

Endocannabinoids in Liver Disease

Joseph Tam, Jie Liu, Bani Mukhopadhyay, Resat Cinar, Grzegorz Godlewski, and George Kunos

Endocannabinoids are lipid mediators of the same cannabinoid (CB) receptors that mediate the effects of marijuana. The endocannabinoid system (ECS) consists of CB receptors, endocannabinoids, and the enzymes involved in their biosynthesis and degradation, and it is present in both brain and peripheral tissues, including the liver. The hepatic ECS is activated in various liver diseases and contributes to the underlying pathologies. In patients with cirrhosis of various etiologies, the activation of vascular and cardiac CB₁ receptors by macrophage-derived and platelet-derived endocannabinoids contributes to the vasodilated state and cardiomyopathy, which can be reversed by CB₁ blockade. In mouse models of liver fibrosis, the activation of CB₁ receptors on hepatic stellate cells is fibrogenic, and CB₁ blockade slows the progression of fibrosis. Fatty liver induced by a high-fat diet or chronic alcohol feeding depends on the activation of peripheral receptors, including hepatic CB₁ receptors, which also contribute to insulin resistance and dyslipidemias. Although the documented therapeutic potential of CB₁ blockade is limited by neuropsychiatric side effects, these may be mitigated by using novel, peripherally restricted CB₁ antagonists. (HEPATOLOGY 2011;53:346-355)

Marijuana has been used for its psychoactive and medicinal properties for millennia. Like other plant-derived substances, marijuana has been slow to yield its secrets, with insights into its mechanism of action beginning to emerge only during the last decades. The existence of specific cannabinoid (CB) receptors in mammalian tissues was first revealed by radioligand binding, and this was followed by the

molecular cloning of two G protein-coupled CB receptors.¹ CB₁ receptors are the most abundant receptors in the mammalian brain, but they are also expressed in peripheral tissues, including various cell types of the liver, at much lower yet functionally relevant concentrations.²⁻⁸ CB₂ receptors are expressed primarily in immune and hematopoietic cells and have also been detected in the liver in certain pathological states.^{9,10} Additional CB receptors may exist,¹¹ but their potential role in liver biology is unknown.

Abbreviations: 2-AG, 2-arachidonoyl glycerol; ACC, acetyl coenzyme A carboxylase; AEA, arachidonoyl ethanolamide; AFLD, alcoholic fatty liver disease; AM630, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]-(4-methoxyphenyl)methanone; AM6545, 5-(4-(4-cyanobut-1-ynyl)phenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(1,1-dioxo-thiomorpholino)-1H-pyrazole-3-carboxamide; ApoE, apolipoprotein E; CB, cannabinoid; CBD, cannabidiol; CPT1, carnitine palmitoyltransferase 1; DIO, diet-induced obesity; ECS, endocannabinoid system; FA, fatty acid; FAAH, fatty acid amide hydrolase; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; HU-308, 4-[4-(1,1-dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-methanol; I/R, ischemia/reperfusion; JWH-133, (6aR,10aR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; LCB₁^{-/-}, liver cannabinoid receptor 1 knockout; LPL, lipoprotein lipase; MTP, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; RAR, retinoid A receptor; SREBP1c, sterol regulatory element binding protein 1c; TG, triglyceride; THC, tetrahydrocannabinol; VLDL, very low density lipoprotein.

From the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD.

Received August 2, 2010; accepted November 5, 2010.

Address reprint requests to: George Kunos, M.D., Ph.D., National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 5625 Fishers Lane, MSC-9413, Bethesda, MD 20892-9413. E-mail: gkunos@mail.nih.gov; fax: 301-480-0257.

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DOI 10.1002/hep.24077

Potential conflict of interest: Nothing to report.

The discovery of CB receptors triggered a search for endogenous ligands. Arachidonoyl ethanolamide (AEA), also known as anandamide, was the first such ligand discovered,¹² with 2-arachidonoyl glycerol (2-AG) identified 3 years later.^{13,14} Additional endogenous ligands have since been identified¹ but have received less attention. AEA and 2-AG are generated on demand in response to a rise in intracellular calcium or metabotropic receptor activation.¹ Their biosynthesis from membrane phospholipid precursors may proceed along multiple, parallel pathways.^{15,16} Once released, they remain largely membrane-associated because of their hydrophobic nature, and they can be taken up by cells via a high-affinity uptake mechanism¹⁷; this is followed by their enzymatic degradation. AEA is metabolized primarily by membrane-associated fatty acid amide hydrolase (FAAH),¹⁸ whereas 2-AG is preferentially degraded by monoglyceride lipase.¹⁹

The psychoactive properties of CBs and the abundance of CB₁ receptors in the brain could suggest that

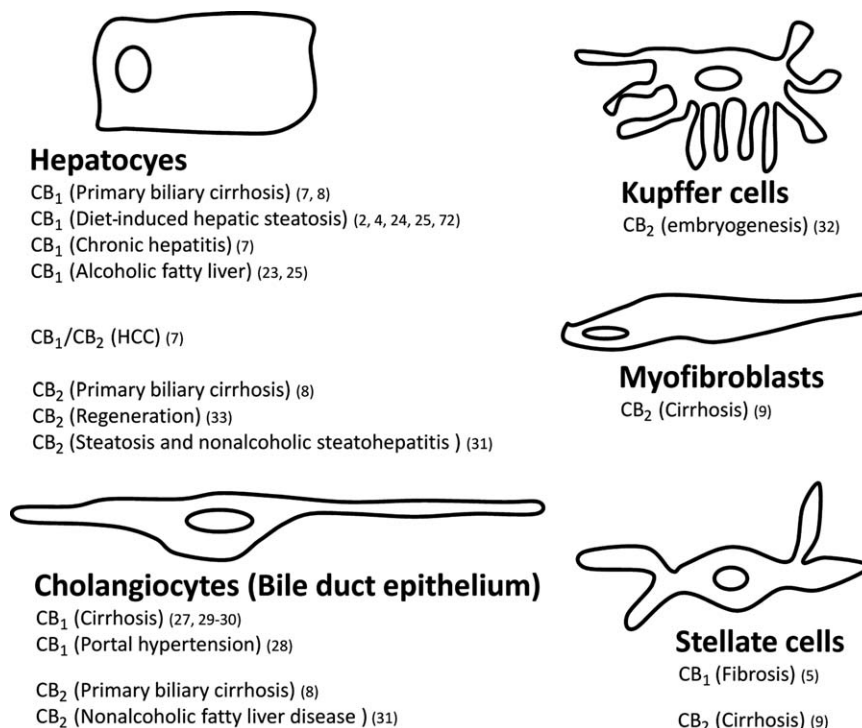


Fig. 1. Cellular distribution of hepatic CB₁ and CB₂ receptors and their involvement in liver diseases. Numbers in parentheses refer to the original reports listed in the References section.

the endocannabinoid system (ECS) is primarily a neuronal signaling system; therefore, evidence for the presence and functional importance of the ECS in the liver² was unexpected. Indeed, early studies of brain CB₁ receptors used the liver as a negative control.²⁰ However, recent reports have documented low-level CB₁ expression in the whole liver,^{2-4,21-23} hepatocytes,^{6,23-25} stellate cells,^{5,26} and hepatic vascular endothelial cells²⁷⁻³⁰ (see Fig. 1). CB₁ receptors are present in human hepatocytes²⁵ and in the whole human liver, with increased expression noted in patients with hepatocellular carcinoma (HCC)⁷ or primary biliary cirrhosis.⁸ CB₂ receptors are undetectable in the normal liver but are induced in pathological conditions such as nonalcoholic fatty liver disease (NAFLD),³¹ the embryonic state,³² liver fibrosis,⁹ the regenerating liver,³³ and HCC.⁷ Hepatic endocannabinoids levels are similar to those in the brain,^{2,26} whereas FAAH expression is higher in the liver versus the brain. Evidence implicating the ECS in the regulation of hepatic hemodynamics, fibrogenesis, and lipid metabolism and in the dysregulation of these functions in pathological states such as cirrhosis, NAFLD, alcoholic fatty liver, and ischemia/reperfusion (I/R) injury is discussed next.

Endocannabinoids and Altered Hemodynamics in Cirrhosis

The potent hypotensive action of Δ^9 tetrahydrocannabinol (THC) has long been recognized, and attempts

have even been made to exploit it for the treatment of hypertension.³⁴ This and the similar effects of synthetic CB analogues and endocannabinoids are mediated by CB₁ receptors located, in part, in the peripheral cardiovascular system,³⁵ and they play a pathogenic role in various forms of shock,^{36,37} including endotoxic shock.³⁸⁻⁴⁰

Advanced liver cirrhosis is associated with endotoxemia and hypotension, and this suggests endocannabinoid involvement. Indeed, cirrhosis in rats is accompanied by progressive hypotension reversible by CB₁ blockade,²⁷ which also reduces the elevated portal venous pressure and mesenteric blood flow. The likely source of endocannabinoids is activated macrophages, in which lipopolysaccharide induces the CD14-dependent synthesis of AEA.^{38,41} AEA levels are elevated in circulating macrophages of cirrhotic rats or patients, and such macrophages injected into normal rats elicit CB₁-mediated hypotension.^{27,42} Cirrhosis increases CB₁ expression in vascular endothelial cells²⁷ or in mesenteric arteries^{29,43} and increases the vasodilator potency of AEA.^{29,43,44} In patients with cirrhosis, circulating AEA levels, but not 2-AG levels, are increased in peripheral blood but not in hepatic veins or liver tissue, and this suggests that the liver is not its source.⁴⁵

These findings implicate AEA as a mediator of the vasodilated state in cirrhosis. Although in one study of patients with cirrhosis the increase in circulating AEA did not correlate with the degree of hepatic and renal dysfunction,⁴⁶ in another study of patients with

primary biliary cirrhosis, the CB₁ expression in hepatocytes and biliary epithelial cells and the CB₂ expression in hepatocytes and cholangiocytes were positively correlated with the severity of the histological stage.⁸ Cirrhosis is associated with renal sodium retention, which has been attributed, in part, to portal hypertension secondary to liver parenchymal damage and fibrosis.⁴⁷ In cirrhotic rats, rimonabant dose-dependently reduced ascites by ensuring a less positive sodium balance.⁴⁸

AEA induces CB₁-mediated mesenteric vasodilation independently of nitric oxide.⁴⁹ However, the effect of higher doses of AEA is resistant to CB₁ blockade⁴⁹ and may be mediated via putative AEA receptors implicated in the mesenteric vasorelaxant effect of AEA observed in CB₁/CB₂ double-knockout mice,^{11,50} which may also contribute to mesenteric vasodilation in cirrhosis.

The hyperdynamic circulation of advanced cirrhosis is associated with increased cardiac output and tachycardia. However, the cirrhotic heart has an underlying decrease in contractility and β -adrenergic hyposensitivity called *cirrhotic cardiomyopathy*,⁵¹ and this has been attributed to endocannabinoid activation of cardiac CB₁ receptors on the basis of pharmacological studies using isolated myocardial preparations from bile duct-ligated rats.⁵² *In vivo* studies using an intraventricular pressure-volume microcatheter system revealed a profound decrease in baseline cardiac contractility, which was acutely normalized by CB₁ blockade.⁵³ The suppression of cardiac contractility by CB₁ receptor activation may involve inhibition of L-type calcium channels⁵⁴ and/or reductions in the myocardial cyclic adenosine monophosphate content.⁵⁵ Of the 2 major endocannabinoids, AEA is more likely to be involved, as suggested by a cirrhosis-related increase in myocardial AEA levels but not 2-AG levels.⁵³

These findings raise the therapeutic potential of CB₁ blockade in treating the hemodynamic abnormalities of patients with advanced liver cirrhosis. Because the increase in mesenteric blood flow may precipitate the rupture of varicosities and also contributes to ascites formation, CB₁ blockade may avert these potentially fatal complications and thus keep patients alive until a liver transplant becomes available.

Endocannabinoids and Liver Fibrosis

CB₂ receptors, which are normally undetectable in the liver, are prominently expressed in the cirrhotic human liver and are also detectable in nonparenchymal liver cells in the fibrotic mouse liver.⁹ THC suppresses

the proliferation and induces the apoptosis of human hepatic myofibroblasts and stellate cells via CB₂ receptors⁹ and thus may be antifibrotic and hepatoprotective.⁵⁶ Accordingly, CB₂^{-/-} mice had an enhanced response to fibrogenic stimuli.⁹ CB₂ receptor activation by AEA also inhibits the hyperplastic proliferation of cholangiocytes, which is a frequent result of extrahepatic biliary obstruction, cholestasis, and toxic liver injury. This has been associated with the increased production of reactive oxygen species and cell death via the induction of the activator protein 1 complex and thioredoxin 1.³ In cirrhotic rats, chronic treatment with the CB₂-selective agonist JWH-133 attenuated cellular markers of fibrosis⁵⁷ and enhanced the regenerative response to acute liver injury. Accordingly, CB₂^{-/-} mice had delayed liver regeneration in response to CCl₄-induced injury, whereas JWH-133 treatment reduced the injury and accelerated liver regeneration.³³ These findings signal the therapeutic potential of nonpsychoactive CB₂ agonists in the treatment of liver fibrosis.

Paradoxically, in patients with hepatitis C virus infection, daily cannabis use increased fibrosis progression instead of protecting patients against it.⁵⁸ Thus, endocannabinoids also exert a profibrotic effect that is possibly mediated by CB₁ receptors. This is compatible with the finding of increased CB₁ expression in stellate cells and hepatic myofibroblasts in the cirrhotic human liver and in the livers of mice with three different models of fibrosis.⁵ Genetic or pharmacological ablation of CB₁ receptors protected mice against liver injury; this was reflected by the reduced expression of smooth muscle α -actin and transforming growth factor β .⁵ 2-AG is the likely fibrogenic mediator because its hepatic level is preferentially increased by the CCl₄ treatment of mice²⁶ and rats.⁵³ Higher doses of 2-AG induce apoptosis in activated hepatic stellate cells (HSCs) *in vitro* through a receptor-independent, membrane cholesterol-dependent mechanism; this means that 2-AG also has antifibrotic activity.²⁶ AEA showed a similar effect, although the eventual cell death was by necrosis rather than apoptosis.⁵⁹ For both endocannabinoids, these effects occur in the 2 to 50 μ M range. The hepatic concentration of AEA is orders of magnitude below such levels, whereas 2-AG may reach low micromolar concentrations.²⁶ Because the proapoptotic effect of 2-AG is independent of CB receptors, it could contribute to the reduction of fibrotic activity observed after CB₁ blockade.⁴⁸ The profibrotic and adverse hemodynamic effects of CB₁ activation could provide a rationale for the use of CB₁ antagonists in the medical management of advanced liver cirrhosis.

Endocannabinoids and Metabolic Syndrome

The CB₁-mediated, appetite-promoting effect of endocannabinoids⁶⁰ was the primary impetus for the development of brain-penetrating CB₁ receptor antagonists for the treatment of obesity. The first-in-class compound rimonabant caused weight reduction and improved the associated cardiometabolic risk factors, but neuropsychiatric side effects, including depression and anxiety, have prevented its approval in the United States and have led to its withdrawal from the market in other countries (reviewed by Rosenson⁶¹). Accumulating evidence indicates, however, that the metabolic effects of endocannabinoids are mediated, at least in part, by peripheral CB₁ receptors, as discussed in some detail later. Indeed, a non-brain-penetrating CB₁ antagonist was recently reported to retain the beneficial metabolic effects of rimonabant in obese mice without producing the behavioral effects that predict neuropsychiatric side effects in humans; this may revive interest in the therapeutic potential of CB₁ antagonism.⁶²

Reduced food intake is not the primary mechanism of weight reduction by CB₁ blockade in obesity. In mice with diet-induced obesity (DIO), chronic use of rimonabant caused a transient reduction in food intake and sustained weight loss, and this indicated food intake-independent effects on energy balance.^{2,63} Increased *de novo* hepatic lipogenesis has been documented in DIO mice^{2,64,65} and in people with NAFLD^{66,67} and may be mediated by endocannabinoids. Indeed, lipogenic gene expression and the rate of *de novo* hepatic lipogenesis were increased by CB₁ agonists and decreased by CB₁ antagonists in rodents.^{2,4,25,68,69} A high-fat diet increases hepatic CB₁ expression^{2,4,21,25} and the hepatic levels of AEA.² Thus, endogenous AEA acting via hepatic CB₁ receptors contributes to increased *de novo* lipogenesis in mouse models of obesity. CB₁-mediated hepatic lipogenesis may explain the finding that in patients with chronic hepatitis C infection, daily cannabis smoking was an independent risk factor for steatosis severity but not for obesity.⁷⁰

Although lipogenesis via hepatic CB₁ receptors may contribute to lipid accumulation, extrahepatic CB₁ must play a major role because liver-specific CB₁^{-/-} mice are only partially protected from steatosis,²⁴ whereas CB₁^{-/-} mice with selective transgenic re-expression of CB₁ in hepatocytes, similar to their global CB₁ knockout littermates, remain largely resistant to the steatotic effect of a high-fat diet.⁶² The source of liver fat may be adipose tissue because the activation of CB₁ receptors in adipocytes promotes

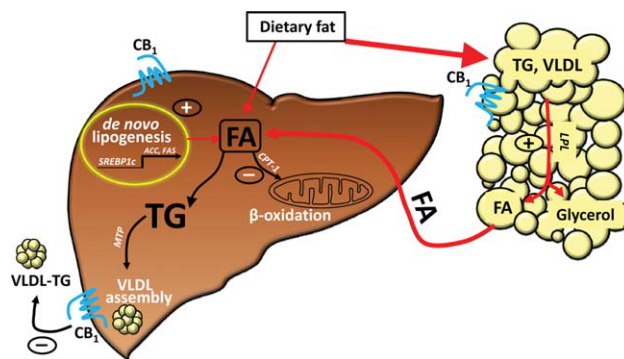


Fig. 2. CB₁ mechanisms involved in liver fat accumulation. The major pathway is CB₁ activation of LPL in adipose tissue, which results in increased FA release and transfer to the liver.⁷¹ Additional mechanisms mediated via hepatic CB₁ receptors include increased *de novo* hepatic lipogenesis, decreased FA oxidation,² and decreased secretion of TG-rich VLDL.⁶² Abbreviations: FA, fatty acid; LPL, lipoprotein lipase. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

lipogenesis,⁷¹ and the released fatty acids may be taken up and converted to triglycerides (TGs) by the liver.⁴ On the other hand, the rapid depletion of excess hepatic TGs after CB₁ blockade may involve hepatic CB₁ receptors, as indicated by the increased rate of secretion of TG-rich very low density lipoprotein (VLDL) from the livers of both DIO and *ob/ob* mice after treatment with a peripherally restricted CB₁ antagonist⁶² (see Fig. 2).

Endocannabinoids are also involved in the diet-induced decrease in fatty acid oxidation. The activity of hepatic carnitine palmitoyltransferase 1 (CPT1), the rate-limiting enzyme in mitochondrial fatty acid β -oxidation, is suppressed by either a high-fat diet or treatment with a CB₁ agonist, and both effects are prevented by rimonabant.²⁴ Conversely, hepatic CPT1 activity is increased in CB₁^{-/-} mice²⁴ and in DIO mice after chronic CB₁ blockade.^{24,62,72} Adiponectin is a key stimulator of fatty acid β -oxidation, and CB₁ blockade increases plasma adiponectin.⁷³ The improved insulin sensitivity following CB₁ blockade has been found to have both adiponectin-dependent^{74,75} and adiponectin-independent components,⁷⁵ although the role of adiponectin in the effects of CB₁ blockade on hepatic mitochondrial function and fatty acid oxidation has not been explored. Increased energy expenditure due to increased fat oxidation after CB₁ blockade has been documented with indirect calorimetry in rats⁷⁶⁻⁷⁸ and mice.⁶² These effects likely contribute to the food intake-independent sustained weight loss^{62,79} as well as the reversal of hepatic steatosis^{62,80,81} after chronic CB₁ blockade.

The DIO-related hypertriglyceridemia was modestly attenuated, whereas the accompanying increase in plasma LDL cholesterol and decrease in high-density lipoprotein cholesterol were absent in both $CB_1^{-/-}$ and $LCB_1^{-/-}$ mice on a high-fat diet. This suggests that hepatic CB_1 mediates diet-induced changes in hepatic lipoprotein metabolism and/or secretion. In a recent study, the treatment of mice with an inhibitor of monoglyceride lipase resulted in elevated hepatic levels of 2-AG, increased hepatic expression of sterol regulatory element binding protein 1c (SREBP1c) and FAS, hypertriglyceridemia, and an accumulation in plasma of apolipoprotein E (ApoE)-depleted, TG-rich apolipoproteins.⁶⁸ These changes were absent in $CB_1^{-/-}$ and $ApoE^{-/-}$ mice and could be prevented by CB_1 blockade. Furthermore, despite the elevated hepatic lipogenic gene expression, TG secretion rates were unchanged, but TG clearance from plasma was inhibited.⁶⁸ In contrast, in DIO mice with long-term up-regulation of the ECS, peripheral CB_1 blockade did increase TG secretion,⁶² as discussed previously.

High-fat diets also result in elevated plasma insulin and leptin levels accompanied by hyperglycemia, which indicates insulin resistance,^{24,82} as well as leptin resistance.^{83,84} Interestingly, both $CB_1^{-/-}$ and $LCB_1^{-/-}$ mice remained glucose-tolerant and insulin-sensitive and did not display the hyperleptinemia associated with high-fat diets.²⁴ Moreover, the insulin and leptin resistance of DIO mice was normalized by the peripheral CB_1 antagonist AM6545.⁶² There is also evidence that THC induces glucose intolerance in humans⁸⁵ and rodents via activation of CB_1 receptors.⁸⁶ Thus, endocannabinoids and hepatic CB_1 play an important role in diet-induced insulin and leptin resistance.

Diet-induced insulin resistance involves adipose tissue, skeletal muscle, and the liver as well as interactions between the three tissues through neurogenic⁸⁷ and/or humoral factors.⁸⁸ In mice, a high-fat diet induces CB_1 expression in skeletal muscle,⁸⁹ and CB_1 blockade increases insulin-induced glucose uptake and phosphorylation in the skeletal muscle of genetically obese mice.⁷⁸ The possibility that the activation of hepatic CB_1 may influence the insulin sensitivity of extrahepatic tissues via the release of soluble mediators remains to be explored.

CB_2 receptors may also be involved in diet-induced hormonal and metabolic changes. In rats, the selective CB_2 agonist JWH-133 improved glucose tolerance, whereas the CB_2 antagonist AM630 had the opposite effect and also prevented the effect of JWH-133.⁹⁰ These effects are the opposite of the glucose intolerance induced by CB_1 receptor activation (discussed previously) and could minimize the effects of mixed

CB_1/CB_2 agonists on glucose homeostasis. The well-documented insulin sensitization by chronic CB_1 blockade^{91,92} may be due to a reversal of the action of AEA, which has low CB_2 efficacy.⁹³ This is also consistent with findings that high-fat diet-induced glucose intolerance and insulin resistance are associated with increases in hepatic AEA levels but not 2-AG levels.²

In a recent study,³¹ CB_2 expression was strongly induced by both steatosis and nonalcoholic steatohepatitis, and this suggests CB_2 involvement in hepatic fat metabolism. Indeed, a modest increase in CB_2 expression was reported in hepatocytes from both *ob/ob* and DIO mice. On the other hand, $CB_2^{-/-}$ mice were resistant to diet-induced steatohepatitis and were less insulin-resistant than wild-type littermates on the same diet. Furthermore, JWH-133 increased the hepatic accumulation of TGs in DIO mice.⁹⁴ The CB_2 -induced insulin resistance suggested by these findings in mice is the opposite of the insulin-sensitizing effect of CB_2 agonists in rats.⁹⁰ Further studies are needed to resolve this discrepancy.

Alcoholic Fatty Liver

Chronic alcoholism may lead to steatosis that can further progress to steatohepatitis, liver cirrhosis, and HCC. Ethanol enhances hepatic lipogenesis^{95,96} and decreases fatty acid oxidation.⁹⁷ The similar mechanisms of diet-induced and alcohol-induced steatosis, together with ethanol's ability to increase endocannabinoid levels, at least in the brain,⁹⁸ suggest ECS involvement in the alcoholic fatty liver. Indeed, the exposure of male mice to a low-fat, liquid ethanol diet for 4 weeks increased hepatic CB_1 expression and 2-AG levels but not AEA levels. 2-AG was increased in HSCs but not in hepatocytes. The expression of diacylglycerol lipase β was also increased in HSCs,²³ and this suggests increased biosynthesis of 2-AG. Rimona-bant treatment attenuated ethanol-induced steatosis without affecting alcohol intake and blood ethanol levels, and this suggests CB_1 involvement. This was further supported by the resistance of both $CB_1^{-/-}$ and $LCB_1^{-/-}$ mice to ethanol-induced steatosis.²³

The hepatic nuclear expression of SREBP1c and its target FAS was increased, whereas CPT1 expression and activity decreased in ethanol-fed mice, in agreement with earlier findings.⁹⁶ In both $CB_1^{-/-}$ and $LCB_1^{-/-}$ mice, the effects of ethanol on SREBP1c, FAS, and CPT1 were blunted or absent. Furthermore, CPT1 activity was increased and resistant to suppression by ethanol in both CB_1 knockout strains.²³ This supports the notion that in alcoholic fatty liver disease

(AFLD), hepatic lipogenesis is increased and fatty acid oxidation is decreased via CB₁ activation.

CB₁^{-/-} hepatocytes are resistant to ethanol-induced steatosis, whereas ethanol increases 2-AG exclusively in HSCs. This suggests a paracrine mechanism by which HSC-derived 2-AG activates CB₁ receptors on adjacent hepatocytes to stimulate lipogenesis and inhibit fatty acid oxidation in the latter. Indeed, coculturing HSCs from alcohol-fed mice with hepatocytes from control mice resulted in increased lipogenic gene expression in the latter. The paracrine effect of ethanol-primed HSCs was blunted when the hepatocytes in the coculture were from LCB₁^{-/-} mice, and this confirmed the role of CB₁ receptors.²³ This paracrine interaction, together with high levels of retinoic acid in HSCs and its well-known role in the control of gene expression, prompted a study of the possible role of retinoic acid and its receptors in regulating hepatic CB₁ expression. CB₁ expression in isolated mouse or human hepatocytes was up-regulated by retinoid A receptor γ (RAR γ) or pan-RAR agonists, and the effect could be attenuated by small interfering RNA knockdown of RAR γ but not other RAR subtypes.²⁵ Both CB₁ and RAR γ were up-regulated in hepatocytes from mice fed either a high-fat diet or a liquid alcohol diet. Furthermore, 2-AG up-regulated CB₁ in normal hepatocytes but not in retinaldehyde dehydrogenase 1^{-/-} hepatocytes, which are deficient in retinoic acid. Thus, CB₁ autoinduction may also involve retinoic acid.²⁵ Interestingly, autoinduction of hepatic CB₁ receptors is also suggested by the finding that chronic rimonabant treatment of DIO mice reversed the diet-induced up-regulation of hepatic CB₁.⁴ These reviewed findings suggest that CB₁ antagonists could be effective in the treatment of both AFLD and NAFLD.

Endocannabinoids and I/R Injury

I/R injury may develop in conditions in which the blood and oxygen supply to a tissue is transiently disrupted and then restored. Hepatic I/R injury is a potentially fatal complication of liver surgery (including liver transplantation). Substances that improve hypoxia tolerance may also protect against I/R injury. CBs induce hypomotility and hypothermia, both of which result in reduced oxygen demand. The metabolic effects of CBs that promote energy storage and reduce energy expenditure (discussed previously) may also reduce oxygen demand. There is evidence that endocannabinoids acting via CB₂ protect against hepatic I/R injury.^{99,100}

In mice, segmental ischemia followed by reperfusion (but not ischemia alone) markedly increased the he-

patic levels of AEA and 2-AG, which correlated with the severity of tissue damage.⁹⁹ I/R-induced tissue damage, including neutrophil infiltration and lipid peroxidation, was attenuated by pretreatment with JWH-133 in wild-type mice but not in CB₂^{-/-} mice, in which the damage was more severe than that in wild-type littermates.⁹⁹ Another potent and selective CB₂ agonist, HU-308, caused similar effects and also attenuated I/R-induced hepatocyte apoptosis and mitigated the tumor necrosis factor- α -induced expression of cell adhesion molecules (intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1) in hepatic sinusoidal endothelial cells.¹⁰⁰ Thus, CB₂ agonists may afford protection at multiple levels against I/R injury; this highlights their therapeutic potential.

CB₁ receptor blockade also protects the liver from I/R injury and superimposed endotoxaemia.¹⁰¹ In one study, rats subjected to lipopolysaccharide plus I/R had an immediate increase in CB₁ expression in perisinusoidal hepatocytes. Rimonabant treatment reduced both tissue necrosis and serum alanine aminotransferase levels in the late phase of reperfusion and attenuated the oxidative injury.¹⁰¹ Further studies with peripherally restricted CB₁ receptor antagonists could reinforce the therapeutic potential of this approach.

Hepatic Encephalopathy and Autoimmune Hepatitis

Hepatic encephalopathy is a neuropsychiatric syndrome that may accompany acute liver failure. The underlying mechanisms are not completely understood, although there is evidence for the pathogenic role of ammonia, alterations in various central neurotransmitter systems, and altered cerebrovascular function. Mice with thioacetamide-induced fulminant liver failure have elevated brain 2-AG levels. The treatment of such mice with 2-AG or the CB₂ agonist HU-308 improved the neurological score and cognitive function, and these effects were blocked by a CB₂ antagonist. The beneficial effects of CB₂ agonists could be mimicked by treatment with the CB₁ antagonist rimonabant.¹⁰² In another study by the same group, thioacetamide treatment or bile duct ligation induced CB₂ expression in the brain and also resulted in the activation of adenosine monophosphate-activated protein kinase. The absence of both effects in CB₂^{-/-} mice indicated the role of CB₂ receptors,¹⁰³ although there is also evidence for the additional involvement of transient receptor potential cation channel V1 receptors.¹⁰⁴

Cannabidiol (CBD) is a nonpsychoactive constituent of marijuana with no significant CB₁ or CB₂

activity. CBD was found to improve cognitive and motor function as well as the neuroinflammation found in hepatic encephalopathy.¹⁰⁵ The cerebral inflammatory response of mice to bile duct ligation was reduced by CBD treatment, and the effect was attributed to indirect activation of hippocampal A2A adenosine receptors. It is possible that a combined treatment with a CB₂ agonist and CBD would offer additive therapeutic benefits to patients with hepatic encephalopathy.

In a murine model of concanavalin A–induced autoimmune hepatitis, THC was found to attenuate the hepatitis on the basis of decreased plasma levels of liver enzymes and inflammatory cytokines and reduced tissue injury.¹⁰⁶ Interestingly, FAAH^{-/-} mice responded with reduced hepatic damage to concanavalin A treatment, and this suggests hepatoprotection by endogenous AEA.¹⁰⁶ In contrast, the results of another study suggest that hepatoprotection may be achieved by blocking CB₁ receptors.¹⁰⁷

Concluding Remarks

The ECS is present in the liver and is involved in the control of various hepatic functions with important therapeutic implications. Increased CB₁ activity contributes to the hemodynamic abnormalities and promotes fibrosis in liver cirrhosis, whereas CB₁ blockade attenuates and delays these changes. Endocannabinoids acting via hepatic CB₁ receptors have emerged as mediators of both diet-induced fatty liver and alcoholic fatty liver, which together account for the majority of cirrhosis cases in Western societies. Additionally, hepatic CB₁ activation contributes to obesity-related insulin and leptin resistance and dyslipidemias. This provides a strong rationale for the therapeutic use of CB₁ antagonists in patients with these conditions. Although neuropsychiatric side effects limit the therapeutic potential of brain-penetrating CB₁ antagonists, the recent emergence of second-generation, peripherally restricted CB₁ antagonists may mitigate this problem. Additionally, nonpsychoactive CB₂ agonists may offer therapeutic benefits by attenuating liver injury and promoting tissue repair in the fibrotic liver.

References

1. Pacher P, Batkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* 2006;58:389-462.
2. Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, et al. Endocannabinoid activation at hepatic CB₁ receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 2005;115:1298-1305.

3. DeMorrow S, Francis H, Gaudio E, Ueno Y, Venter J, Onori P, et al. Anandamide inhibits cholangiocyte hyperplastic proliferation via activation of thioredoxin 1/redox factor 1 and AP-1 activation. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G506-G519.
4. Jourdan T, Djaouti L, Demizieux L, Gresti J, Verges B, Degraze P. CB₁ antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice. *Diabetes* 2010;59:926-934.
5. Teixeira-Clerc F, Julien B, Grenard P, Tran Van Nhieu J, Deveaux V, Li L, et al. CB₁ cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nat Med* 2006;12:671-676.
6. Michalopoulos GK, Bowen WC, Mule K, Luo J. HGF-, EGF-, and dexamethasone-induced gene expression patterns during formation of tissue in hepatic organoid cultures. *Gene Expr* 2003;11:55-75.
7. Xu X, Liu Y, Huang S, Liu G, Xie C, Zhou J, et al. Overexpression of cannabinoid receptors CB₁ and CB₂ correlates with improved prognosis of patients with hepatocellular carcinoma. *Cancer Genet Cytogenet* 2006;171:31-38.
8. Floreani A, Lazzari R, Macchi V, Porzionato A, Variola A, Colavito D, et al. Hepatic expression of endocannabinoid receptors and their novel polymorphisms in primary biliary cirrhosis. *J Gastroenterol* 2009;45:68-76.
9. Julien B, Grenard P, Teixeira-Clerc F, Van Nhieu JT, Li L, Karsak M, et al. Antifibrogenic role of the cannabinoid receptor CB₂ in the liver. *Gastroenterology* 2005;128:742-755.
10. Ashton JC, Rahman RM, Nair SM, Sutherland BA, Glass M, Appleton I. Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB₂ receptor in the brain. *Neurosci Lett* 2007;412:114-117.
11. Begg M, Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L, Mo FM, et al. Evidence for novel cannabinoid receptors. *Pharmacol Ther* 2005;106:133-145.
12. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;258:1946-1949.
13. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 1995;215:89-97.
14. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 1995;50:83-90.
15. Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, et al. A biosynthetic pathway for anandamide. *Proc Natl Acad Sci U S A* 2006;103:13345-13350.
16. Simon GM, Cravatt BF. Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. *J Biol Chem* 2008;283:9341-9349.
17. Fowler CJ. The pharmacology of the cannabinoid system—a question of efficacy and selectivity. *Mol Neurobiol* 2007;36:15-25.
18. McKinney MK, Cravatt BF. Structure and function of fatty acid amide hydrolase. *Annu Rev Biochem* 2005;74:411-432.
19. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A* 2002;99:10819-10824.
20. Galiegue S, Mary S, Marchand J, Dussosoy D, Carriere D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 1995;232:54-61.
21. Quarta C, Bellocchio L, Mancini G, Mazza R, Cervino C, Brulke LJ, et al. CB₁(1) signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. *Cell Metab* 2010;11:273-285.
22. Lee TY, Lee KC, Chang HH. Modulation of the cannabinoid receptors by andrographolide attenuates hepatic apoptosis following bile duct ligation in rats with fibrosis. *Apoptosis* 2010;15:904-914.

23. Jeong WI, Osei-Hyiaman D, Park O, Liu J, Batkai S, Mukhopadhyay P, et al. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. *Cell Metab* 2008;7:227-235.
24. Osei-Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey-White J, Jeong WI, et al. Hepatic CB(1) receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest* 2008;118:3160-3169.
25. Mukhopadhyay B, Liu J, Osei-Hyiaman D, Godlewski G, Mukhopadhyay P, Wang L, et al. Transcriptional regulation of cannabinoid receptor-1 expression in the liver by retinoic acid acting via retinoic acid receptor-gamma. *J Biol Chem* 2010;285:19002-19011.
26. Siegmund SV, Qian T, de Minicis S, Harvey-White J, Kunos G, Vinod KY, et al. The endocannabinoid 2-arachidonoyl glycerol induces death of hepatic stellate cells via mitochondrial reactive oxygen species. *FASEB J* 2007;21:2798-2806.
27. Batkai S, Jarai Z, Wagner JA, Goparaju SK, Varga K, Liu J, et al. Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nat Med* 2001;7:827-832.
28. Biecker E, Sagesser H, Reichen J. Vasodilator mRNA levels are increased in the livers of portal hypertensive NO-synthase 3-deficient mice. *Eur J Clin Invest* 2004;34:283-289.
29. Domenicali M, Ros J, Fernandez-Varo G, Cejudo-Martin P, Crespo M, Morales-Ruiz M, et al. Increased anandamide induced relaxation in mesenteric arteries of cirrhotic rats: role of cannabinoid and vanilloid receptors. *Gut* 2005;54:522-527.
30. Moezi L, Gaskari SA, Liu H, Baik SK, Dehpour AR, Lee SS. Anandamide mediates hyperdynamic circulation in cirrhotic rats via CB(1) and VR(1) receptors. *Br J Pharmacol* 2006;149:898-908.
31. Mendez-Sanchez N, Zamora-Valdes D, Pichardo-Bahena R, Barredo-Prieto B, Ponciano-Rodriguez G, Bermejo-Martinez L, et al. Endocannabinoid receptor CB2 in nonalcoholic fatty liver disease. *Liver Int* 2007;27:215-219.
32. Buckley NE, Hansson S, Harta G, Mezey E. Expression of the CB1 and CB2 receptor messenger RNAs during embryonic development in the rat. *Neuroscience* 1998;82:1131-1149.
33. Teixeira-Clerc F, Belor MP, Manin S, Deveaux V, Cadoudal T, Choibert MN, et al. Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. *HEPATOLOGY* 2010;52:1046-1059.
34. Zaugg HE, Kyncl J. New antihypertensive cannabinoids. *J Med Chem* 1983;26:214-217.
35. Lake KD, Compton DR, Varga K, Martin BR, Kunos G. Cannabinoid-induced hypotension and bradycardia in rats mediated by CB1-like cannabinoid receptors. *J Pharmacol Exp Ther* 1997;281:1030-1037.
36. Wagner JA, Varga K, Ellis EF, Rzigalinski BA, Martin BR, Kunos G. Activation of peripheral CB1 cannabinoid receptors in haemorrhagic shock. *Nature* 1997;390:518-521.
37. Wagner JA, Hu K, Bauersachs J, Karcher J, Wiesler M, Goparaju SK, et al. Endogenous cannabinoids mediate hypotension after experimental myocardial infarction. *J Am Coll Cardiol* 2001;38:2048-2054.
38. Varga K, Wagner JA, Bridgen DT, Kunos G. Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. *FASEB J* 1998;12:1035-1044.
39. Wang Y, Liu Y, Sarker KP, Nakashima M, Serizawa T, Kishida A, et al. Polymyxin B binds to anandamide and inhibits its cytotoxic effect. *FEBS Lett* 2000;470:151-155.
40. Kohro S, Imaizumi H, Yamakage M, Masuda Y, Namiki A, Asai Y, et al. Anandamide absorption by direct hemo-perfusion with polymyxin B-immobilized fiber improves the prognosis and organ failure assessment score in patients with sepsis. *J Anesth* 2006;20:11-16.
41. Liu J, Batkai S, Pacher P, Harvey-White J, Wagner JA, Cravatt BF, et al. Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-kappaB independently of platelet-activating factor. *J Biol Chem* 2003;278:45034-45039.
42. Ros J, Claria J, To-Figueras J, Planaguma A, Cejudo-Martin P, Fernandez-Varo G, et al. Endogenous cannabinoids: a new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat. *Gastroenterology* 2002;122:85-93.
43. Yang YY, Lin HC, Huang YT, Lee TY, Hou MC, Wang YW, et al. Role of Ca²⁺-dependent potassium channels in in vitro anandamide-mediated mesenteric vasorelaxation in rats with biliary cirrhosis. *Liver Int* 2007;27:1045-1055.
44. Orliac ML, Peroni R, Celuch SM, Adler-Graschinsky E. Potentiation of anandamide effects in mesenteric beds isolated from endotoxemic rats. *J Pharmacol Exp Ther* 2003;304:179-184.
45. Caraceni P, Viola A, Piscitelli F, Giannone F, Berzigotti A, Cescon M, et al. Circulating and hepatic endocannabinoids and endocannabinoid-related molecules in patients with cirrhosis. *Liver Int* 2010;30:816-825.
46. Fernandez-Rodriguez CM, Romero J, Petros TJ, Bradshaw H, Gasalla JM, Gutierrez ML, et al. Circulating endogenous cannabinoid anandamide and portal, systemic and renal hemodynamics in cirrhosis. *Liver Int* 2004;24:477-483.
47. Bernardi M. Renal sodium retention in preascitic cirrhosis: expanding knowledge, enduring uncertainties. *HEPATOLOGY* 2002;35:1544-1547.
48. Domenicali M, Caraceni P, Giannone F, Pertosa AM, Principe A, Zambuni A, et al. Cannabinoid type 1 receptor antagonism delays ascites formation in rats with cirrhosis. *Gastroenterology* 2009;137:341-349.
49. Garcia N Jr, Jarai Z, Mirshahi F, Kunos G, Sanyal AJ. Systemic and portal hemodynamic effects of anandamide. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G14-G20.
50. Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, et al. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci U S A* 1999;96:14136-14141.
51. Gaskari SA, Honar H, Lee SS. Therapy insight: cirrhotic cardiomyopathy. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:329-337.
52. Gaskari SA, Liu H, Moezi L, Li Y, Baik SK, Lee SS. Role of endocannabinoids in the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats. *Br J Pharmacol* 2005;146:315-323.
53. Batkai S, Mukhopadhyay P, Harvey-White J, Kechrid R, Pacher P, Kunos G. Endocannabinoids acting at CB1 receptors mediate the cardiac contractile dysfunction in vivo in cirrhotic rats. *Am J Physiol Heart Circ Physiol* 2007;293:H1689-H1695.
54. Gebremedhin D, Lange AR, Campbell WB, Hillard CJ, Harder DR. Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca²⁺ channel current. *Am J Physiol* 1999;276:H2085-H2093.
55. Howlett AC. Cannabinoid receptor signaling. *Handb Exp Pharmacol* 2005;168:53-79.
56. Lotersztajn S, Teixeira-Clerc F, Julien B, Deveaux V, Ichigotani Y, Manin S, et al. CB2 receptors as new therapeutic targets for liver diseases. *Br J Pharmacol* 2008;153:286-289.
57. Munoz-Luque J, Ros J, Fernandez-Varo G, Tugues S, Morales-Ruiz M, Alvarez CE, et al. Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *J Pharmacol Exp Ther* 2008;324:475-483.
58. Hezode C, Roudot-Thoraval F, Nguyen S, Grenard P, Julien B, Zafrani ES, et al. Daily cannabis smoking as a risk factor for progression of fibrosis in chronic hepatitis C. *HEPATOLOGY* 2005;42:63-71.
59. Yang Q, Liu HY, Zhang YW, Wu WJ, Tang WX. Anandamide induces cell death through lipid rafts in hepatic stellate cells. *J Gastroenterol Hepatol* 2010;25:991-1001.
60. Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2001;410:822-825.
61. Rosenson RS. Role of the endocannabinoid system in abdominal obesity and the implications for cardiovascular risk. *Cardiology* 2009;114:212-225.
62. Tam J, Vemuri VK, Liu J, Batkai S, Mukhopadhyay B, Godlewski G, et al. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest* 2010;120:2953-2966.
63. Ravinet Trillou C, Delgorge C, Menet C, Arnone M, Soubrie P. CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int J Obes Relat Metab Disord* 2004;28:640-648.

64. Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, et al. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1 β coactivation of SREBP. *Cell* 2005;120:261-273.
65. Biddinger SB, Almind K, Miyazaki M, Kokkotou E, Ntambi JM, Kahn CR. Effects of diet and genetic background on sterol regulatory element-binding protein-1c, stearoyl-CoA desaturase 1, and the development of the metabolic syndrome. *Diabetes* 2005;54:1314-1323.
66. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005;115:1343-1351.
67. Westerbacka J, Kotronen A, Fielding BA, Wahren J, Hodson L, Perttinen J, et al. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. *Gastroenterology*; doi:10.1053/j.gastro.2010.06.064.
68. Ruby MA, Nomura DK, Hudak CS, Mangravite LM, Chiu S, Casida JE, et al. Overactive endocannabinoid signaling impairs apolipoprotein E-mediated clearance of triglyceride-rich lipoproteins. *Proc Natl Acad Sci U S A* 2008;105:14561-14566.
69. Son MH, Kim HD, Chae YN, Kim MK, Shin CY, Ahn GJ, et al. Peripherally acting CB1-receptor antagonist: the relative importance of central and peripheral CB1 receptors in adiposity control. *Int J Obes (Lond)* 2010;34:547-556.
70. Hezode C, Zafrani ES, Roudot-Thoraval F, Costentin C, Hessami A, Bouvier-Alias M, et al. Daily cannabis use: a novel risk factor of steatosis severity in patients with chronic hepatitis C. *Gastroenterology* 2008;134:432-439.
71. Cota D, Marsicano G, Tschöp M, Grubler Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 2003;112:423-431.
72. Flamment M, Gueguen N, Wetterwald C, Simard G, Maltheri Y, Ducluzeau PH. Effects of the cannabinoid CB1 antagonist, rimonabant, on hepatic mitochondrial function in rats fed a high fat diet. *Am J Physiol Endocrinol Metab* 2009;297:E1162-E1170.
73. Despres JP, Golay A, Sjostrom L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* 2005;353:2121-2134.
74. Migrenne S, Lacombe A, Lefevre AL, Pruniaux MP, Guillot E, Galzin AM, et al. Adiponectin is required to mediate rimonabant-induced improvement of insulin sensitivity but not body weight loss in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R929-R935.
75. Watanabe T, Kubota N, Ohsugi M, Kubota T, Takamoto I, Iwabu M, et al. Rimonabant ameliorates insulin resistance via both adiponectin-dependent and adiponectin-independent pathways. *J Biol Chem* 2009;284:1803-1812.
76. Herling AW, Gossel M, Haschke G, Stengelin S, Kuhlmann J, Müller G, et al. CB1 receptor antagonist AVE1625 affects primarily metabolic parameters independently of reduced food intake in Wistar rats. *Am J Physiol Endocrinol Metab* 2007;293:E826-E832.
77. Kunz I, Meier MK, Bourson A, Fisseha M, Schilling W. Effects of rimonabant, a cannabinoid CB1 receptor ligand, on energy expenditure in lean rats. *Int J Obes (Lond)* 2008;32:863-870.
78. Liu YL, Connolly IP, Wilson CA, Stock MJ. Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep(ob)/Lep(ob) mice. *Int J Obes (Lond)* 2005;29:183-187.
79. Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, et al. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 2003;284:R345-R353.
80. Gary-Bobo M, Elachouri G, Gallas JF, Janiak P, Marini P, Ravinet-Trillou C, et al. Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker *fa/fa* rats. *HEPATOLOGY* 2007;46:122-129.
81. DeLeve LD, Wang X, Kanel GC, Atkinson RD, McCuskey RS. Prevention of hepatic fibrosis in a murine model of metabolic syndrome with nonalcoholic steatohepatitis. *Am J Pathol* 2008;173:993-1001.
82. Poirier B, Bidouard JP, Cadrouvele C, Marniquet X, Staels B, O'Connor SE, et al. The anti-obesity effect of rimonabant is associated with an improved serum lipid profile. *Diabetes Obes Metab* 2005;7:65-72.
83. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L. Overfeeding rapidly induces leptin and insulin resistance. *Diabetes* 2001;50:2786-2791.
84. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 2000;105:1827-1832.
85. Hollister LE, Reaven GM. Delta-9-tetrahydrocannabinol and glucose tolerance. *Clin Pharmacol Ther* 1974;16:297-302.
86. Bermudez-Siva FJ, Serrano A, Diaz-Molina FJ, Sanchez Vera I, Juan-Pico P, Nadal A, et al. Activation of cannabinoid CB1 receptors induces glucose intolerance in rats. *Eur J Pharmacol* 2006;531:282-284.
87. Uno K, Katagiri H, Yamada T, Ishigaki Y, Ogihara T, Imai J, et al. Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity. *Science* 2006;312:1656-1659.
88. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005;436:356-362.
89. Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* 2006;27:73-100.
90. Bermudez-Silva FJ, Sanchez-Vera I, Suarez J, Serrano A, Fuentes E, Juan-Pico P, et al. Role of cannabinoid CB2 receptors in glucose homeostasis in rats. *Eur J Pharmacol* 2007;565:207-211.
91. Doyon C, Denis RG, Baraboi ED, Samson P, Lalonde J, Deshaies Y, et al. Effects of rimonabant (SR141716) on fasting-induced hypothalamic-pituitary-adrenal axis and neuronal activation in lean and obese Zucker rats. *Diabetes* 2006;55:3403-3410.
92. Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet* 2006;368:1660-1672.
93. Gonsiorek W, Lunn C, Fan X, Narula S, Lundell D, Hipkin RW. Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Mol Pharmacol* 2000;57:1045-1050.
94. Deveaux V, Cadoudal T, Ichigotani Y, Teixeira-Clerc F, Louvet A, Manin S, et al. Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. *PLoS One* 2009;4:e5844.
95. Lieber CS, Schmid R. The effect of ethanol on fatty acid metabolism; stimulation of hepatic fatty acid synthesis in vitro. *J Clin Invest* 1961;40:394-399.
96. You M, Fischer M, Deeg MA, Crabb DW. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory element-binding protein (SREBP). *J Biol Chem* 2002;277:29342-29347.
97. You M, Matsumoto M, Pacold CM, Cho WK, Crabb DW. The role of AMP-activated protein kinase in the action of ethanol in the liver. *Gastroenterology* 2004;127:1798-1808.
98. Basavarajappa BS, Saito M, Cooper TB, Hungund BL. Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specific neuromodulators in cerebellar granule neurons. *Biochim Biophys Acta* 2000;1535:78-86.
99. Batkai S, Osei-Hyiaman D, Pan H, El-Assal O, Rajesh M, Mukhopadhyay P, et al. Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. *FASEB J* 2007;21:1788-1800.
100. Rajesh M, Pan H, Mukhopadhyay P, Batkai S, Osei-Hyiaman D, Hasko G, et al. Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. *J Leukoc Biol* 2007;82:1382-1389.
101. Caraceni P, Pertosa AM, Giannone F, Domenicali M, Grattagliano I, Principe A, et al. Antagonism of the cannabinoid CB-1 receptor protects rat liver against ischaemia-reperfusion injury complicated by endotoxaemia. *Gut* 2009;58:1135-1143.

102. Avraham Y, Israeli E, Gabbay E, Okun A, Zolotarev O, Silberman I, et al. Endocannabinoids affect neurological and cognitive function in thioacetamide-induced hepatic encephalopathy in mice. *Neurobiol Dis* 2006;21:237-245.
103. Dagon Y, Avraham Y, Ilan Y, Mechoulam R, Berry EM. Cannabinoids ameliorate cerebral dysfunction following liver failure via AMP-activated protein kinase. *FASEB J* 2007;21:2431-2441.
104. Avraham Y, Zolotarev O, Grigoriadis NC, Poutahidis T, Magen I, Vorobiev L, et al. Cannabinoids and capsaicin improve liver function following thioacetamide-induced acute injury in mice. *Am J Gastroenterol* 2008;103:3047-3056.
105. Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM. Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation. *J Hepatol* 2009;51:528-534.
106. Hegde VL, Hegde S, Cravatt BF, Hofseth LJ, Nagarkatti M, Nagarkatti PS. Attenuation of experimental autoimmune hepatitis by exogenous and endogenous cannabinoids: involvement of regulatory T cells. *Mol Pharmacol* 2008;74:20-33.
107. Kojima M, Kato N, Hirano D, Ochi T, Nii A, Shinjo K, et al. Selective CB1 cannabinoid receptor antagonist, SR141716A, attenuates liver injury induced by concanavalin A. *Hepatol Res* 2009;39:408-414.