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Insulin resistance in clinical and experimental alcoholic liver disease

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Abstract

Alcoholic liver disease (ALD) is the number one cause of liver failure worldwide; its management costs billions of health care dollars annually. Since the advent of the obesity epidemic, insulin resistance and diabetes have become common clinical findings in patients with ALD; and the development of insulin resistance predicts the progression from simple steatosis to cirrhosis in ALD patients. Both clinical and experimental data implicate the impairment of several mediators of insulin signaling in ALD, and experimental data suggest that insulin-sensitizing therapies improve liver histology. This review explores the contribution of impaired insulin signaling in ALD and summarizes the current understanding of the synergistic relationship between alcohol and nutrient excess in promoting hepatic inflammation and disease.

Keywords

alcoholic liver disease; insulin resistance; metabolic syndrome; NAFLD/NASH; steatohepatitis

Introduction

Alcoholic liver disease (ALD) is a major cause of liver failure worldwide and its management consumes billions of dollars annually.[1, 2] ALD is diagnosed in two million Americans each year; accounts for 44% of all liver-related deaths; and is the second leading cause of liver transplantation in the United States,[1, 3] making this disease a significant public health burden. The histologic spectrum of ALD includes simple hepatic steatosis (an accumulation of hepatocellular lipid droplets), steatohepatitis (steatosis with inflammation), and cirrhosis. Although the majority of patients with alcoholic steatosis do not develop cirrhosis,[4] the factors that mediate progression to advanced disease are not well understood. Common risk factors for advanced ALD include prolonged duration and excessive quantity of alcohol consumption (i.e. on average 15 and 8 alcoholic drinks per week for men and women, respectively), female gender, advanced age, concomitant viral hepatitis, obesity, and insulin resistance (IR).[5] Insulin resistance in ALD develops from alcohol's effects on hepatic and non-hepatic tissues and may be exacerbated by the

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steatogenic effects of overnutrition on the liver. The combined hepatic effect of alcohol overconsumption and overnutrition is a rather recent area of investigation.

This review will discuss the contribution of impaired insulin signaling to the pathogenesis of ALD by outlining the (1) epidemiologic and clinical data associating IR to ALD progression; (2) the alcohol-mediated mechanisms responsible for IR in ALD; (3) the impact of insulin-sensitizing medications in experimental ALD; and (4) and the interaction of alcohol and nutrients in promoting ALD.

ALD and IR epidemiology

A meta-analysis of studies examining the relationship of alcohol consumption with prevalence of the metabolic syndrome, insulin resistance, and/or frank diabetes revealed that alcohol consumption less than 40 g/day in men and 20 g/day in women reduces the risk of the metabolic syndrome, while greater consumption increases risk.[6] There are far fewer quality studies, however, that examine the prevalence of IR and/or diabetes in patients who develop liver disease from chronic alcohol consumption. In an analysis of cirrhotic patients with hepatitis C compared with cirrhotic patients of other etiologies, Zein, *et al*. observed that 19% of the 53 patients with ALD had a concomitant diagnosis of diabetes.[7] This prevalence parallels that observed in hepatitis C cirrhotic patients, but is nearly four-times the prevalence of diabetes in patients with cholestatic etiologies of cirrhosis, suggesting that alcohol confers an additional risk factor for diabetes in the cirrhotic patient. In a prospective study of 268 ALD patients specifically examining risk factors for fibrosis development, Raynard, *et al*. observed that both body mass index and fasting glucose are independently associated with risk of fibrosis in cirrhotic and non-cirrhotic ALD patients.[5] In the study's subset of cirrhotic patients (n = 200), fasting glucose, but not body mass index, is associated with increased fibrosis risk. The prevalence of IR and/or diabetes was not presented in this study, and to our knowledge there have been no long-term prospective studies of the incidence of IR in ALD patients.

Normal insulin signaling

Understanding how IR develops in ALD requires a review of normal insulin signaling (Fig. 1A). Under normal physiological conditions, insulin secretion from pancreatic beta cells is stimulated by post-prandial increases in blood glucose and circulates systemically to normalize blood glucose levels. Insulin prevents hyperglycemia by stimulating glucose uptake by skeletal muscle and adipose tissue, while suppressing glucose production and glycogenolysis in the liver. Insulin also stimulates the liver to convert excess glucose into glycogen and triglyceride for storage.

Insulin exerts its effects on target tissues by binding the insulin receptor and stimulating receptor auto-phosphorylation and internalization, which in turn recruits and activates insulin receptor substrate proteins 1 and 2 (IRS1/2). IRS1/2 can activate phosphatidylinositol 3 (PI3)-kinase which converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Akt binds PIP3 at the cell membrane through its pleckstrin homology domain and is subsequently phosphorylated and activated. In muscle and adipose tissue, insulin-mediated Akt activation stimulates the

translocation of glucose transporters to the membrane to allow glucose uptake. In the liver, insulin binding to the insulin receptor has three major actions: (1) suppression of gluconeogenesis in part by inactivating the gluconeogenic gene transcription factor forkhead box O1 (FOXO1); (2) promotion of fatty acid synthesis through activation of sterol regulatory element–binding protein (SREBP) 1, a transcription factor that drives production of genes involved in fatty acid synthesis; and (3) stimulation of production of glycogen (a storage form of glucose) through phosphorylation and inhibition of glycogen synthase kinase (GSK), the repressor of glycogen synthesis.[8]

Insulin resistance is defined as a state of relative insulin insufficiency due to reduced tissue insulin responsiveness.[9] Insulin resistance can be measured clinically and experimentally in a number of ways,[10] including by use of the glucose tolerance test (GTT) and the insulin tolerance test (ITT) in which serial glucose measurements are made after administration of either glucose or insulin, respectively; differences in glucose kinetics are determined by comparing the areas under the respective curves. The gold standard for assessing insulin resistance is the euglycemic–hyperinsulinemic clamp, where insulin levels are acutely raised and maintained while simultaneously infusing glucose to maintain glucose levels. When coupled with glucose tracers, the euglycemic–hyperinsulinemic clamp allows accurate assessment both of glucose disposal in peripheral tissues and changes in glucose production by the liver.[11]

ALD and IR in human studies

The few studies that have specifically investigated the mechanisms of IR in patients with ALD were performed prior to the advent of the obesity epidemic and include only a small sample of cirrhotic patients. To our knowledge, the first of these studies was a euglycemic–hyperinsulinemic clamp performed in six patients with alcoholic cirrhosis and six control patients.[12] These researchers observed that patients with alcoholic cirrhosis have a higher calculated insulin affinity constant: the concentration of insulin required to reduce maximum glucose metabolism by 50% thus reflecting lower insulin sensitivity. Cirrhotic patients also demonstrate a significantly lower maximum rate of glucose metabolism (i.e. utilization), resulting in a net effect of impaired insulin signaling in alcoholic cirrhotic patients compared with control patients.

The respective contributions of hepatic and peripheral insulin sensitivity to glucose utilization were explored in a study by Shmueli, *et al.*[13] These researchers quantified whole-body, splanchnic, and peripheral glucose uptake in fourteen alcoholic cirrhotic men and six age- and weight-matched control patients. The cirrhotic patients had clinical evidence of portal hypertension and impaired glucose tolerance at baseline. Patients underwent an oral glucose tolerance test and hyperglycemic clamp with and without somatostatin to measure insulin and insulin-independent glucose uptake. In addition, indirect calorimetry was performed to estimate glucose oxidation. Alcoholic cirrhotic patients had basal insulin levels six times that of control patients, but normal fasting glucose, suggesting the cirrhotic patients had impaired tissue responsiveness to insulin. In addition, cirrhotic patients had higher C-peptide/insulin ratios, suggesting reduced hepatic and/or renal degradation of insulin. Plasma non-esterified fatty acids were also approximately 1.5×

higher in the cirrhotic patients than in controls, consistent with impaired insulin action at the tissue level. The glucose infusion rate, a surrogate measure of whole body glucose uptake during clamp conditions, was 60% lower in alcoholic cirrhotic patients compared with controls; and forearm glucose uptake was 30% of normal levels. Both hepatic glucose production and splanchnic glucose uptake were similar in the two groups, and the reduction in glucose uptake was predominantly due to impairment of glucose non-oxidative disposal (typically due to glycogen synthesis), [13, 14] suggesting that post-insulin receptor defects contribute to IR in ALD patients.

Subsequently, Magnusson *et al*. performed intravenous GTT and ITT in eight non-diabetic patients with alcoholic cirrhosis and eight control patients.[15] Serial glucose measurements demonstrated impaired response to both glucose and insulin in alcoholic cirrhotic patients compared with controls. Additionally, the early response to insulin, rather than insulin clearance, accounted for the differences between groups. This impairment of the early insulin response suggested that alcoholic cirrhotic patients have pancreatic beta cell dysfunction. Indeed, a study of diabetic and non-diabetic patients with alcoholic cirrhosis and healthy control patients (six in each group) revealed a four-times higher fasting insulin in non-diabetic and diabetic alcoholic cirrhotics compared with controls, and a diminished insulin response to glucose during the euglycemic–hyperinsulinemic clamp in diabetic alcoholic cirrhotic patients due to reduced pancreatic beta cell function.[16]

Both Magnusson's and Tranberg's studies provide evidence for impaired pancreatic insulin secretion in alcoholic cirrhotic patients; but inappropriate glucagon response to hyperglycemia is also implicated in ALD patients. Glucagon, insulin's counter-regulatory hormone, is two- to four-times higher in fasting non-diabetic and diabetic alcoholic cirrhotic patients, respectively, compared with control patients.[16] Shmueli, *et al*. reported both higher fasting and clamped glucagon levels in alcoholic cirrhotic patients compared with control patients. Additionally, Kruszynska, *et al*. also observed that alcoholic cirrhotic patients have an increased glucagon response to the secretagogue arginine compared with controls, as well as impaired glucagon response to hyperglycemia. Together, these early studies suggested that impaired insulin secretion, tissue response to insulin, non-oxidative glucose disposal, and glucagon response to glucose all contribute to IR in alcoholic cirrhotic patients. Although these studies have not been replicated in humans with earlier stages of ALD, they have been experimentally modeled in rodents with alcoholic steatosis and steatohepatitis, suggesting that IR can develop in ALD in the absence of cirrhosis.

Modeling ALD *in vivo* and *in vitro*

Investigating the specific mechanisms of IR in ALD is challenging because there are no experimental models that fully recapitulate the histologic spectrum of ALD. Traditional *in vitro* hepatocyte systems have limited utility because expression of the alcohol metabolizing enzymes, alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (Cyp2E1), is required for studying the effects of alcohol oxidation on the liver.[17] Hepatoma cell lines such as HepG2 cells do not express alcohol metabolizing enzymes,[18] and primary hepatocytes rapidly lose the ability to metabolize ethanol in culture,[19] making them suitable for studies of acute effects of alcohol but not chronic effects.[20, 21]

Use of *in vivo* models is critical for understanding the effects of chronic alcohol consumption on liver disease and insulin signaling, but *in vivo* models (at least thus far) fail to replicate the full spectrum of ALD. In fact, the majority of models used to study effects of alcohol on liver health and biology produce only steatosis accompanied by mild inflammation and, in some cases, fibrosis [22] and cirrhosis [23], but never severe disease such as alcoholic hepatitis.[24, 25]

The majority of *in vivo* rodent models incorporate chronic alcohol feeding with or without additional dietary modifications to induce disease. In choosing an alcohol feeding model, alcohol delivery route, alcohol content, and duration of feeding are all critical considerations, as different feeding regimens model different features of human disease. The most widely used model of steatosis and steatohepatitis is the Lieber–DeCarli diet, in which a liquid ethanol or calorie-matched control liquid is pair-fed to rodents; it is an *ad libitum* liquid diet formulation typically containing an alcohol content up to 36% percent of total calories,[26] but can be adapted for use with higher alcohol content. One advantage of the Lieber–DeCarli model is that caloric composition and intake is similar between ethanol and control-fed animals, which is critical for studying the effects of alcohol on metabolism. Animals fed the Lieber–DeCarli diet have blood alcohol levels of 100–150 mg/dl (22-35 mM),[27] which correspond to levels that produce impairment in humans.

Wild-type mice chronically fed a Lieber–DeCarli diet with 15% ethanol caloric content for four weeks develop glucose intolerance.[11] We have shown a temporal relationship between the onset of alcoholic steatosis and development of IR in mice fed the alcohol diet in the absence of differences in body weight, fat mass, or food intake compared with control-fed mice.[11, 28] Insulin resistance in our model is due to both hepatic and peripheral insulin resistance, as measured by the euglycemic–hyperinsulinemic clamp.

A similar finding was found in rats fed the Lieber–DeCarli diet. Male Sprague-Dawley rats fed 35% ethanol diet for two weeks develop both hepatic and peripheral IR, however, it is unclear if two weeks of ethanol feeding in these rats causes significant ALD pathology as histology was not examined.[29] Eight week alcohol feeding in Long-Evans rats has been shown to promote significant steatosis and steatohepatitis.[30] De la Monte *et al*. observed that a Lieber–DeCarli diet with alcohol content of 37% of total calories administered to Long-Evans rats for eight weeks causes IR due to downregulation of several insulin signaling genes (detailed below).[30, 31]

Notably, choice of rodent and strain within a single species can also impact alcohol-mediated insulin resistance, as rats are more susceptible than mice to hepatic effects of alcohol due to reduced alcohol clearance,[32] and Long-Evans rats are more susceptible to IR than Sprague-Dawley rats.[31]

To achieve an alcohol caloric intake of greater than 36% total calories, the intra-gastric feeding (or Tsukamoto–French) model is often used, where the alcohol is delivered through a surgically implanted gastric tube, allowing for up to 41.4% of total calories to be from alcohol.[33-35] This feeding model leads to extensive steatosis and IR[36] accompanied by

mild inflammation and low levels of fibrosis.[23, 34] To study the acute effects of alcohol, a single bolus can be delivered by oral gavage to induce IR.[37]

All of the above model systems have been used to increase understanding of the mechanisms of IR in ALD, providing insight into pathways that may mediate ALD progression.

Mechanisms of IR in ALD

Chronic alcohol consumption promotes ALD through its pleiotropic effects on the insulin signaling cascade. The following sections summarize what is understood about alcohol's effects on insulin signaling, from the proximal binding of insulin to its cognate receptor, to the downstream actions on effector molecules (summarized in Fig. 1B). We also review the current understanding of the role of adipokines, lipid metabolites, genetics, and intestinal dysbiosis in the pathogenesis of IR in ALD.

Alcohol, the insulin receptor, insulin receptor substrates, and PI3K

Alcohol has several effects on the insulin receptor in the liver. Chronic alcohol feeding has been shown to both reduce and have no effect on insulin receptor binding. De la Monte *et al*. examined Long-Evans rats fed a Lieber–DeCarli 37% caloric-content alcohol diet for eight weeks and found reduced insulin receptor binding in alcohol-fed rats compared with controls.[30] Using competitive binding saturation assays in the same rat model, Pang *et al*. extended those findings by showing reduced receptor binding and reduced binding affinity in the alcohol-fed rats compared with pair-fed control animals.[38] Notably, the finding of reduced insulin receptor binding and affinity does not appear to be species specific, as similar insulin receptor impairment with chronic alcohol feeding also occurs in Sprague-Dawley rats.[39]

Despite recent studies reporting that chronic alcohol feeding impairs insulin receptor binding affinity, earlier studies did not report similar findings. Tuma *et al*. measured insulin receptor binding in primary hepatocytes isolated from Sprague-Dawley rats fed a Lieber–DeCarli 36% caloric-content alcohol diet for five to six weeks and did not observe a reduction in insulin receptor binding. Rather, they observed a decrease in the number of insulin receptors and reduced internalization of the insulin–insulin receptor complex.[40] Patel *et al*. observed similar results in primary hepatocytes isolated from Sprague-Dawley rats fed a 37% caloric-content alcohol diet for four weeks.[41] Impairment of insulin–insulin receptor internalization may be a specific feature of hepatic injury from *chronic* alcohol exposure, as *acute* ethanol treatment of hepatocytes from chow-fed animals does not worsen insulin–insulin receptor internalization.[42]

Phosphorylation of the insulin receptor and its downstream effectors has been investigated in *in vitro* and *in vivo* models of ALD. Banerjee *et al*. examined effects of alcohol in FOCUS cells (human hepatocellular carcinoma cells that overexpress IRS1) and observed that alcohol treatment significantly reduces the levels of insulin-stimulated tyrosyl phosphorylation of the insulin receptor beta subunit.[43] While some *in vivo* data indicate a similar reduction in insulin receptor phosphorylation and activation,[44] other data

demonstrate enhanced insulin receptor phosphorylation in chronic ethanol–fed animals in response to insulin stimulation without enhancement of downstream signaling events,[29] suggesting additional post-receptor mechanisms exist. Onishi *et al*. investigated the effects of acute portal vein injection of insulin on hepatic insulin signaling in both acute and chronic alcohol-fed Sprague-Dawley rats. Despite developing both hepatic and peripheral IR in response to acute- and chronic-alcohol feeding, both groups of alcohol-fed rats had increased tyrosine phosphorylation of the insulin receptor. The explanation for this finding is unclear but may relate to differences in the mode of hepatic insulin exposure, i.e. endogenous production versus exogenous administration.

Impaired phosphorylation of insulin substrate proteins and kinase activity of PI3K are also implicated in ALD pathology. Alcohol reduces IRS protein levels[45] and inhibits IRS phosphorylation.[46] The mechanism of alcohol's inhibitory action on IRS may be through the activation of c-terminal Jun kinase (JNK) by the ethanol-metabolizing enzyme Cyp2E1. IRS proteins have an inhibitory phosphorylation site targeted by JNK, and overexpression of Cyp2E1 in a non-alcoholic steatohepatitis mouse model activates JNK, which in turn inhibits IRS.[47]

Data regarding alcohol's effect on downstream effectors PI3K, PIP2, and PIP3 are mixed, but may relate to differences in models used and concentrations of alcohol administered. Alcohol treatment can upregulate phosphatase and tensin homolog (PTEN), which opposes the action of PI3K, converting PIP3 to PIP2 and inhibiting Akt membrane translocation.[48] Low-dose ethanol can also enhance PI3K activity by post-transcriptional downregulation of the regulatory p55γ subunit, leading to enhanced insulin sensitivity.[32]

Alcohol, Akt, and Akt target proteins

Akt phosphorylation is central to canonical insulin signaling; but recent data challenge the importance of Akt in hepatic insulin signaling, as it is dispensable for insulin action in the absence of FOXO1.[49] Nevertheless, studies examining alcohol's effects on insulin signaling in ALD demonstrate that alcohol's inhibitory effects on Akt phosphorylation promote IR. Alcohol increases the Tribbles homolog TRB3, which binds and inhibits the pleckstrin homology domain of Akt, thus blocking Akt membrane translocation and activation.[50]

Alcohol also has effects on several Akt target proteins, namely FOXO1, GSK, and SREBP-1. FOXO1 is a transcription factor that activates gluconeogenic genes. Alcohol feeding increases FOXO1 mRNA and protein,[51, 52] as well as phosphorylation of FOXO1.[51] Activation of FOXO1 promotes differentiation of quiescent hepatic stellate cells to collagen-producing myofibroblasts to promote fibrosis,[53] findings of particular relevance to mechanisms of ALD progression.

Basally active GSK α and β isoforms prevent glycogen synthesis by phosphorylating and inactivating glycogen synthase. In the fed state, insulin-stimulated Akt phosphorylation of GSK promotes glycogen synthesis by preventing suppression of glycogen synthase.[54] Ethanol increases liver GSK levels through ROS production[55] and signaling through the

glucocorticoid receptor.[56] This increase of GSK activity by ethanol is consistent with the observation that alcohol-treated rats and mice have reduced glycogen content.[57, 58]

SREBP-1 plays an important role in regulating the transcription of genes involved in hepatic lipogenesis (such as fatty acid synthase, stearoyl-CoA desaturase, and ATP citrate lyase). A role for SREBP-1 has been clearly established in ALD pathogenesis and was recently reviewed in Liu *et al*.[59] SREBP-1 can be both upregulated and downregulated by ethanol, depending on the model used[32, 60] and, possibly, the degree of insulin resistance that develops.[61] These effects on SREBP-1 provide a mechanistic link between the actions of alcohol on insulin signaling and lipid synthetic pathways.

Alcohol and adipokines

Adipokines are white adipose tissue–derived hormones that regulate glucose, lipid and energy homeostasis.[62, 63] Leptin and adiponectin are two major adipokines that promote insulin sensitivity and are implicated in several liver diseases,[64] including in ALD pathogenesis.

The level of leptin, a product of the gene *ob*, positively correlates with fat mass. Leptin regulates food intake and energy expenditure and mice with genetic leptin deficiency have hyperphagia, obesity, hepatic steatosis and hyperinsulinemia.[65, 66] Wild-type mice chronically fed a 38% Lieber–DeCarli ethanol diet for up to eight weeks develop steatohepatitis and have reduced white adipose tissue mass and serum leptin levels compared with control-fed mice; exogenous leptin administration in these mice improves steatohepatitis.[67] In humans, patients with acute alcoholic steatohepatitis (a clinical syndrome of ALD that results in high short-term mortality)[68] have reduced serum leptin levels compared with alcoholic cirrhotic patients despite similar body mass indices,[69] suggesting that factors independent of fat mass may play a role in leptin secretion in these patients. Importantly, high levels of the adipokine resistin (little studied in ALD) are associated with increased mortality in this study.

Adiponectin is a 30 kD insulin sensitizing hormone whose effects on the liver include activation of the nuclear receptors PPARα and PPARγ co-activator alpha, inhibition of the *de novo* lipogenesis regulatory protein SREBP1, and activation of AMP-activated kinase (AMPK).[70-73] The question of whether reduced adiponectin levels contribute to ALD pathology remains unsettled, as studies demonstrate both reduced and increased adiponectin levels in human and experimental ALD. One study of male FVB/n alcohol-fed mice showed a temporal correlation between reduction of serum adiponectin, increase of tumor necrosis factor α (TNF-α), and development of hepatic steatosis.[74] Other studies in mice, rats, and micropigs have similarly demonstrated that alcohol feeding is associated with hypo-adiponectinemia[73, 75, 76] and reduced expression of adiponectin receptors in the liver.[77, 78]

We and others have demonstrated increased, rather than decreased, adiponectin levels in experimental ALD.[67, 79, 80] The reasons for these discrepancies are unknown but may relate to both temporal factors, as Tan and colleagues observed only a transient increase in serum adiponectin,[67] and Xu *et al*. found no change in adiponectin levels for the first three

weeks of alcohol feeding[74]; biologic factors may also be relevant, as experimental models fall short of reproducing the full spectrum of ALD seen in humans (discussed above).

Two studies of human ALD patients suggest that adiponectin levels are indeed increased.[81, 82] Buechler, *et al*. measured serum adiponectin levels in patients with (1) chronic alcoholism but no advanced liver disease; (2) alcoholic cirrhosis with daily alcohol intake of 100 g/day for over ten years; (3) hepatitis C chronic infection with and without advanced liver disease; and (4) no liver disease but a single episode of 140–500 g of excessive alcohol intake in a 24 hr period. They found that alcoholic patients with and without advanced liver disease have significantly higher serum adiponectin levels compared with patients infected with hepatitis C, regardless of stage of liver disease. Moreover, although healthy patients have no change in serum adiponectin levels after a single binge episode of alcohol consumption, a two-week period of abstinence in chronic alcoholics without advanced liver disease causes a fall in serum adiponectin levels. In a similar study, Kasztelan-Szczerbinska, *et al*. measured adipokine levels in 147 adult patients and 30 healthy controls.[82] Compared with control patients, adiponectin levels in ALD patients were significantly higher and positively correlated with liver disease severity. Importantly, there was no association between adiponectin and serologic inflammatory markers. Although these human studies, and our mouse study, suggest adiponectin increases in ALD, the data do not necessarily contradict prior studies. Adiponectin levels may increase with alcohol consumption through direct action of alcohol on adipocytes, but in the liver there may remain relative adiponectin resistance, as evidenced by reduced hepatic expression of adiponectin receptors.[77, 78] Therefore, the net effect would be reduced hepatic adiponectin signaling, promotion of hepatic steatosis, and impairment of insulin signaling.[77, 78] (Fig. 2)

Alcohol, lipid metabolites, and IR

Bioactive lipids may also play a role in IR in ALD. Specifically, diacylglycerol (DAG) and ceramides are two major lipid metabolites that have been implicated in the pathogenesis of IR in ALD. DAG causes hepatic insulin resistance through activation of a specific novel PKC isoform in liver, protein kinase C epsilon (PKCε). Upon activation, PKCε binds and inhibits the intracellular kinase domain of the insulin receptor,[8] thereby inhibiting insulin-stimulated activation of IRS1/2 and Akt. Both ethanol and its metabolite acetaldehyde can increase DAG levels *in vitro*, although the effect appears to be acute[21, 83] and variable depending on culture conditions.[84] Whether this is a mechanism contributing to alcohol-mediated insulin resistance *in vivo* remains to be investigated.

Ceramides are sphingolipids and cell membrane components that function as second messengers in the cell, influencing diverse functions such as cell growth, apoptosis, and insulin signaling.[85, 86] Elevated cellular ceramides can promote insulin resistance by activating protein kinase C zeta (PKCζ), which inhibits Akt translocation and activation.[87, 88] Ceramides also stimulate protein phosphatase 2A (PP2A), which de-phosphorylates Akt,[89] and increased PP2A activation has been observed in mice chronically fed a Lieber–DeCarli alcohol diet[28]. Ceramides are increased in the livers of alcohol fed mice,[11, 58, 90-93] and hepatic ceramide reduction is associated with improved insulin sensitivity in rats and mice chronically-fed alcohol[28, 58, 94] through restoration of Akt phosphorylation.

[94] Moreover, pharmacological reduction of liver ceramide levels reduces steatosis and improves glucose intolerance and insulin sensitivity.[44, 80]

A number of mechanisms have been identified that link increased hepatic ceramide with IR in ALD (Fig. 2). Alcohol intake increases adipose tissue lipolysis,[95] thereby increasing circulating palmitate, a fatty acid substrate required for the rate limiting step of *de novo* ceramide synthesis in the liver.[96] Alcohol also increases expression of the lipid droplet protein perilipin 2 (Plin2),[11] which is required for alcohol-mediated increases in liver ceramides[58] and promotion of glucose intolerance and IR.[58, 97] Alcohol may reduce either the levels of adiponectin or hepatic response to adiponectin, and adiponectin has been shown to reduce liver ceramides through activation of ceramidase activity.[80, 98] Alcohol also increases levels of the inflammatory cytokine TNF-α, which stimulates sphingomyelinase,[99] an enzyme required for the hydrolysis of sphingomyelin to ceramide.

Together, the above data link IR with ALD pathogenesis, implicate multiple mechanisms of IR in ALD, and provide potential novel targets to ameliorate disease in ALD patients.

Genetics, IR, and ALD

The interaction of the above effectors of insulin signaling with the host genome is an area of ongoing investigation in ALD. One candidate gene associated with ALD severity is the patatin-like phospholipase domain–containing protein 3 (*PNPLA3*, also known as adiponutrin, *ADPN*). PNPLA3 is a member of the patatin-like phospholipase family.[100] The rs738409 *PNPLA3* single nucleotide variant (so called G allele) creates an isoleucine to methionine mutation at residue 148 (I148M) of the human protein. Investigators with the Dallas Heart Study demonstrated an association of the rs738409 SNP with non-alcoholic fatty liver disease (NAFLD) susceptibility. [101] Since this initial report, several other human studies have verified the link of the rs738409 variant with NAFLD susceptibility and severity, including increased risk of cirrhosis (reviewed in Ref. [102]).

The contribution of the rs738409 *PNPLA3* variant has been investigated in two major ALD studies. The initial report was based on an examination of patients enrolled in the Dallas Heart Study.[103] Tian, *et al*. evaluated Mestizo patients with a history of significant alcohol abuse. They genotyped 305 patients with normal liver function and history of alcohol dependence, 434 patients with intermediate ALD, and 482 patients with clinically-apparent alcoholic cirrhosis. Patients with cirrhosis were on average older, with longer alcohol consumption duration than the other groups. They observed that the rs738409 variant was more common in cirrhotic patients than in the other groups, accounted for 49% of cirrhosis susceptibility risk, and was associated with cirrhosis severity as measured by the Child-Pugh classification system. This association with ALD severity was similarly seen in two independent German cohorts of patients.[104]

The function of the PNPLA3 gene product and how it promotes ALD pathogenesis is unknown. PNPLA3 is highly expressed in human liver,[105, 106] but in rodents the highest expression is in white adipose tissue.[107] Both lipids [107] and carbohydrates [105] increase hepatic PNPLA3 expression; while fasting reduces its expression.[106, 107] PNPLA3 localizes to both the cell membrane and lipid droplets,[108] which is reminiscent

of the aforementioned Plin2 lipid droplet protein. In fact, the kinetics of PNPLA3 upregulation in response to oleic acid parallel those of Plin2.[105] PNPLA3 is a target of the lipogenic nuclear receptor SREBP-1c [109], but also demonstrates triglyceride hydrolase activity, with the I148M variant exhibiting reduced lipolytic activity compared with wild-type PNPLA3 protein. [108] Adenoviral overexpression of wild-type mouse PNPLA3 does not induce hepatic steatosis but does increase VLDL secretion.[109] Conversely, adenoviral hepatic overexpression of the mutant PNPLA3 protein increases liver triglyceride content in mice through impaired hydrolase activity.[108] These observations suggest that wild-type PNPLA3 expression prevents hepatic steatosis through positive regulation of lipolysis and targeting of lipids for hepatic secretion as VLDL; however, two independent reports failed to find an effect of complete *PNPLA3* deletion (KO) on hepatic steatosis or lipid homeostasis,[110, 111] leading some to hypothesize that the rs738409 variant is a gain-of-function mutation;[112] another alternative is that other unidentified pathways may compensate for the absence of PNPLA3 in such models. For example, there is compensatory upregulation of PNPLA5 (a neutral lipase and family member of PNPLA3) in both the white adipose tissue and livers of *PNPLA3* KO mice exposed to high carbohydrate diets.[110, 111] This upregulation may mitigate the global loss of *PNPLA3* by augmenting autophagy,[113] thus, reducing cellular lipid droplet content. Studies using liver-specific *PNPLA3* KO may be helpful in understanding these and other compensatory pathways.

The role of PNPLA3 is also unclear in glucose homeostasis. For example, overexpression of wild-type PNPLA3 in mouse liver impairs glucose tolerance, and knockdown of PNPLA3 expression in leptin-deficient and -resistant mouse models improves glucose tolerance.[109] However, complete knockout of *PNPLA3* in mice has no effect on glucose tolerance,[110, 111] and PNPLA3 is not associated with insulin resistance in patients with NAFLD.[101] Possible explanations for these discrepancies include differences in study designs, nutritional status, and contributions of other pathways when PNPLA3 is absent. As differential composition of hepatic lipids has been observed in patients with the rs738409 variant, and is proposed to explain the lack of association of this variant with insulin resistance in NAFLD patients,[114] future experimental approaches should not only include in-depth analysis of organ-specific PNPLA3 expression but also careful analysis of hepatic lipid composition in order to interpret effects (or lack thereof) on glucose homeostasis. Performance of these studies in alcoholic liver disease models is also important for understanding the role of co-factors on PNPLA3 biology.

ALD and alterations of the gut microflora

Intestinal dysbiosis and the resultant alterations in circulating cytokines and metabolites may contribute to IR in ALD. Patients with ALD have higher urinary recovery of orally-administered polyethylene glycol compared with control subjects, suggesting increased intestinal permeability in ALD patients. This increased intestinal permeability positively correlates with serum levels of lipopolysaccharide,[115] a bacterial outer membrane product that increases production of TNF-α and its intracellular effector nuclear factor kappa B (NF-κB), both demonstrated to promote insulin resistance.[116]

In addition to increased intestinal permeability, human and experimental animal studies have demonstrated that chronic alcohol consumption alters both the family and phyla distribution of intestinal bacteria. [117-119] Indirect evidence that this local alteration of intestinal microbiota may have systemic effects on both glucose and lipid homeostasis includes data from experimental studies showing that inhibition of alcohol's effects on intestinal microbiota with oral lactobacillus or oats prevents alcoholic steatohepatitis.[120-122] Additional indirect evidence include data from a human metabolomic study demonstrating that compared to patients with alcoholic cirrhosis, patients with acute alcoholic hepatitis have elevations in two bacterial derived bile acids (deoxycholate and glycodeoxycholate), lipid profiles suggestive of enhanced adipose lipolysis and impaired hepatic beta-oxidation, as well as increased glucose, fructose, and sugar alcohols, suggesting poor glucose utilization.[123] The results of this metabolomic study provide compelling evidence that metabolomics can have diagnostic utility in addition to serving as potential windows into disease mechanisms. Future studies should combine *in vivo* metabolic phenotyping with metabolomics and microbiome analyses in ALD patients, with and without antibiotics, to help delineate a more direct link between alteration of gut microbiota and systemic glucose and lipid homeostasis in ALD patients.

ALD therapeutics that target IR

The mainstay of management in ALD patients is alcohol cessation, but only a small percentage of patients with alcohol dependence can achieve abstinence.[124, 125] The studies highlighted above demonstrate that alcohol's pleiotropic effects on the liver promote the development of IR in ALD, yet there have been no trials of insulin-sensitizing medications in patients with ALD and, as a result, there are currently no approved pharmacologic treatments that target IR in ALD. A recent retrospective study of 250 predominantly cirrhotic diabetic patients taking metformin (an AMPK-activating biguanide drug widely used in the treatment of type II diabetes) at the time of cirrhosis diagnosis was performed to investigate the benefit of continued metformin treatment after cirrhosis diagnosis.[126] The study population included 29 patients with ALD and revealed a mortality benefit independent of cirrhosis severity in patients with non-alcoholic steatohepatitis (NASH) who continued metformin, compared with patients whose metformin was discontinued within three months of cirrhosis diagnosis. The study also demonstrated a trend toward mortality benefit in patients with cirrhosis of all other etiologies. The lack of significance in non-NASH patients is likely due to low sample size. Therefore, similar studies should be replicated in ALD patients using a larger cohort of patients. While no clinical data, there have been several studies of insulin-sensitizers in experimental models of ALD, which we discuss below (also summarized in Table 1).

Metformin and AMPK activation

AMPK is a cellular energy sensor enzyme that, when activated, reduces lipid synthesis, promotes fat oxidation, and inhibits gluconeogenesis.[127] Several studies have shown that metformin treatment of alcohol-fed mice reduces hepatic steatosis.[128-130] Zhu *et al.* fed alcohol by oral gavage to male Wistar rats with or without metformin twice-daily for 16 weeks and found improvement in liver enzymes, hepatomegaly, and insulin resistance in the

rats treated with metformin compared with rats that did not receive metformin. These improvements were associated with increases in hepatic AMPK and insulin receptor gene expression, and support findings of Tomita *et al*. who demonstrated that administration of AICAR (an AMPK activator) to male Wistar rats fed an alcohol Lieber–DeCarli diet for six weeks reduces hepatic steatosis through downregulation of SREBP-1c and reduction of lipid peroxidation.[131] Ajmo *et al*. also demonstrated a critical role of AMPK in experimental ALD. Wild-type mice were fed a low-fat 29% Lieber–DeCarli alcohol diet with or without resveratrol for four weeks. Resveratrol treatment stimulated AMPK in the livers of the mice and ameliorated hepatic steatosis and hepatitis.[78]

The above experimental ALD studies show histologic benefit with metformin and AMPK-activation, but metformin's mechanism of improving lipid and glucose homeostasis in ALD may also include AMPK-independent mechanisms. Bergheim *et al*. found improvement of hepatic steatosis, hepatitis, and hepatic necrosis in alcohol-fed wild-type mice treated with metformin, but concluded from their studies that the action of metformin does not require AMPK activation, as AMPK levels were comparable to those of mice who did not receive metformin.[129] They instead demonstrated a critical role for metformin-induced inhibition of plasminogen activator inhibitor-1, an acute phase reactant associated with inflammation. The different conclusions from these studies may be due to models used and/or differences in metformin doses.

PPARγ activation for treatment of ALD

Thiazolidinediones (TZDs) are PPARγ agonists and insulin-sensitizers used in the treatment of diabetes and non-alcoholic fatty liver disease (NAFLD). PPARγ is a transcription factor that controls adipocyte differentiation and production of adiponectin. Common TZDs include rosiglitazone, pioglitazone, and troglitazone. Although there are no data on the impact of TZDs on alcohol-mediated IR, there are a number of experimental studies documenting the benefit of TZDs on alcoholic steatosis and inflammation. Pioglitazone administered by oral gavage to alcohol-fed female Wistar rats prevents alcohol-induced hepatic steatosis and inflammation.[132] The benefit of TZD therapy is in part due to reduced Kupffer cell sensitivity to LPS, a known promoter of alcoholic steatohepatitis. This reduced sensitivity to LPS with pioglitazone therapy was previously observed in a study of acute alcohol toxicity in female Sprague-Dawley rats.[133]

Pioglitazone and troglitazone were both shown to improve histologic ALD in male Sprague-Dawley rats chronically fed alcohol by intra-gastric administration. The authors did not observe improvements in serum glucose or insulin in TZD-treated animals; however, it is unclear if these serological measurements were in a fasting state, and there were no *in vivo* assessments of glucose or insulin tolerance.[134]

The beneficial actions of rosiglitazone in ALD are multifactorial but may largely result from its stimulatory effect on adiponectin. Administration of low (3 mg/kg) or high dose (10 mg/kg) rosiglitazone to wild-type mice chronically fed a combined alcohol–polyunsaturated fat diet reduces hepatic steatosis and serum transaminase levels in both low and high dose groups compared with alcohol-treated mice not given rosiglitazone. Notably, these

improvements correlated with increased serum adiponectin levels and hepatic adiponectin receptor gene expression, likely through increased SIRT-1–AMPK activation.[135]

ALD and PPARα therapy

PPARα is a transcription factor whose activation promotes fatty acid oxidation.[136] Reduced PPARα has been linked to ALD pathogenesis. The pharmacologic PPARα agonist Wy14643 improves steatohepatitis, increases PPARα responsive genes, and reduces pro-inflammatory gene expression in wild-type mice chronically fed a 4% alcohol diet.[137] Adult male Long-Evans rats chronically fed alcohol for eight weeks had improved steatohepatitis after a three-week intraperitoneal treatment of GW7647, a PPARα agonist.[138] Notably, these rats had similar improvement of steatohepatitis and upregulation of insulin responsive genes after treatment with PPARγ and PPARδ agonists,[138, 139] suggesting that PPARs as a class may have beneficial effects on ALD.

ALD and adiponectin therapy

Exogenous administration of adiponectin improves experimental ALD;[74, 80] and adiponectin reduces LPS-induced TNF-α production in Kupffer cells isolated from male Wistar alcohol-fed rats.[116] While upregulation of the aforementioned hepatic adiponectin–AMPK pathway plays a role in TZD-related improvements in experimental ALD, AMPK-independent pathways may also contribute to the beneficial effects of adiponectin. For example, we have shown that exogenous adiponectin alleviates hepatic steatosis. While there was no difference in AMPK expression between alcohol-fed wild-type and adiponectin gene–knockout mice, a significant reduction of hepatic ceramide levels was seen in the knockout mice.[80] Identification of the specific pathways that link adiponectin with ceramide biosynthesis in ALD require further exploration, including investigation of the specific ceramide pools and subtypes that promote disease.

The translation of adiponectin into clinical practice has been hampered by adiponectin's high concentration in the serum, short half-life (40–90 min), and complex post-translational modifications and tertiary structure [140]. Adiponectin exerts many of its positive effects by binding to its receptors AdipoR1 and AdipoR2 [141]. Recently, a small molecule agonist (AdipoRon) of both adiponectin receptors was identified.[142] AdipoRon treatment is orally administered and bioavailable at relevant target sites. In both wild-type and leptin receptor–deficient high-fat diet–fed mice, AdipoRon improves dyslipidemia and insulin sensitivity. In the livers of wild-type mice, AdipoRon reduces liver triglyceride levels, upregulates pathways involved in fat oxidation, and reduces expression of inflammatory and gluconeogenic genes. AdipoRon has not been investigated in either clinical or experimental ALD, but its inability to augment AMPK activation in the setting of maximal adiponectin concentration may affect its utility in the subset of ALD patients with hyperadiponectinemia.

ALD and FXR agonism

Agonists of the farnesiod X receptor (FXR) comprise a new class of therapeutic agents that have shown promise for the treatment of NAFLD.[143-145] The natural ligand for FXR, a member of the nuclear hormone transcription factor family, is bile acids. Upon activation, FXR dimerizes with retinoid X receptor (RXR) and drives production of genes involved in

bile acid, glucose, and lipid homeostasis.[146] Two synthetic FXR agonists, WAY-362450 and obeticholic acid (OCA, 6α-ethyl-chenodeoxycholic acid), have been studied in experimental ALD. Wu *et al*. showed that relative to control-fed mice, mice fed a standard Lieber–DeCarli ethanol diet have elevated liver bile acids, reduced FXR association with the nuclear receptor RXR and downregulation of FXR-responsive genes.[147] Treatment of ethanol-fed mice with WAY-362450 normalizes FXR function and reduces steatohepatitis. Livero *et al*. showed that OCA alleviates ethanol-mediated increases in liver triglyceride, hepatic damage, and oxidative stress in Swiss mice fed a low protein diet with 10% ethanol in the drinking water.[148] To date, there are no published human studies of FXR agonism in ALD; however, there is a clinical trial of OCA in patients with acute alcoholic hepatitis that is currently underway.[149] In the phase 2 Trial of Obeticholic Acid in Patients with Moderately Severe Alcoholic Hepatitis,[a] patients will receive either placebo or 10 mg OCA for six weeks and will be followed for changes in clinical measures of liver disease severity and adverse events.

Syndrome of metabolic and alcoholic steatohepatitis (SMASH)

Investigation of therapeutics that aim to improve lipid and glucose homeostasis in ALD underscores the parallels between ALD and NAFLD, a disease largely the result of over-nutrition, and considered by many to be the hepatic manifestation of the metabolic syndrome.[150] Indeed, in both diseases obesity increases liver disease severity;[5, 151] and insulin resistance and diabetes commonly occur in these patients.[7, 152] Current guidelines for diagnosis of NAFLD, however, do not take into account the possibility that both diseases can co-exist in a single patient. To the contrary, diagnosis of NAFLD requires exclusion of patients with “significant alcohol consumption”.[153] We have observed that the clinical presentation of patients with liver disease who have a history of both heavy chronic alcohol consumption and over-nutrition with features of the metabolic syndrome is quite common. Moreover, only 10–35 percent of heavy drinkers develop steatohepatitis, and only 8–20% develop cirrhosis,[154] suggesting that alcohol intake alone is insufficient for development of ALD.

The above clinical observations are supported by epidemiological data. Naveau *et al*. studied 1604 alcoholic patients admitted to a gastroenterology unit for alcoholism or ALD.[155] The majority of these patients had biopsy-proven ALD. Seventeen percent of alcoholic cirrhotic patients were overweight (body mass index ≥ 25 in women; ≥ 27 in men) for at least 10 years prior to study enrollment, while only 7% of non-cirrhotics were overweight during this period. Similarly, compared with patients with normal livers, patients with acute alcoholic hepatitis had a higher prevalence of excess weight over a ten year period (8.2% vs. 2.6%). Patients who were overweight for at least 10 years prior to study enrollment were more likely to have cirrhosis than normal weight patients (60% vs. 35%). Subsequent multivariate analysis revealed that an alcoholic patient who is overweight for at least ten years is 2.15 times more likely to have cirrhosis, three times more likely to have acute alcoholic hepatitis, and 2.5 times more likely to have hepatic steatosis than normal weight patients. The prior study may in fact underrepresent the true proportion of obese patients with ALD, as

[a] https://clinicaltrials.gov/ct2/show/NCT02039219?term=obeticholic+acid&rank=4

NHANES III data from 1988–1994 indicate that approximately 45% of patients with ALD are obese, 40% have either IR or diabetes, and 32% have the metabolic syndrome.[151]

The interaction of obesity and alcohol consumption in promoting liver disease has been explored in several studies. Stepanova *et al*. observed that the presence of IR or diabetes increases overall mortality, while the presence of obesity or metabolic syndrome increases liver related mortality in ALD patients.[151] Similar findings are reported in a Scottish study [156] and supported by earlier histologic data that demonstrate increased body weight and long duration of alcohol consumption independently predict advanced fibrosis and cirrhosis among alcoholic patients without overt liver disease.[157] Additionally, a cross-sectional study of elderly adults with a range of patterns of alcohol consumption demonstrated that the combination of obesity and high alcohol intake (>3 drinks/day) significantly increases the odds of elevated liver enzymes compared with either clinical factor alone.[158]

These epidemiological studies suggest a synergistic relationship between alcohol and over-nutrition in liver disease progression and experimental data corroborate this hypothesis. For example, obese rats are sensitized to the development of steatohepatitis after a single binge of alcohol.[159] Sprague-Dawley rats fed a high-fat and 16% Lieber–DeCarli alcohol diet have increased hepatocellular inflammation and apoptosis than rats fed a high-fat diet alone[160] and this increase in inflammation may relate to suppressed SIRT1 deacetylase activity.[161] Similar histologic findings, as well as fibrosis, have been reported in male Wistar rats fed a combined alcohol and high-fat diet.[162, 163]

Despite these experimental observations, the specific nutritional components that promote SMASH are not well understood. Fructose has been implicated in SMASH, as Sprague-Dawley rats fed a combination of fructose and ethanol develop significantly greater liver damage and metabolic abnormalities than either fructose- or ethanol-fed rats;[164] but the role of fatty acids is less clear because polyunsaturated fatty acids and saturated fatty acids may have opposing effects in NAFLD and ALD. Namely, polyunsaturated fatty acids improve NAFLD through downregulation of SREBP1,[165, 166] while polyunsaturated fatty acids have been found to both promote and ameliorate experimental ALD.[73, 167, 168] Saturated fatty acids worsen experimental NAFLD,[169, 170] but long-chain saturated fatty acids may protect against ALD, based on a recent study by Chen *et al*.[171] These authors demonstrated that chronic alcohol ingestion reduces long-chain saturated fatty acid synthesis by gut microbiota in humans and mice, and that supplementation of saturated fatty acids improves liver histology and gut intestinal epithelial barrier function in alcohol-fed mice.[171] The hypothesis linking nutrients produced by gut microbiota with liver disease risk is an attractive one, as intestinal dysbiosis has been mechanistically linked with both ALD[172] and NAFLD.[173, 174] These studies deserve continued investigation as SMASH patients—obese with concomitant metabolic syndrome and excessive alcohol use—becomes a more common.

Conclusion

ALD is a prevalent disease that results in significant morbidity and mortality, yet little is known about the factors that promote disease progression except for chronic alcohol consumption and a few demographic factors. IR is prevalent in patients with ALD, and it increases risk of advanced disease. There are multiple mechanisms of IR in ALD patients that likely converge to cause disease progression. Some of these mechanisms involve both direct inhibition of the insulin signaling pathway and indirect effects on target proteins, adipokines, and lipid mediators that impair signaling. Additional pathways, for example the role of inflammation in promoting IR, have not been explored in this review. Although the variability of experimental approaches has limited their interpretation, most of the data suggest that targeting insulin signaling pathways pharmacologically may ameliorate disease. To date, however, there are no studies in humans supporting the clinical use of insulin sensitizers with ALD. A focus on management of IR in patients with ALD may be timely because although patients with ALD have been historically malnourished,[175] the advent of the obesity epidemic has led to a high proportion of ALD patients who suffer from the consequences of obesity. Increasingly, the distinction between ALD and NAFLD patients is blurred, and is reflected in the recognition that the combination of over-nutrition and excessive alcohol use act synergistically to promote liver disease. Future studies should examine both biological and environmental factors that play roles in the syndrome of metabolic and alcoholic hepatitis in order to uncover novel therapeutic targets.

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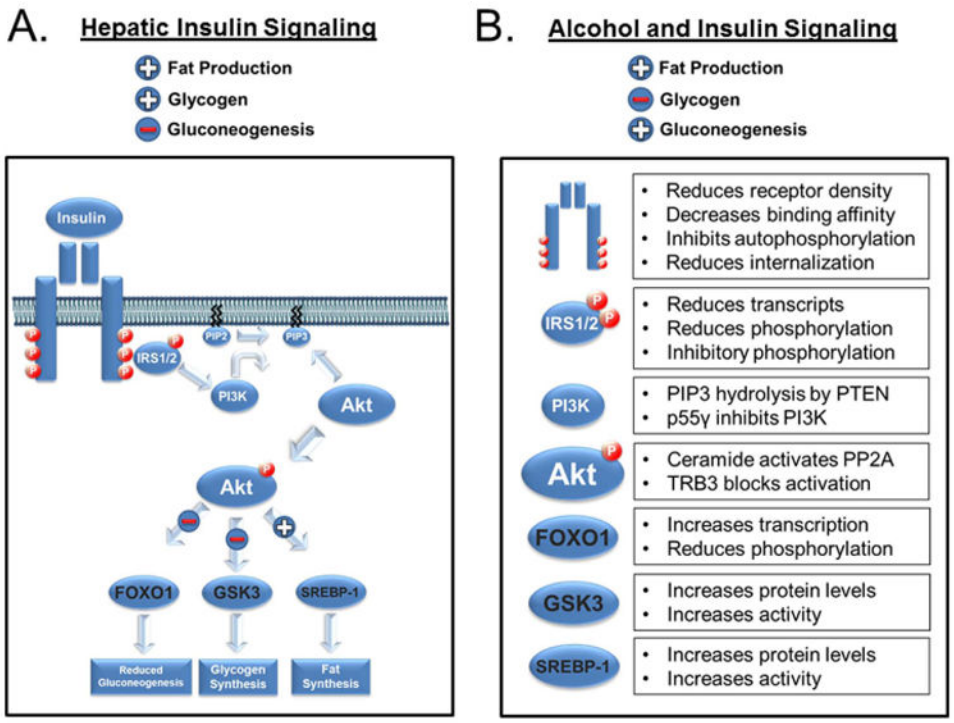

Figure 1.

Model for intra-cellular insulin signaling and alcohol's effect on insulin signaling. (A) Schematic of insulin's effects on intra-cellular signaling and effects on metabolism. (B) Known effects of alcohol on insulin signaling molecules and effects on metabolism.

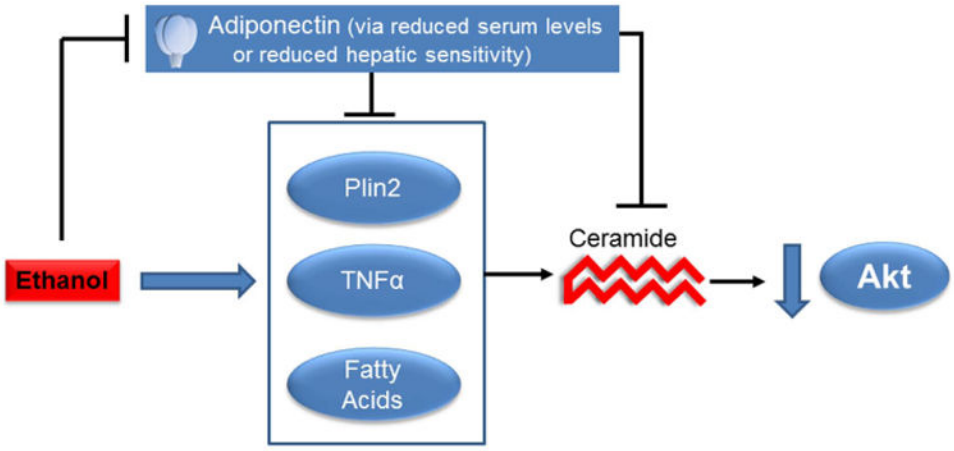

Figure 2.

Mechanisms of insulin resistance in ALD. Putative model for molecular mediators of insulin resistance in ALD.

Table 1
Insulin-sensitizing therapies in experimental ALD

Drug	Reference	Model system	Beneficial effects
Metformin	Zhu *et al.*[135]	Male Wistar Rats gavaged daily with 5-9 g/kg ethanol with or without metformin (200mg/kg) for 16 weeks	Alcoholic animals treated with metformin have reduced HOMA-IR, blood glucose and insulin. Metformin also associated with reduced liver TG and increased serum adiponectin
	Bergheim *et al.* 136	Acute ethanol treatment (12 h) in C57Bl/6 male mice via IP ethanol injection following 4 days of metformin treatment (200 mg/kg IP)	Metformin treatment reduces liver TG accumulation associated with reduction of inflammatory molecule PAI-1
	You *et al.* 137	Ethanol-treated rat hepatoma cells (H4IIEC3, McA-RH-7777) treated with 1 mM metformin	Metformin blocks alcohol-mediated increases in SREBP-1 and decreases in fat oxidation.
AICAR	Tomita *et al.* 138	Male Wistar rats pair-fed standard Lieber–DeCarli diet for six weeks treated with AICAR daily (0.5 mg/g, subcutaneous).	AICAR treatment reduces ethanol-mediated increases in hepatic steatosis, triglyceride, SREBP-1 and lipid peroxidation
Resveratrol	Ajmo *et al.* 2008	C57Bl/6 male mice fed a low fat Lieber–DeCarli diet with 29% of calories from alcohol for 4 weeks. Resveratrol (200 or 400 mg/kg) was administered in the diet for the final two weeks of feeding	Resveratrol alleviates alcohol-mediated liver steatosis, triglyceride and ALT associated with increased serum adiponectin and hepatic Sirt1, pAMPK and reduced nuclear SREBP-1
TZDs	Enomoto *et al.* 2003	Female Wistar rats fed ethanol by intra-gastric feeding (5g/kg) for 8 weeks. Pioglitazone was also administered IG (500 μg/kg). Using same feeding protocol for 4 weeks, LPS-induced liver damage and kupffer cell sensitization assessed *in vitro*	Increased liver steatosis, ALT and AST observed following 8 week ethanol and 4 week + LPS treatment; reversed by pioglitazone treatment. Kupffer cells isolated from 4 week fed animals display increased LPS sensitivity that was diminished by pioglitazone treatment
	Ohata *et al.* 2004	Acute intra-gastric ethanol treated (5 g/kg) female Sprague-Dawley rats received pioglitazone (1 mg/kg, IP) or vehicle at time of ethanol treatment and 24 h later. LPS given 24 h after treatment.	Ethanol-treated animals are sensitized to LPS-induced increases in ALT, AST, focal necrosis, inflammation and serum TNF-α. Sensitization reversed by pioglitazone treatment
	Tomita *et al.* 2004	Male sprague-dawley rats fed ethanol by daily gavage (4 g/kg) given daily doses of pioglitazone (10 mg/kg) and troglitazone (200 mg/kg)	Ethanol-treated animals have increased liver triglyceride and ALT, reversed by pioglitazone and troglitazone treatment. Pioglitazone and troglitazone reverse alcohol-mediated suppression of hepatic c-Met mRNA
	Shen *et al.* 2010	Male C57Bl/6 mice pair fed Lieber–DeCarli (29% ethanol-derived calories) diet for 4 weeks +/- rosiglitazone (3 and 10 mg/kg) for last 2 weeks	Rosiglitazone treatment blocks alcohol-mediated increases in liver steatosis, triglyceride, AST and ALT. Rosiglitazone elevates serum adiponectin and liver adiponectin receptor mRNA levels in control and ethanol-treated mice. Changes in rosiglitazone-treated animals associated with increased nuclear SIRT1, reduced FOXO1 and histone H3 acetylation
Adiponectin	Correnti *et al.* 2014	Male adiponectin KO mice pair fed a modified Lieber–DeCarli diet (Reyes chocolate-flavor) with increasing ethanol concentrations ending with 7 days of 34% of calories from ethanol. Adiponectin administered by subcutaneous osmotic pump for last 5 days of feeding	Adiponectin treatment of alcohol-fed mice reduces liver triglyceride, steatosis, hepatomegaly and inflammatory cytokine message for TNF-α and MCP-1
	Xu *et al.* 2003	Male FVB/n mice were pair-fed a high-fat/low-carbohydrate liquid diet with 34% of calories from ethanol for 5 weeks. Adiponectin was administered by subcutaneous osmotic pump for last two weeks of feeding	Adiponectin treatment of alcohol-fed mice reduces serum ALT, liver triglyceride, steatosis, hepatomegaly and TNF-α. Associated with increased liver fat oxidation and reduced fatty acid synthase activity
PPARα	Kong *et al.* 2011	Male C57Bl/6 mice pair-fed 4% (v/v) Lieber–DeCarli liquid alcohol diet for 12 weeks with Wy 14,643 (50 mg/kg) in the diet	Wy 14,643 treatment of alcohol-fed mice reduces ALT, AST, steatosis and necrosis associated with normalization of hepatic levels of PPARα, PPARα-responsive genes; and lipid metabolizing enzymes
	de la Monte *et al.* 2011	Male Long-Evans rats pair-fed a Lieber–Decarli diet with 37% of calories from alcohol (9.2%, v/v) for 8	GW7647 treatment reduces alcohol-induced liver steatosis and improves liver histology

Drug	Reference	Model system	Beneficial effects
		weeks. GW7647 (25 μg/kg, IP) was administered twice weekly for the last 5 weeks	
	Lebrun *et al*. 2013	Female C57BL/6J mice pari-fed a Lieber–DeCarli ethanol diet (ramp from 4-35% ethanol for 10 d, then 35% for 10 d) with or without Wy 14,643 (12 mg/100 ml liquid diet)	Wy 14,643 treatment normalizes liver TG, glucose infusion rate and hepatic glucose production; and reduces inflammatory gene transcripts
PPARδ	de la Monte *et al*. 2011	Male Long-Evans rats pair-fed a Lieber–DeCarli diet with 37% of calories from alcohol (9.2%, v/v) for 8 weeks. PPARδ (L-160043; 2 μg/kg, IP) was administered twice weekly for the last 5 weeks	PPARδ agonist treatment of alcohol-fed rats reduces steatosis, improves liver histology and normalizes expression of insulin-responsive genes
	Ramirez *et al*. 2012	Male Long-Evans rats pair-fed a Lieber–DeCarli diet with 37% of calories from alcohol (9.2%, v/v) for 8 weeks. PPARδ (L-160043; 2 μg/kg, IP) was administered twice weekly for the last 5 weeks	PPARδ agonist treatment of alcohol-fed rats improves alcohol-mediated impairment of hepatic insulin/Akt signaling
PPARγ	de la Monte *et al*. 2011	Male Long-Evans rats pair-fed a Lieber–DeCarli diet with 37% of calories from alcohol (9.2%, v/v) for 8 weeks. PPARγ agonist (F-L-Leu; 20 μg/Kg, IP) was administered twice weekly for the last 5 weeks	PPARγ agonist treatment of alcohol-fed rats reduces steatosis and liver histology and normalizes expression of insulin-responsive genes
FXR	Wu *et al*. 2014	Male C57Bl/6 mice pair-fed Lieber–DeCarli liquid diet with 35.5% of calories from alcohol for 4 weeks. WAY-362450 (30mg/kg/d, IG) was given for 4 weeks.	WAY-362450 treatment WAY-362450 normalizes FXR function, reduces hepatic bile acids, steatosis, and liver damage
	Livero *et al*. 2014	Male Swiss mice fed a low-protein diet with 10% ethanol in the drinking water for 6 weeks. Animals received FXR agonist (OCA, 3 mg/kg IG) for last two weeks	OCA treatment alleviates ethanol-mediated increases in liver triglyceride, damage, and oxidative stress