number of cells in the liver. They recognize exogenous and endogenous molecular signals, such as microbes or cytokines, and they initiate several responses in

reaction to this. These include the release of cytokines and chemokines, the recruitment of new macrophages or other immune cells, engulfment of cells and cell debris, and the presentation of antigens to the adaptive immune system. Kupffer cells also respond to alterations in lipid accumulation and activate inflammatory pathways (reviewed by [95]). These cells are therefore thought to be particularly important in maintaining liver homeostasis and regulating liver inflammation.

Kupffer cells probably induce insulin resistance through interactions with hepatocytes. For example, classically activated (M1) Kupffer cells were shown to inhibit insulin signaling in hepatocytes [96]. This effect was probably mediated through TNFα secretion, as inhibition of TNFα attenuated the effect of Kupffer cells on hepatocytes [96]. Several other studies showed a decrease in pro-inflammatory cytokine expression in the liver after depletion of Kupffer cells [97,98]. These pro-inflammatory cytokines activate IKK2 and JNK in hepatocytes and thereby induce insulin resistance. In addition, activation of isolated Kupffer cells was shown to induce insulin resistance by the secretion of oncostatin M (OSM). OSM activated the suppressor of cytokine signaling 3 (SOCS3) in hepatocytes, which attenuated the phosphorylation of AKT and thereby inhibited insulin signaling [99].

Many studies have investigated the role of Kupffer cell depletion in the development of insulin resistance with conflicting results (Table 4). Several papers show that this improves hepatic insulin resistance [96–98], whereas others have shown a deterioration of hepatic and systemic insulin resistance [100,101]. It was also shown that depleting Kupffer cells before the start of a high fat diet could prevent hepatic and systemic insulin resistance, but the depletion did not improve insulin resistance once it had developed [102]. In some studies, differences in body weight gain and/or fat mass following Kupffer cell depletion could not be excluded as a confounding factor (Table 4). It is possible that the activation status of the Kupffer cells determines whether depletion can impair or improve insulin signaling. Alternative M2 activation of Kupffer cells was shown to improve hepatic and systemic insulin resistance in mice [103]. In the two studies in which insulin resistance was exacerbated by Kupffer cell depletion, Kupffer cells were alternatively activated before depletion [100,101].

Nevertheless, studies investigating Kupffer cell activation and insulin resistance raise further controversy. Liver-specific ablation of interleukin-6 receptor α (Il-6Rα) resulted in an exaggerated inflammatory response and glucose intolerance in mice. Depletion of their Kupffer cells ameliorated glucose intolerance [104]. In another study, it was shown that Kupffer cells became activated within 4 weeks of a high fat diet, through reduced nitric oxide signaling [105]. However, this did not simultaneously result in the development of hepatic insulin resistance at the level of IRS-1/AKT signaling, which only started after 8 weeks of high fat diet [105]. After 8 weeks the Kupffer cells were activated to a similar extent as at 4 weeks, but inflammation in the whole liver was now observed [105]. This may indicate that Kupffer cell activation does not by itself induce insulin resistance, but that it depends on the interaction between Kupffer cells and hepatocytes and the subsequent activation of inflammation in hepatocytes.

In contrast, we recently found that severe inflammation in the whole liver does not induce insulin resistance [106]. Low-density lipoprotein receptor knockout mice fed a high fat, high cholesterol diet developed severe liver inflammation, driven by cholesterol-induced Kupffer cell activation [107,108], but they did not develop hepatic or systemic insulin resistance following 2 and 15 weeks on such a diet [106]. Thus, Kupffer cell activation does not always lead to insulin resistance. Whether Kupffer cells induce insulin resistance, may depend on how they are activated and on their inflammatory status. In addition, their effect on insulin resistance is likely to be indirect, as it could depend on their ability to activate inflammatory pathways within hepatocytes.

#### 3.1.5. Other immune cells in the liver and insulin resistance

Studies have reported that neutrophils have infiltrated the liver in patients with NASH [109]. This was later confirmed in mice fed on a high fat diet [110]. Ablation of the expression of neutrophil elastase reduced the amount of neutrophils in the liver and resulted in a reduction in hepatic inflammation and hepatic as well as systemic insulin resistance [110]. However, in this study, adipose tissue neutrophils and macrophages were also reduced, so the improvements in glucose tolerance could be due to changes in the liver, or adipose tissue, or both.

Lastly, the role of hepatic natural killer T (NKT)-cells in the development of insulin resistance was investigated by Hua et al. [111]. They reported that a diet enriched in MUFAs or SFAs resulted in fewer NKT cells in the liver. This was associated with a more pro-inflammatory cytokine release and the development of glucose intolerance. These results agree with those from a recent study by Martin-Murphy et al., who found a depletion of NKT cells by knockdown of *Cd1d* resulted in hepatic inflammation and systemic insulin resistance [112]. However, differences in steatosis and body weight cannot be excluded as confounding factors in these studies [111,112].

### 3.2. Hepatic inflammation and insulin resistance: unresolved questions

Despite the tight association between low-grade chronic inflammation and insulin resistance, evidence from numerous mouse models suggests that inflammation in the liver does not drive insulin resistance (Tables 3 and 4, Fig. 2). Some mice that have a reduction in hepatic inflammation are even more susceptible to insulin resistance, indicating that factors besides inflammation cause insulin resistance in the mice. Similar to the effect of lipids, whether hepatic inflammation induces insulin resistance seems to depend on the circumstances under which it occurs. Conditions that are likely to be of importance are the duration and the level of inflammation. In addition, changes in the inflammatory pathways may affect glucose metabolism through mechanisms other than insulin resistance. In several mouse models, alterations in glucose metabolism were mediated by glucagon [85,87]. Because these alterations appear to be similar to insulin resistance at first sight, it is important to thoroughly investigate glucose homeostasis and determine if the insulin signaling is affected. The discrepancies between the studies may also be explained by effects on adipose tissue inflammation, as these have not always been reported (Table 3). In addition, the role of Kupffer cells in the development of insulin resistance is still controversial. Although, in some cases, the differences between the effects of Kupffer cell depletion could be explained by their activation status, a pro-inflammatory activation does not necessarily induce insulin resistance. This can be partly explained by the ability of Kupffer cells to activate inflammatory pathways in hepatocytes.

## 4. Concluding remarks

The role of NAFLD in the development of insulin resistance remains controversial, even when comparing studies in which body weight gain has been ruled out as a confounding factor. In general, we believe that NAFLD cannot be considered to be one of the main drivers of insulin resistance in mice, but there is more likely to be a dangerous liaison between the two. Whether this is also the case in human NAFLD remains to be determined. As alterations in body weight or fat mass appear to be a driving factor in the intricate relationship between NAFLD and systemic as well as hepatic insulin resistance in many studies, this supports the idea that adiposity is more important than NAFLD in the etiology of insulin resistance. On the one hand steatosis, inflammation and insulin resistance may simply be correlated via the common driver of obesity, suggesting that there is no causal relationship in either direction, while, on the other hand, it is possible that NAFLD only causes insulin resistance under certain circumstances. These circumstances are likely to include environmental factors, such as gut microbiota and dietary composition. In addition, the intracellular localization of lipids, fatty acid composition in the liver, and the amount and duration of inflammation may determine if NAFLD causes insulin resistance. Moreover, it may also depend on whether adipose tissue inflammation is affected.

To further investigate the circumstances under which NAFLD causes insulin resistance, we recommend taking lipid species and their localization into account as markers of steatosis in future studies. We further consider it important to measure inflammatory endpoints (such as cytokines, chemokines, and markers for macrophages and Kupffer cells) to determine whether modulations in the pathways involved in inflammation actually alter the levels of inflammation. Since inflammation is tightly controlled, the many negative-feedback pathways that regulate it could interfere with the outcome and detailed measurements might lead to surprising results. In this respect, it is also important to measure if inflammation in adipose tissue is affected. Moreover, discrepancies between the various reports offer an opportunity to investigate the underlying differences and to learn more about the role of environmental factors in the disease process. For example, NAFLD is associated with insulin resistance in some models of the disease, but not in all. It would be interesting to investigate what underlies the differences between these NAFLD models. Finally, by studying more liver-specific models and gain-of-function models, greater insight into the role of NAFLD in insulin resistance will be obtained. We are certain that, with the techniques now available, many factors that play a role in the etiology of insulin can be elucidated in the near future.

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