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Cannabinoid Receptors in Metabolic Regulation and Diabetes

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There is an urgent need for developing effective drugs to combat the obesity and Type 2 diabetes mellitus epidemics. The endocannabinoid system plays a major role in energy homeostasis. It comprises the cannabinoid receptors 1 and 2 (CB_1 and CB_2), endogenous ligands called endocannabinoids and their metabolizing enzymes. Because the CB_1 receptor is overactivated in metabolic alterations, pharmacological blockade of the CB_1 receptor arose as a promising candidate to treat obesity. However, because of the wide distribution of CB_1 receptors in the central nervous system, their negative central effects halted further therapeutic use. Although the CB_2 receptor is mostly peripherally expressed, its role in metabolic homeostasis remains unclear. This review discusses the potential of CB_1 and CB_2 receptors at the peripheral level to be therapeutic targets in metabolic diseases. We focus on the impact of pharmacological intervention and/or silencing on peripheral cannabinoid receptors in organs/tissues relevant for energy homeostasis. Moreover, we provide a perspective on novel therapeutic strategies modulating these receptors. Targeting CB_1 with peripherally restricted antagonists, neutral antagonists, inverse agonists, or monoclonal antibodies could represent successful strategies. CB_2 agonism has shown promising results at preclinical level. Beyond classic antagonism and agonism targeting orthosteric sites, the recently described crystal structures of CB_1 and CB_2 open new possibilities for therapeutic interventions with negative and positive allosteric modulators. The challenge of simultaneously targeting CB_1 and CB_2 might be possible by developing dualsteric ligands. The future will tell whether these promising strategies result in a renaissance of the cannabinoid receptors as therapeutic targets in metabolic diseases.

cannabinoid receptors; diabetes; metabolic regulation; obesity; therapeutic targets

From the Endocannabinoid System to the Endocannabinoidome

The cannabinoid (CB) receptors are described as G protein-coupled receptors (GPCRs), whose ligands include the phytocannabinoid Δ(9)-tetrahydrocannabinol (THC), endogenous ligands derived from arachidonid acid called endocannabinoids (ECs), and multiple synthetic compounds (1).

ECs are lipid mediators that are able to counteract satiety signals in the hypothalamus and the gastrointestinal tract (2). Exogenous cannabinoids and ECs signal through the CB receptors, promoting overfeeding and lipid biosynthesis and storage (2). The CB receptors were identified and named in order of discovery: cannabinoid receptor 1 (CB_1 receptor) and cannabinoid receptor 2 (CB_2 receptor). One decade after their discovery, these receptors were cloned, and the ECs anandamide (N-arachidonoyl-ethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) were identified. This finding led to coining of the term endocannabinoid system (3). To sum up, the EC system comprises the receptors CB_1 and CB_2, the ECs AEA and 2-AG, and their five metabolizing enzymes:

N-acylphosphatidylethanolamine-phospholipase D (NAPE-PLD), diacylglycerol lipase (DAGL)-α and β, fatty acid amide hydrolase (FAAH), and monoacylglycerol lipase (MAGL) (FIGURE 1). Although other molecules have been proposed as part of the EC system, their physiological role is under discussion. Nowadays, the concept of the EC system has been expanded to the endocannabinoidome, comprising multiple lipid mediators, their inactivating or synthesizing enzymes, and their molecular targets (such as nuclear receptors, ligand-activated ion channels and orphan GPCRs) (4). To date only CB_1 and CB_2 are consider bona fide receptors (5).

Both preclinical and clinical evidence have demonstrated that impaired energy balance in obesity and hyperglycemia leads to an overactivation of the EC system due to an increase in the expression of cannabinoid receptors, ECs, as well as the enzymes regulating their synthesis and degradation (6–8). Besides circulating levels of ECs, hypothalamic ECs are increased in obese animals with leptin deficiency (*ob/ob* mice) or impaired leptin signaling (such as *db/db* mice and Zucker (*fa/fa*) rats) (9).

CB_1 receptor acts as a master regulator of whole-body and cell energy metabolism controlling food intake, lipogenesis, glucose uptake, insulin secretion, and gluconeogenesis (10). Although activation of CB_2 receptor has been classically anti-inflammatory and antioxidant properties, increasing evidence suggests a potential role for CB_2 receptor expression and

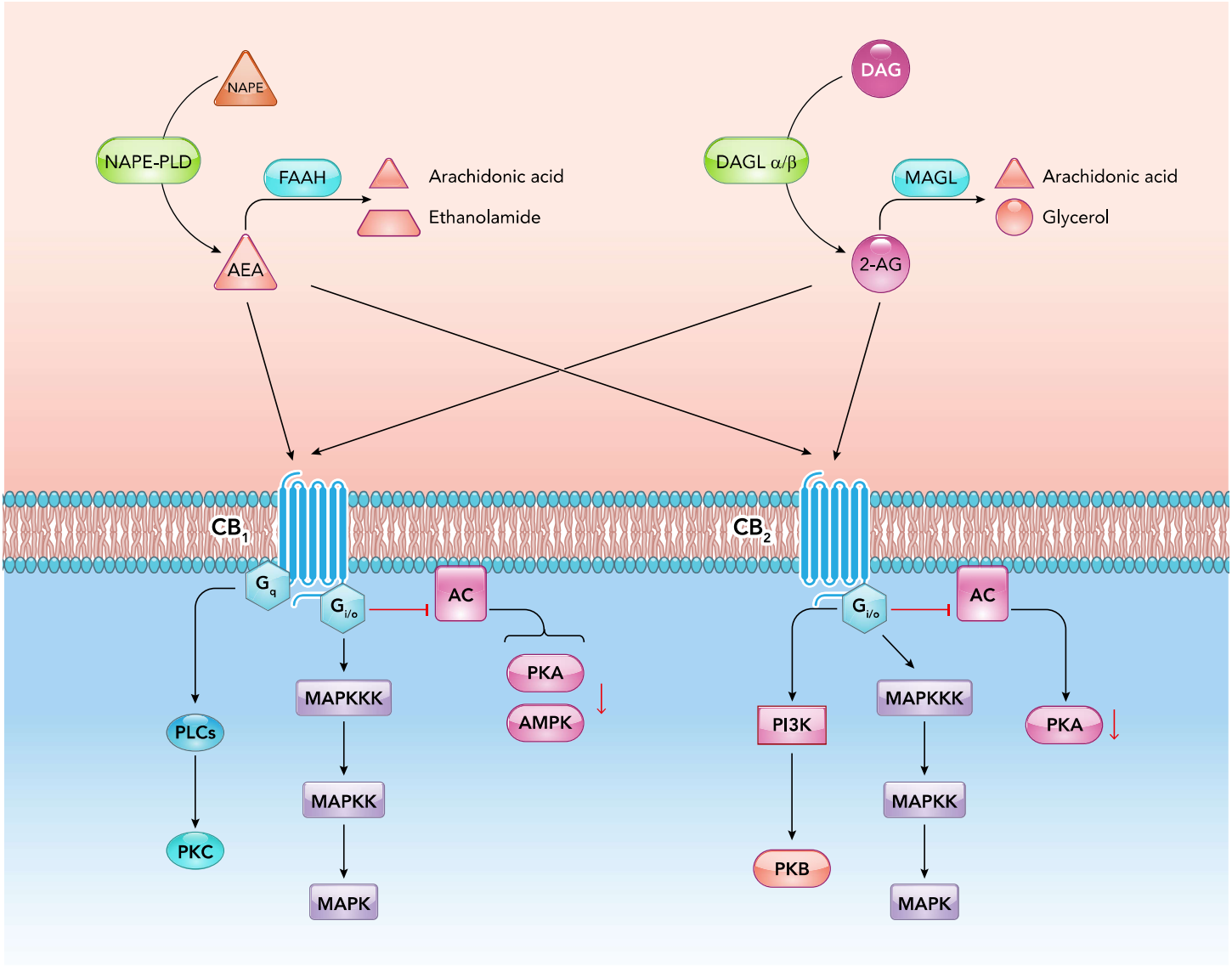

FIGURE 1. **Overview of the endocannabinoid system**
The endocannabinoid system includes endogenous cannabinoids (2-AG, AEA), their synthesizing (NAPE-PLD, DAGL) and metabolizing (FAAH, MAGL) enzymes, the receptors CB1 and CB2, as well as some of the receptor-modulated major signaling pathways. 2-AG, 2-arachidonoylglycerol; AC, Adenylat cyclase; AEA, anandamide; AMPK, adenosine monophosphate-activated protein kinase; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; NAPE-PLD, *N*-acylphosphatidyl-ethanolamine-phospholipase D; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C. Created with BioRender.com.

activation in metabolic regulation at central and peripheral level, as we will later discuss in more detail.

The search for pharmacological interventions to treat obesity and type 2 diabetes mellitus (T2DM) epidemics has put the EC system in the spotlight as a master regulator of food intake, energy expenditure, and fat mass expansion. In the last 30 years, drug development and pharmacological research on cannabinoid receptors has been mostly focused on ligands binding the orthosteric site, such as agonists, neutral antagonists, and inverse agonists. Compounds acting as orthosteric ligands compete for the receptor and principally cannot bind simultaneously. Nevertheless, the relative novel crystal structure of the CB_1 and CB_2 receptor has demonstrated that the CB_1 receptor allows binding of nonorthosteric ligands, the so-called allosteric modulators (11, 12). Allosteric modulators do not compete with endogenous ligands for binding to the receptor, and they rather modulate the action of the ligand whether by increasing it as positive allosteric modulators (PAMs) or decreasing it as negative allosteric modulators (NAMs). Both exogenous and endogenous allosteric modulators open new possibilities for drug development targeting the CB_1 and CB_2 receptors. Tackling obesity and metabolic regulation through the central and peripheral effects of CB receptors and ECs on food intake deserves a full review in itself. Therefore, in this review we will focus on peripheral effects of CB_1 and CB_2 receptors and drugs targeting them, devoid of central effects, which could represent novel therapeutic approaches in metabolic disorders.

CB_1 Receptors in Diabetes and Its Complications

Within the EC system, CB_1 receptors play a major role in energy homeostasis. Although CB_1 receptors are mainly expressed in the brain, they are also found in metabolically active tissues/organs key for non-central neurologic metabolic control, such as the pancreas, liver, adipose tissue (AT), and skeletal muscle.

Overall EC system activity, understood as increased signal transduction, is enhanced in obesity and the metabolic syndrome. A cause of this overactivation are the enhanced levels of circulating ECs (10). This overactivation leads, in turn, to increased lipogenesis and energy storage in the aforementioned tissues and organs. Besides its activity, CB_1 receptor expression is also enhanced in obesity in adipose tissue (13), liver (14), and skeletal muscle (15). Obese and T2DM patients also display enhanced tisular levels of CB_1 receptor (16). Food intake can have an impact on EC system activation. Thus, EC levels change during food ingestion through the impact of regulators of food intake, such as leptin and ghrelin. During feeding, leptin levels are enhanced, inhibiting the formation of precursors of AEA and 2-AG in the hypothalamus (9). During fasting, the levels of leptin decrease, while ghrelin levels increase, leading to enhanced ECs (17). In turn, pharmacological blockade of the CB_1 receptor with rimonabant reduces circulating levels of ghrelin (18). In genetic animal models of obesity where leptin is altered, hypothalamic EC levels are increased, while CB_1 receptor is downregulated (9, 19). CB_1 receptor genetic deficiency resulted in lean, diet-induced obesity-resistant animals with increased leptin sensitivity and less circulating leptin levels (20). Analogously, pharmacological blockade of the CB_1 receptor with rimonabant resulted in food intake and body weight reduction (2, 9, 20). This effect was due to blockade of CB_1 central orexigenic effects and lipogenesis at peripheral levels (2). Peripheral CB_1 receptor blockade in animals under a high-fat diet (HFD) has also proven useful in restoring leptin sensitivity (21).

Besides genetic and pharmacological modulation, environmental factors, such as polyunsaturated fatty acid content in the food (22) or the anticipated pleasure of eating, can impact central and peripheral EC levels (23). In that line, lower circulating AEA levels have been reported in patients with anorexia nervosa (24).

In light of all this evidence, CB_1 receptor antagonism or inverse agonism came across as a potentially beneficial therapeutic strategy in obesity and T2DM. Phase III clinical trials with CB_1 receptor antagonists resulted in weight loss, reduction of features of the metabolic syndrome, and cardiovascular risk factors (4). In 2006, the CB_1 receptor selective antagonist/inverse agonist SR141716A (rimonabant, Acomplia) was launched to fight obesity and metabolic syndrome. Although initially proven safe in several clinical trials, depression and suicidal ideation led to withdrawal of rimonabant and other antagonists/inverse agonists under clinical development at that time (taranabant, otenabant, and ibinabant) (25–27). As mentioned earlier, CB_1 receptor is most abundant in the brain compared with other peripheral tissues controling energy homeostasis such as liver, adipose tissue, skeletal muscle, and the endocrine pancreas. The rimonabant experience underpinned the problem of central effects of CB receptors. In 2005, it was discovered that the CB_1 receptor contains allosteric binding sites, which can be recognized by small molecules, or allosteric modulators (28), opening new possibilities for CB_1 receptor regulation. Since peripheral CB_1 receptor modulation offers therapeutic opportunities in metabolic regulation, we summarize the main actions of CB_1 receptor in noncentral nervous system (CNS) key tissues/organs related to metabolic homeostasis (FIGURE 2).

CB_1 Receptors in Adipose Tissue

In 2003, two independent groups described the presence of functional CB_1 receptors in mature adipocytes and demonstrated how CB_1 receptor activation led to

increased lipogenesis in the AT (2, 13). In vitro CB_1 receptor activation in white adipocytes led to enhanced glucose uptake, increased activity of the lipogenic enzyme lipoprotein lipase, and reduced lipogenesis, resulting in fatty acid synthesis and triglyceride accumulation (29, 30). Furthermore, cyclic adenosine monophosphate (cAMP) release is inhibited, and adenosine monophosphate-activated protein kinase (AMPK) activation is reduced, which both lead to reduced mitochondrial biogenesis. Decreased mitochondrial biogenesis, in turn, reduces lipolysis and fatty acid oxidation and makes the white versus the "beige" adipocyte phenotype prevail. CB_1 receptor agonism increases adipogenesis due to increased activation of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ). The EC AEA acts as a PPARγ agonist, amplifying the EC system-induced adipogenesis (31). CB_1 receptor activation also results in the inhibition of the anti-inflammatory and insulin sensitizing adipokine adiponectin (29). These deleterious effects of CB_1 activation on adipocytes have been shown to be prevented with several strategies. The inverse CB_1 receptor agonist JD5037 prevented enhanced glucose uptake and leptin resistance in mice exposed to diet-induced obesity (DIO) (21). Accordingly, CB_1 receptor knockout (KO) mice display reduced fat mass and higher energy expenditure than corresponding WT littermates, independently of food intake (32). Both CB_1 receptor genetic deletion or pharmacological blockade with SR141716 (rimonabant) rescued mitochondrial biogenesis under HFD. CB_1 receptor blockade with the neutral antagonist AM6545 increased energy expenditure due to fatty acid oxidation in AT (33). The novel peripheral CB_1 receptor antagonist AJ5012 has been proposed to improve insulin resistance in obese mice by reducing AT inflammation (34).

CB_1 receptor expression has also been reported in supraclavicular brown adipose tissue (BAT) in humans. Obese individuals display reduced CB_1 receptor expression in BAT, which could reflect reduced BAT activity (35). The novel peripheral restricted CB_1 receptor antagonist BPR0912 has been proposed to activate BAT-mediated thermogenesis in rodents (36). In iPS-derived human brown adipocytes, pharmacological blockade of the CB_1 receptor with rimonabant increased glucose uptake, whereas CB_2 receptor agonists and antagonists exerted no effect. Therefore, CB_1 receptor antagonism has arisen as a potential tool to modulate BAT activitiy peripherally (35).

CB_1 Receptors in Liver

CB_1 receptor expression in hepatocytes was first described by Osei-Hiyaman et al. (37). In this study, CB_1 receptor activation by the endogenous EC anandamide increased de novo lipogenesis through the induction of the lipogenic transcription factor sterol regulatory element-binding protein 1c and its target enzymes acetyl-CoA carboxylase1 and fatty acid

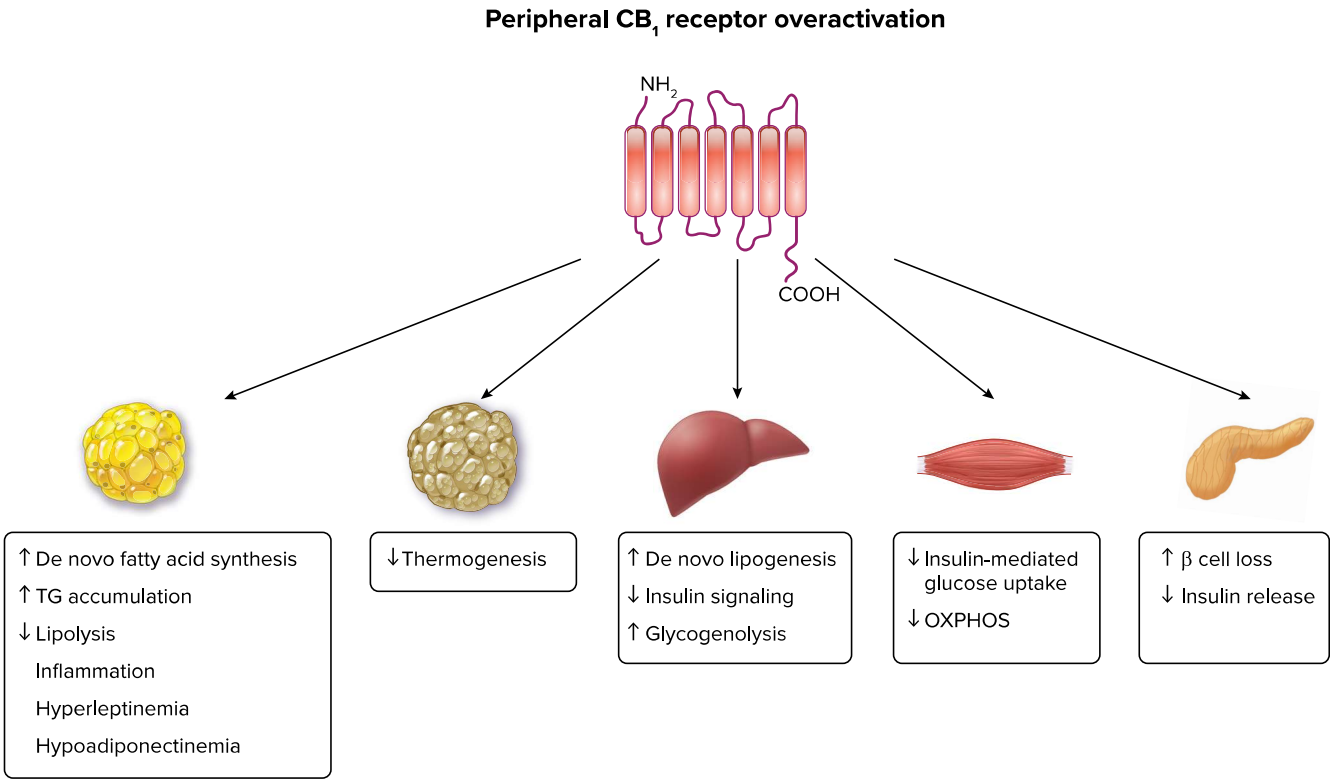

FIGURE 2. **Diagram depicting the main effects of cannabinoid 1 (CB1) receptor overactivation at peripheral level in white adipose tissue, brown adipose tissue, skeletal muscle, liver, and endocrine pancreas**

synthase in DIO mice leading to steatosis. HFD-induced CB_1 receptor activation in the liver triggers de novo lipogenesis and hepatic insulin resistance (38, 39). DIO-induced CB_1 receptor activation by ECs in mice resulted in endoplasmic reticulum stress-induced synthesis of specific ceramide subspecies in the liver, resulting in hepatic insulin resistance. The peripherally restricted CB_1 receptor inverse agonist JD5037 was able to prevent both hepatic insulin resistance and ceramide synthesis (40). CB_1 receptor expression is upregulated in nonalcoholic fatty liver disease (NAFLD) (41). Moreover, an increased EC system activity was observed in an HFD-induced NAFLD mouse model (42). Thus, pharmacological blockade of the CB_1 receptor could be a potential therapeutic approach in NAFLD. Via the CB_1 receptor, ECs also promote liver fibrosis (39, 43), characteristic for nonalcoholic steatohepatitis (NASH). These findings are in accordance with results in CB_1 receptor knockout mice, where the observed anti-inflammatory effects are similar to treatment with CB_1 receptor antagonists due to a reduction of proinflammatory cytokine production (44). The fact that pharmacological blockade of the CB_1 receptors in NAFLD attenuates hepatic oxidative stress parameters substantiates the therapeutic potential of CB_1 receptor blockade to prevent NAFLD pathogenesis (45).

CB_1 Receptors in Skeletal Muscle

The expression of CB_1 receptor and some of the enzymatic components of the EC system, such as DAGLα/β and MAGL, have been reported in human and rodent skeletal muscle (46, 47). Regarding their localization, CB_1 receptors have been reported in the mitochondria of striated muscles in similar levels as in rat brain (48). In mice, CB_1 receptor in skeletal muscle has been proposed to be mostly localized in mitochondria, where it might have an impact on mitochondrial oxidative activity (49).

In obesity, the chronic activation of CB_1 receptor in muscle is likely contributing to alter body composition, promoting fat formation and reducing insulin sensitivity and physical endurance. CB_1 receptor activation blocks insulin-mediated glucose uptake in skeletal muscle (14, 50). Our group demonstrated that both AEA and the conditioned medium from human adipocytes that contained AEA, among other factors, impaired insulin signaling in human skeletal muscle cells. AEA augmented insulin receptor substrate (IRS-1) (Ser-307) phosphorylation probably via the extracellular-signal-regulated kinases (ERK)1/2 and p38 activation, resulting in impaired glucose uptake. AEA-induced insulin resistance was prevented by the CB_1 receptor antagonist rimonabant (47). CB_1 receptors might become overactivated in the skeletal muscle during obesity due to increased levels of ECs (51). In line, specific deletion of CB_1 receptor in skeletal muscle of *ob/ob* mice prevented diet-induced insulin resistance and increased energy expenditure (50). Skeletal muscle-specific KO mice were protected not only against diet but, importantly, against age-induced insulin resistance by upregulating insulin signaling, thus, increasing myogenesis and the production of the beneficial myokine interleukin 6 (52). Furthermore, González-Mariscal et al. (52), suggest that skeletal muscle-specific KO mice display improved physical endurance, likely due to an increase of type I slow-twitch (oxidative) fibers versus type II fibers (glycolytic) fibers.

CB_1 Receptors in Pancreas

It has been shown that β-cells express CB_1 receptor, which often appears membrane-bound (53). Some authors have claimed that CB_1 receptor immunoreactivity was not observed in α cells of human islets (53, 54). However, another group demonstrated CB_1 receptor expression in glucagon-expressing α-cells in mouse and rat pancreatic islets (55). Tharp et al. (56) reported CB_1 receptor is present predominantly in δ-cells in pancreatic islets of mice, rats, and humans, independent of obesity or diabetes. Besides the discussion about the specific cell type expressing CB_1 receptors within the pancreas (57), there is still an ongoing debate about the cell-specific presence of the EC-synthesizing (NAPE-PLD and DAGL) or metabolizing enzymes (FAAH and MAGL) (57–59). Biosynthesizing enzymes are mostly localized in α-cells, whereas degrading enzymes appear to be mostly localized in insulin-secreting β-cells (55). We will now focus on the main effects of CB_1 receptor pharmacological modulation at the β-cell level, where more data are available. CB_1 receptor activation leads to impaired insulin signaling and release (53, 58). In Zucker diabetic fatty rats, the CB_1 receptor antagonist idipinabant prevented β-cell loss (60). Zucker diabetic fatty rats treated with the inverse agonist JD5037 were euglycemic, although they displayed higher plasma insulin and C-peptide levels. Thus, blocking the CB_1 receptor in infiltrating macrophages prevented the nucleotide-binding domain-like receptor protein 3-apoptosis-associated speck-like protein containing CARD inflammasome activation, leading to overproduction of interleukin 1β and β-cell loss (61). Another more complex mechanism proposed for CB_1 receptor-mediated impairment of insulin signaling and β-cell loss is that the CB_1 receptor can form a heterotrimeric complex with the insulin receptor (62).

CB_2 Receptors in Diabetes

Contrarily to CB_1, CB_2 receptors are mainly peripherally distributed, most specifically in the immune system (63), where they mediate immunomodulatory functions (64). Compared to CB_1, CB_2 receptors have been classicaly suggested to play a minor role in metabolic homeostasis (63). In line, there is still controversy on the real impact of CB_2 receptors in metabolic regulation. CB_2 receptor

stimulation promotes antiobesity effects by reducing food intake and weight gain (65), whereas an overexpression of CB_2 receptors in the brain induces hyperglycemia in mice (66). In a seminal study, CB_2 receptor knockout mice ($CB_2R^{-/-}$) showed nonsignificant morphological differences (64). Another study showed that $CB_2R^{-/-}$ mice revealed increased food intake and obesity with age (67). Finally, a more recent study compared the effect of DIO on CB_1 and CB_2 receptor double-KO mice to the single-KO models. $CB_2R^{-/-}$ displayed signs of impaired glucose clearance, while insulin sensitivity was improved in CB_1 and CB_2 receptor double-KO mice when tested by the glucose tolerance test, suggesting a compensatory interplay between both receptors in DIO (68). Here, we summarize the main noncentral actions of CB_2 receptor on the main tissues/organs related to metabolic homeostasis (Table 1).

CB_2 Receptors in Adipose Tissue

The CB_2 receptor, which is present in AT (79), is mostly linked to the regulation of inflammation (80) or energy homeostasis (69). Verty et al. (69) demonstrated that the treatment with the CB_2 receptor agonist JWH-015 improved obesity-associated inflammation and body weight in DIO mice. In detail, an increased expression of the anti-inflammatory cytokine interleukin-10 and reduced expression of the proinflammatory cytokine tumor necrosis factor α in white AT were found after injection of JWH-015. In accordance, treatment with the CB_2 receptor inverse agonist SR144528 showed anti-inflammatory effects in human adipocytes (73), whereas the inverse agonist JTE-907 led to an upregulation of inflammatory genes in human adipocytes (72). Moreover, it was observed that JWH-015 had no effect on uncoupling protein 1 (UCP1) expression (69). While stimulation of the CB_2 receptor with another agonist, JWH-133, led to an increase of UCP1 expression in adipocytes derived from lean patients (70). Therefore, it has been proposed that CB_2 receptor activation might promote browning (65). On the contrary, the genetic ablation of CB_2 receptor results in increased adiposity (81). Furthermore, it is suggested that JWH-133 may reduce inflammation, leptin levels, as well as lipid droplet number and size (70). However, Deveaux et al. (71) showed that treatment with the CB_2 agonist JWH-133 potentiated adipose tissue inflammation in HFD-fed mice.

CB_2 Receptors in Liver

The CB_2 receptor is also found in different cell types within the liver. Although under physiological conditions the CB_2 receptor expression in the liver is low (75, 82). CB_2 receptors were detected in cultured hepatic myofibroblasts of human cirrhotic liver and in activated hepatic stellate cells (75), but predominantly in immune cells like Kupffer cells (82). Some groups have reported that mice hepatocytes do not express CB_2 receptors (71, 83, 84). On the contrary, Méndez-Sánchez et al. localized CB_2 receptors in hepatocytes, cholangiocytes and hepatic stellate cells in patients with NAFLD (85). It has been suggested that the CB_2 expression in the liver might be associated with the disease progression of NAFL and regulated by the interplay of inflammation, fibrosis and fat deposition (85, 86). The overexpression of CB_2 in NAFLD patients might be a defense mechanism, since CB_2 activation has been shown to reduce collagen synthesis in hepatic stellate cells. The reduced collagen synthesis can be achieved by activating caspase 3-like activity in hepatic stellate cells inducing apoptosis of fibrotic cells (75) and by reducing DNA synthesis of hepatic stellate cells, which inhibits their proliferation (86). Therefore, CB_2 receptor agonism in NAFLD might represent a therapeutic approach.

Not all signaling pathways mediated by CB_2 receptor activation are fully understood yet. However, its activation might be triggered during liver injury (87). These protective properties mostly rely on antifibrogenic and anti-inflammatory signals generated by CB_2 receptor activation in immune cells and hepatic myofibroblasts (87). Especially in Kupffer and endothelial cells, several CB_2 receptor agonists might lead to decreased inflammation and a lower generation rate of oxidative and nitrosative stress (74). In activated hepatic stellate cells, CB_2 receptor activation with JWH-015 promoted antifibrotic effects, namely, apoptosis. In the same study, it was further shown that blocking of the CB_2 receptor of human hepatic myofibroblasts with the inverse agonist SR144528 prevented apoptosis induced by THC. The reduced apoptotic effect was shown in an increased cell viability rate (75).

In an in vivo study, the CB_2 agonist JWH-133 enhanced HFD-induced hepatic steatosis and adipose tissue inflammation in wild-type mice (71). An in vitro study showed that the CB_2 agonist AM1241 increased the expression of CB_1 in hepatocytes treated with oleic acid, which might suggest a cross-regulation between CB_2 and CB_1 (88).

CB_2 Receptors in Skeletal Muscle

Cavuoto et al. demonstrated for the first time the expression of CB_2 receptor in both human and rodent skeletal muscle, as well as in human primary skeletal muscle myotubes (15). In addition, it was discovered that CB_2 receptor is time-dependently expressed in skeletal muscle, myofibroblasts, and macrophages during the process of skeletal muscle wound healing in rats (89). In a following study, this group suggested a beneficial role of the CB_2 receptor on skeletal muscle regeneration after ischemia-reperfusion injury in mice, partly by regulating macrophage M1/M2 polarization (90). Information about the CB_2 receptor modulation in skeletal muscle are scarcely reported. Agudo et al. (67) demonstrated that CB_2 receptor KO mice are protected from diet-induced and age-related

Table 1. ***Summary of the main effects of CB_2 receptor agonism and antagonism at peripheral level (adipose tissue, skeletal muscle, liver and endocrine pancreas)***

Tissue	CB2 Receptor Agonism	CB2 Receptor Antagonism/Inverse Agonism
Adipose tissue	– JWH-015 reduces inflammation in DIO mice (69) – JWH-133 reduces inflammation, leptin levels, lipid droplet number, and size in obesity-derived adipocytes (70) – JWH-133 potentiates adipose tissue inflammation in HFD-fed mice (71) – JWH-133 increases UCP1 expression in obesity- and in mesenchymal stem cell-derived adipocytes (70) and might potentially induce browning (65)	– JTE-907 upregulates inflammatory and angiogenic genes in human adipocytes (72) – SR144528 enhances anti-inflammatory effects in human adipocytes (73)
Liver	– JWH-133 and HU-308 reduces inflammation in human liver sinusoidal endothelial cells (74) – JWH-015 promotes apoptosis of rat-activated hepatic stellate cells (75) – JWH-133 enhances HFD-induced hepatic steatosis (71)	– SR144528 reduces apoptotic rate in human hepatic myofibroblasts (75)
Skeletal muscle		– SR144528 enhances insulin sensitivity in mice (67)
Endocrine pancreas	– Trans-caryophyllene increases insulin secretion in MIN6 β-cells (76) – JWH-015 increases glucose-induced insulin secretion in isolated human Langerhans islets (77) – SER601 increases insulin sensitivity in HFD/STZ-induced diabetic mice (78)	– JTE-907 stimulates insulin secretion in human islets (77)

DIO, diet-induced obesity; HFD, high fat diet; STZ, streptozotocin; UCP1, uncoupling protein 1.

insulin resistance. Moreover, mice treated with the CB_2 receptor inverse agonist SR144528 displayed enhanced insulin sensitivity in skeletal muscle with no changes in body weight.

CB_2 Receptors in Pancreas

Besides the ongoing discussion about the specific cell type expressing CB_1 receptors within the pancreas, the presence of CB_2 receptor in β-cells is also controversial. Some studies confirmed the presence of CB_2 receptor in β-cells (8, 55, 77), while other studies discarded CB_2 receptor expression in β-cells (91, 92). One study described the CB_2 receptor expression in somatostatin-secreting δ cells of human islets (91).

For the endocrine pancreas, it has been reported that different CB_2 receptor agonists stimulate insulin release in vitro and in vivo (93). Trans-caryophyllene is an agonist that selectively binds to the CB_2 receptor (94). It has been found to affect glucose-stimulated insulin secretion in MIN6 β-cells (76). In isolated human Langerhans islets, the CB_2 receptor agonist JWH-015 increased glucose-induced insulin secretion (77). In HFD/streptozotocin (STZ)-induced diabetic mice, the synthetic agonist SER601 increased insulin sensitivity (78) via Ca^{2+} signal regulation (95). However, the CB_2 receptor inverse agonist JTE-907 also improved insulin secretion in human islets (77).

CB_1 Receptors as a Therapeutic Target in Metabolic Diseases

The rimonabant case underpinned the need to prevent undesired inactivation of CB_1 in the CNS. This led to a second generation of CB_1 receptor antagonists and inverse agonists with low blood-brain barrier penetration. Chronic treatment with the neutral antagonist

AM6545 reduced high fat diet-induced weight gain and improved glucose homeostasis, insulin resistance, fatty liver, and plasma lipid profile (33). However, AM6545 requires intraperitoneal administration (33).

The antagonist with brain-limited penetrance TM38837 showed promising effects at the preclinical level, attenuating diet-induced obesity in rodent models (96, 97). Nevertheless, this compound displays a very low elimination rate (98) and has been recently reported to exert fear responses in mice at high doses (99).

The CB_1 receptor inverse agonist, JD5037, was tested in preclinical studies and showed low blood-brain barrier penetration, specificity for peripheral CB_1 receptors, and reverse leptin resistance to maintain weight loss in diet-induced obese mice (100). So far, JD5037 has already undergone preclinical safety studies (101), and it has been approved by the Food and Drug Administration to implement clinical trials (102).

Since obesity and the metabolic syndrome are complex disease entities, a third generation of CB_1 antagonists that combine a multitarget approach might prove a useful therapeutic option in these diseases (30, 103). A very elegant example is the hybrid compound MRI-1867, which combined a CB_1 receptor antagonist with an inducible nitric oxide synthase (iNOS) inhibitor. Being that iNOS is a proinflammatory and profibrotic enzyme hyperactivated in metabolic diseases and the cornerstone of many diabetic complications, this compound has proven to be effective in liver fibrosis and chronic kidney disease (104, 105).

Beyond drug discovery, on the basis of ligands targeting orthosteric sites, NAMs of the CB_1 receptor appear to be a potential therapeutic option. Thus, the receptor can be modulated under hyperactivaton by agonists instead of being blocked. Endogenous NAMs have been described, such as hemopressin (106), pregnenolone, and the family of cannabinoid peptides named pepcans (107). Pepcans might be useful for downregulating CB_1 receptor activity. In this line, the active peptide Pep19 (DIIADDEPLT) has been developed as an oral available CB_1 receptor inverse agonist. In diet-induced obese Wistar rats, Pep19 reduced body weight, adiposity, and improved serum glucose, triacylglycerol, and cholesterol without adverse CNS effects (108). The phytocannabinoid cannabidiol has also been proposed to act as a NAM of the CB_1 receptor (109, 110).

Another recent novel strategy based on the allosteric modulators is CB_1 receptor signaling of specific inhibitors. Contrary to antagonists, CB_1 receptor signaling of specific inhibitors does not block the receptor, but selectively modulates the cellular activity under hyperactivation as in pathological scenarios.

Besides using antagonists and NAMs to block or counterbalance CB_1 receptor signaling, the use of biological monoclonal antibodies against CB_1 receptor has arisen as a therapeutic option.

Nimacimab (Namacizumab; RYI-018 or JNJ2463) has recently undergone phase I for NAFLD/NASH and diabetic kidney disease. Another novel monoclonal antibody directed toward CB_1 receptor, IM-102, is currently undergoing testing as an Investigational New Drug, enabling studies to be performed by Integral Molecular for therapeutic application in patients with diabetic neuphropathy and obesity-related kidney complications.

However, as recently emphasized, there are no preclinical data on the pharmacokinetics or pharmacodynamics of these biological drugs, and we know nothing about their potential efficacy (45). Targeting the CB_1 receptor ligands instead of the CB_1 receptor itself is another strategy currently under development in obesity to counteract CB_1 receptor overactivation (111).

CB_2 Receptors as a Therapeutic Target in Metabolic Diseases

CB_2 receptor agonists have been attributed immunomodulatory anti-inflammatory and antioxidant properties, as well as stimulatory effects on insulin secretion (93). Regarding diabetes, the use of CB_2 receptor agonists has been mainly focused on counteracting inflammation in diabetic neuropathy and nephropathy (16). Thus, the use of CB_2 receptor agonists has been scarcely explored in metabolic regulation itself, and preclinical evidence has not been translated into clinical success yet (93).

Deveaux et al. observed that the CB_2 receptor agonist JWH-133 enhanced insulin resistance, as assessed by an insulin tolerance test in wild-type mice exposed to a HFD for 6 wk, while CB_2 receptor KO mice ($Cnr2^{-/-}$) on HFD displayed reduced insulin resistance and inflammation in AT and the liver (71). In contrast, Bermúdez-Silva et al. (112) showed improved glucose tolerance after glucose load following administration of JWH-133 in rats.

It has been proposed that numerous natural or synthetic CB_2 receptor agonists can exert protective effects in animal models of diabetes (93). Thus, the phytocannabinoid β-caryophyllene improved insulin secretion/glucose homeostasis and reduced oxidative stress and circulating proinflammatory cytokines in STZ-diabetic rats (113), while the synthetic agonist SER601 improved pancreatic β-cell function in HFD and STZ-diabetic mice (78). β-caryophyllene might represent a good candidate for a polypharmacological multitargeted strategy.

As discussed above, the expression of CB_2 receptors in AT, liver, pancreas, and skeletal muscle opens new possibilities for peripheral CB_2 receptor agonists or antagonists. However, the precise role of CB_2

receptors in metabolic diseases remains controversial and underlines the need for further studies.

CB_1 and CB_2 Receptor Dual Targeting as a Therapeutic Target in Metabolic Diseases

Another promising approach would be combining CB_1 receptor antagonism with CB_2 agonism. Combining the peripherally restricted CB_1 receptor neutral antagonist AM6545 with the CB_2 receptor agonist AM1241 has proven useful in animal models of diabetic nephropathy (114). To design a single molecule with high affinity as CB_1 receptor antagonist and CB_2 receptor agonist would be the ideal approach. In line, the compounds dual-targeting CB_1 antagonism and CB_2 receptor agonism such as URB447 (115) reduced food intake and body weight gain without central side effects. Moreover, the selective CB_2 agonists AM1710 and GW4058 (116), can antagonize CB_1 receptor signaling in human embryonic kidney 293 cells. However, these dual compounds displayed a lower affinity for CB_1 receptors (45), probably due to the fact that the CB_2 receptor antagonist and agonist binding pockets display a smaller size than the CB_1 antagonist binding pocket (12).

Another potential strategy for this dual modulation of CB_1 and CB_2 receptors is the use of pepcans with dual function acting as NAM for CB_1 and PAM for CB_2. Thus, pepcan-12 (RVD-hemopressin) is secreted by the liver and adrenal glands upon tissue damage (117) and has been proposed to act as a PAM for CB_2 and a NAM for CB_1.

Drug repurposing of already approved compounds that might exert dual actions could be another possibility. The PPARα agonist fenofibrate is an antilipemic drug, which has been proposed to act as a CB_2 receptor agonist and as a CB_1 NAM at high concentrations (118).

The crystal structure of the CB_2 receptor in active conformation remains still uncharacterized. If our knowledge on allosteric binding sites of CB receptors increases, therapeutic options might broaden and the possibility of dualsteric ligands binding both allosteric and orthosteric sites, or determining whether efficient dual modulation of CB_1 and CB_2 receptors is possible. Sex differences have an impact on the effects of cannabinoids (119). Thus, males are more sensitive to the hyperphagic effect of CB_1 receptor agonists and the hypophagic effect of respective antagonists. However, conflicting results have been attributed to the impact of sex steroid hormones on cannabinoid sensitivity (120). Central expression of CB_1 and endocannabinoids have been shown to change with the stages of the hormonal cycle (119). Further investigations on the impact of sex on pharmacological modulation of the CB receptors, especially at the peripheral level, are needed. A better understanding of the pharmacokinetic and pharmacodynamic of cannabinoids in humans would also be helpful for the development of personalized therapies targeting the CB receptors.

Regarding the influence of aging in the EC system, there are only scarce reports assessing this important issue. In this line, skeletal muscle-specific CB_1 receptor KO mice displayed improved whole body metabolism, muscle mass, insulin action, and mitochondrial function in aged mice (52). In line, CB_1 receptor inhibition in aged mice has a preventive effect on muscle loss (121, 122). Because obesity, diabetes, mitochondrial diseases, and aging have sarcopenia as a common factor, CB_1 might represent an interesting therapeutic target in age and metabolism-related sarcopenia. Intriguingly, CB_2 receptor KO mice are protected from age-related insulin resistance (67).

Conclusions

In recent years, several research groups and pharmaceutical companies have developed different compounds based on heterocyclic scaffolds entering modifications to increase CB_1/CB_2 receptor selectivity and availability, and to reduce CNS permeability (123). In the near future, we will see whether the observed peak in patents for compounds targeting CB_1 (124) translates to real therapeutic options in metabolic diseases after successful results in clinical trials. ■

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References

1. Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross, RA. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. *Pharmacol Rev* 62: 588–631, 2010. doi:10.1124/pr.110.003004.

2. Cota D, Marsicano G, Tschöp M, Grübler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thöne-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst ACE, Pasquali R, Lutz B, Stalla GK, Pagotto, U. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 112: 423–431, 2003. doi:10.1172/JCI17725.

3. Di Marzo V, Fontana, A. Anandamide, an endogenous cannabinomimetic eicosanoid: 'killing two birds with one stone'. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 53: 1–11, 1995. doi:10.1016/0952-3278(95)90077-2.

4. Di Marzo V. New approaches and challenges to targeting the endocannabinoid system. *Nat Rev Drug Discov* 17: 623–639, 2018. doi:10.1038/nrd.2018.115.

5. Abood M, Alexander SPH, Barth F, Bonner TI, Bradshaw H, Cabral G, Casellas P, Cravatt BF, Devane WA, Di Marzo V, Elphick MR, Felder CC, Greasley P, Herkenham M, Howlett AC, Kunos G, Mackie K, Mechoulam R, Pertwee RG, Ross, RA. Cannabinoid receptors (version 2019.4) in the IUPHAR/BPS Guide to Pharmacology Database. *GtoPdb CITE*, 2019: 2019. doi:10.2218/gtopdb/F13/2019.4.

6. Blüher M, Engeli S, Klöting N, Berndt J, Fasshauer M, Bátkai S, Pacher P, Schön MR, Jordan J, Stumvoll, M. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 55: 3053–3060, 2006. doi:10.2337/db06-0812.

7. Engeli S, Böhnke J, Feldpausch M, Gorzelniak K, Janke J, Bátkai S, Pacher P, Harvey-White J, Luft FC, Sharma AM, Jordan, J. Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 54: 2838–2843, 2005. doi:10.2337/diabetes.54.10.2838.

8. Matias I, Gonthier M-P, Orlando P, Martiadis V, Petrocellis L, de Cervino C, Petrosino S, Hoareau L, Festy F, Pasquali R, Roche R, Maj M, Pagotto U, Monteleone P, Di Marzo, V. Regulation, function, and dysregulation of endocannabinoids in models of adipose and β-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocrinol Metab* 91: 3171–3180, 2006. doi:10.1210/jc.2005-2679.

9. Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, Kunos, G. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410: 822–825, 2001. doi:10.1038/35071088.

10. Mazier W, Saucisse N, Gatta-Cherifi B, Cota, D. The endocannabinoid system: pivotal orchestrator of obesity and metabolic disease. *Trends Endocrinol Metab* 26: 524–537, 2015. doi:10.1016/j.tem.2015.07.007.

11. Hua T, Vemuri K, Nikas SP, Laprairie RB, Wu Y, Qu L, Pu M, Korde A, Jiang S, Ho J-H, Han GW, Ding K, Li X, Liu H, Hanson MA, Zhao S, Bohn LM, Makriyannis A, Stevens RC, Liu, Z-J. Crystal structures of agonist-bound human cannabinoid receptor CB_1. *Nature* 547: 468–471, 2017. doi:10.1038/nature23272.

12. Li X, Hua T, Vemuri K, Ho J-H, Wu Y, Wu L, Popov P, Benchama O, Zvonok N, Locke K, Qu L, Han GW, Iyer MR, Cinar R, Coffey NJ, Wang J, Wu M, Katritch V, Zhao S, Kunos G, Bohn LM, Makriyannis A, Stevens RC, Liu, Z-J. Crystal structure of the human cannabinoid receptor CB2. *Cell* 176: 459–467.e13, 2019. doi:10.1016/j.cell.2018.12.011.

13. Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, Soubrié, P. The cannabinoid CB_1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese *fa/fa* rats and in cultured adipocyte cells. *Mol Pharmacol* 63: 908–914, 2003. doi:10.1124/mol.63.4.908.

14. Osei-Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey-White J, Jeong W-I, Bátkai S, Marsicano G, Lutz B, Buettner C, Kunos, G. Hepatic CB_1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest* 118: 3160–3169, 2008. doi:10.1172/JCI34827.

15. Cavuoto P, McAinch AJ, Hatzinikolas G, Janovská A, Game P, Wittert, GA. The expression of receptors for endocannabinoids in human and rodent skeletal muscle. *Biochem Biophys Res Commun* 364: 105–110, 2007. doi:10.1016/j.bbrc.2007.09.099.

16. Gruden G, Barutta F, Kunos G, Pacher, P. Role of the endocannabinoid system in diabetes and diabetic complications. *Br J Pharmacol* 173: 1116–1127, 2016. doi:10.1111/bph.13226.

17. Tucci SA, Rogers EK, Korbonits M, Kirkham, TC. The cannabinoid CB_1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin. *Br J Pharmacol* 143: 520–523, 2004. doi:10.1038/sj.bjp.0705968.

18. Cani PD, Montoya ML, Neyrinck AM, Delzenne NM, Lambert, DM. Potential modulation of plasma ghrelin and glucagon-like peptide-1 by anorexigenic cannabinoid compounds, SR141716A (rimonabant) and oleoylethanolamide. *Br J Nutr* 92: 757–761, 2004. doi:10.1079/BJN20041256.

19. Harrold JA, Elliott JC, King PJ, Widdowson PS, Williams, G. Down-regulation of cannabinoid-1 (CB-1) receptors in specific extrahypothalamic regions of rats with dietary obesity: a role for endogenous cannabinoids in driving appetite for palatable food? *Brain Res* 952: 232–238, 2002. doi:10.1016/S0006-8993(02)03245-6.

20. Ravinet Trillou C, Delgorge C, Menet C, Arnone M, Soubrié, P. CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int J Obes* 28: 640–648, 2004. doi:10.1038/sj.ijo.0802583.

21. Tam J, Cinar R, Liu J, Godlewski G, Wesley D, Jourdan T, Szanda G, Mukhopadhyay B, Chedester L, Liow J-S, Innis RB, Cheng K, Rice KC, Deschamps JR, Chorvat RJ, McElroy JF, Kunos, G. Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metab* 16: 167–179, 2012. doi:10.1016/j.cmet.2012.07.002.

22. Watanabe S, Doshi M, Hamazaki, T. n-3 Polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice. *Prostaglandins Leukot Essent Fatty Acids* 69: 51–59, 2003. doi:10.1016/S0952-3278(03)00056-5.

23. Kirkham TC, Williams CM, Fezza F, Di Marzo, V. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol* 136: 550–557, 2002. doi:10.1038/sj.bjp.0704767.

24. Piccolo M, Claussen MC, Bluemel S, Schumacher S, Cronin A, Fried M, Goetze O, Martin-Soelch C, Milos, G. Altered circulating endocannabinoids in anorexia nervosa during acute and weight-restored phases: A pilot study. *Eur Eat Disorders Rev* 28: 46–54, 2020. doi:10.1002/erv.2709. doi:10.1002/erv.2709.

25. Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup, A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *The Lancet* 370: 1706–1713, 2007. doi:10.1016/S0140-6736(07)61721-8.

26. Després J-P, Golay A, Sjöström, L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* 353: 2121–2134, 2005. doi:10.1056/NEJMoa044537.

27. van Gaal L, Pi-Sunyer X, Després J-P, McCarthy C, Scheen, A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the Rimonabant in Obesity (RIO) program. *Diabetes Care* 31: S229–40, 2008. doi:10.2337/dc08-s258.

28. Nguyen T, Li J-X, Thomas BF, Wiley JL, Kenakin TP, Zhang, Y. Allosteric modulation: an alternate approach targeting the cannabinoid CB1 receptor. *Med Res Rev* 37: 441–474, 2017. doi:10.1002/med.21418.

29. Silvestri C, Di Marzo, V. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. *Cell Metab* 17: 475–490, 2013. doi:10.1016/j.cmet.2013.03.001.

30. Simon V, Cota, D. MECHANISMS IN ENDOCRINOLOGY: Endocannabinoids and metabolism: past, present and future. *Eur J Endocrinol* 176: R309–R324, 2017. doi:10.1530/EJE-16-1044.

31. Bouaboula M, Hilairet S, Marchand J, Fajas L, Le Fur G, Casellas, P. Anandamide induced PPARγ transcriptional activation and 3T3-L1 preadipocyte differentiation. *Eur J Pharmacol* 517: 174–181, 2005. doi:10.1016/j.ejphar.2005.05.032.

32. Ruiz de Azua I, Mancini G, Srivastava RK, Rey AA, Cardinal P, Tedesco L, Zingaretti CM, Sassmann A, Quarta C, Schwitter C, Conrad A, Wettschureck N, Vemuri VK, Makriyannis A, Hartwig J, Mendez-Lago M, Bindila L, Monory K, Giordano A, Cinti S, Marsicano G, Offermanns S, Nisoli E, Pagotto U, Cota D, Lutz, B. Adipocyte cannabinoid receptor CB1 regulates energy homeostasis and alternatively activated macrophages. *J Clin Invest* 127: 4148–4162, 2017. doi:10.1172/JCI83626.

33. Tam J, Vemuri VK, Liu J, Bátkai S, Mukhopadhyay B, Godlewski G, Osei-Hyiaman D, Ohnuma S, Ambudkar SV, Pickel J, Makriyannis A, Kunos, G. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest* 120: 2953–2966, 2010. doi:10.1172/JCI42551.

34. Han JH, Shin H, Park J-Y, Rho JG, Son DH, Kim KW, Seong JK, Yoon S-H, Kim, W. A novel peripheral cannabinoid 1 receptor antagonist, AJ5012, improves metabolic outcomes and suppresses adipose tissue inflammation in obese mice. *FASEB J* 33: 4314–4326, 2019. doi:10.1096/fj.201801152RR. doi:10.1096/fj.201801152RR.

35. Lahesmaa M, Eriksson O, Gnad T, Oikonen V, Bucci M, Hirvonen J, Koskensalo K, Teuho J, Niemi T, Taittonen M, Lahdenpohja S, U Din M, Haaparanta-Solin M, Pfeifer A, Virtanen KA, Nuutila, P. Cannabinoid type 1 receptors are upregulated during acute activation of brown adipose tissue. *Diabetes* 67: 1226–1236, 2018. doi:10.2337/db17-1366.

36. Hsiao W-C, Shia K-S, Wang Y-T, Yeh Y-N, Chang C-P, Lin Y, Chen P-H, Wu C-H, Chao Y-S, Hung, M-S. A novel peripheral cannabinoid receptor 1 antagonist, BPR0912, reduces weight independently of food intake and modulates thermogenesis. *Diabetes Obes Metab* 17: 495–504, 2015. doi:10.1111/dom.12447. doi:10.1111/dom.12447.

37. Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, Harvey-White J, Mackie K, Offertáler L, Wang L, Kunos, G. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 115: 1298–1305, 2005. doi:10.1172/JCI23057.

38. Liu J, Zhou L, Xiong K, Godlewski G, Mukhopadhyay B, Tam J, Yin S, Gao P, Shan X, Pickel J, Bataller R, O'Hare J, Scherer T, Buettner C, Kunos, G. Hepatic cannabinoid receptor-1 mediates diet-induced insulin resistance via inhibition of insulin signaling and clearance in mice. *Gastroenterology* 142: 1218–1228.e1, 2012. doi:10.1053/j.gastro.2012.01.032.

39. Teixeira-Clerc F, Julien B, Grenard P, van Tran Nhieu J, Deveaux V, Li L, Serriere-Lanneau V, Ledent C, Mallat A, Lotersztajn, S. CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nat Med* 12: 671–676, 2006. doi:10.1038/nm1421. doi:10.1038/nm1421.

40. Cinar R, Godlewski G, Liu J, Tam J, Jourdan T, Mukhopadhyay B, Harvey-White J, Kunos, G. Hepatic cannabinoid-1 receptors mediate diet-induced insulin resistance by increasing de novo synthesis of long-chain ceramides. *Hepatology* 59: 143–153, 2014. doi:10.1002/hep.26606.

41. Jourdan T, Demizieux L, Gresti J, Djaouti L, Gaba L, Vergès B, Degrace, P. Antagonism of peripheral hepatic cannabinoid receptor-1 improves liver lipid metabolism in mice: evidence from cultured explants. *Hepatology* 55: 790–799, 2012. doi:10.1002/hep.24733.

42. Jorgačević B, Vučević D, Vesković M, Mladenović D, Vukićević D, Vukićević RJ, Todorović V, Radosavljević, T. The effect of cannabinoid receptor 1 blockade on adipokine and proinflammatory cytokine concentration in adipose and hepatic tissue in mice with nonalcoholic fatty liver disease. *Can J Physiol Pharmacol* 97: 120–129, 2019. doi:10.1139/cjpp-2018-0607.

43. Patsenker E, Stoll M, Millonig G, Agaimy A, Wissniowski T, Schneider V, Mueller S, Brenneisen R, Seitz HK, Ocker M, Stickel, F. Cannabinoid receptor type I modulates alcohol-induced liver fibrosis. *Mol Med* 17: 1285–1294, 2011. doi:10.2119/molmed.2011.00149.

44. Mukhopadhyay P, Rajesh M, Bátkai S, Patel V, Kashiwaya Y, Liaudet L, Evgenov OV, Mackie K, Haskó G, Pacher, P. CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. *Cardiovasc Res* 85: 773–784, 2010. doi:10.1093/cvr/cvp369.

45. Cinar R, Iyer MR, Kunos, G. The therapeutic potential of second and third generation CB1R antagonists. *Pharmacol Ther* 208: 107477, 2020. doi:10.1016/j.pharmthera.2020.107477.

46. Crespillo A, Suárez J, Bermúdez-Silva FJ, Rivera P, Vida M, Alonso M, Palomino A, Lucena MA, Serrano A, Pérez-Martín M, Macias M, Fernández-Llébrez P, Rodríguez de Fonseca, F. Expression of the cannabinoid system in muscle: effects of a high-fat diet and CB1 receptor blockade. *Biochem J* 433: 175–185, 2011. doi:10.1042/BJ20100751.

47. Eckardt K, Sell H, Taube A, Koenen M, Platzbecker B, Cramer A, Horrighs A, Lehtonen M, Tennagels N, Eckel, J. Cannabinoid type 1 receptors in human skeletal muscle cells participate in the negative crosstalk between fat and muscle. *Diabetologia* 52: 664–674, 2009. doi:10.1007/s00125-008-1240-4.

48. Arrabal S, Lucena MA, Canduela MJ, Ramos-Uriarte A, Rivera P, Serrano A, Pavón FJ, Decara J, Vargas A, Baixeras E, Martín-Rufián M, Márquez J, Fernández-Llébrez P, Roos B, de Grandes P, Rodríguez de Fonseca F, Suárez, J. Pharmacological blockade of cannabinoid CB1 receptors in diet-induced obesity regulates mitochondrial dihydrolipoamide dehydrogenase in muscle. *PLoS One* 10: e0145244, 2015. doi:10.1371/journal.pone.0145244.

49. Mendizabal-Zubiaga J, Melser S, Bénard G, Ramos A, Reguero L, Arrabal S, Elezgarai I, Gerrikagoitia I, Suarez J, Rodríguez de Fonseca F, Puente N, Marsicano G, Grandes, P. Cannabinoid CB1 receptors are localized in striated muscle mitochondria and regulate mitochondrial respiration. *Front Physiol* 7: 476, 2016. doi:10.3389/fphys.2016.00476.

50. Liu YL, Connoley 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* 29: 183–187, 2005. doi:10.1038/sj.ijo.0802847.

51. Heyman E, Gamelin F-X, Aucouturier J, Di Marzo, V. The role of the endocannabinoid system in skeletal muscle and metabolic adaptations to exercise: potential implications for the treatment of obesity. *Obes Rev* 13: 1110–1124, 2012. doi:10.1111/j.1467-789X.2012.01026.x.

52. González-Mariscal I, Montoro RA, O'Connell JF, Kim Y, Gonzalez-Freire M, Liu Q-R, Alfaras I, Carlson OD, Lehrmann E, Zhang Y, Becker KG, Hardivillé S, Ghosh P, Egan, JM. Muscle cannabinoid 1 receptor regulates Il-6 and myostatin expression, governing physical performance and whole-body metabolism. *FASEB J* 33: 5850–5863, 2019. doi:10.1096/fj.201801145R.

53. Malenczyk K, Jazurek M, Keimpema E, Silvestri C, Janikiewicz J, Mackie K, Di Marzo V, Redowicz MJ, Harkany T, Dobrzyn, A. CB_1 cannabinoid receptors couple to focal adhesion kinase to control insulin release. *J Biol Chem* 288: 32685–32699, 2013. doi:10.1074/jbc.M113.478354.

54. Li C, Bowe JE, Jones, PM. Expression and function of cannabinoid receptors in mouse islets. *Islets* 2: 293–302, 2010. doi:10.4161/isl.2.5.12729.

55. Starowicz K, Cristino M, Matias L, Capasso I, Racioppi RA, Izzo A, Di Marzo, AV. Endocannabinoid dysregulation in the pancreas and adipose tissue of mice fed with a high-fat diet. *Obesity* 16: 553–565, 2008. doi:10.1038/oby.2007.106.

56. Tharp WG, Lee Y-H, Maple RL, Pratley, RE. The cannabinoid CB1 receptor is expressed in pancreatic δ-cells. *Biochem Biophys Res Commun* 372: 595–600, 2008. doi:10.1016/j.bbrc.2008.05.077.

57. Li C, Jones PM, Persaud, SJ. Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. *Pharmacol Ther* 129: 307–320, 2011. doi:10.1016/j.pharmthera.2010.10.006.

58. Horváth B, Mukhopadhyay P, Haskó G, Pacher, P. The endocannabinoid system and plant-derived cannabinoids in diabetes and diabetic complications. *Am J Pathol* 180: 432–442, 2012. doi:10.1016/j.ajpath.2011.11.003.

59. Kim W, Doyle ME, Liu Z, Lao Q, Shin Y-K, Carlson OD, Kim HS, Thomas S, Napora JK, Lee EK, Moaddel R, Wang Y, Maudsley S, Martin B, Kulkarni RN, Egan, JM. Cannabinoids inhibit insulin receptor signaling in pancreatic β-cells. *Diabetes* 60: 1198–1209, 2011. doi:10.2337/db10-1550.

60. Rohrbach K, Thomas MA, Glick S, Fung EN, Wang V, Watson L, Gregory P, Antel J, Pelleymounter, MA. Ibipinabant attenuates β-cell loss in male Zucker diabetic fatty rats independently of its effects on body weight. *Diabetes Obes Metab* 14: 555–564, 2012. doi:10.1111/j.1463-1326.2012.01563.x.

61. Jourdan T, Godlewski G, Cinar R, Bertola A, Szanda G, Liu J, Tam J, Han T, Mukhopadhyay B, Skarulis MC, Ju C, Aouadi M, Czech MP, Kunos, G. Activation of the Nlrp3 inflammasome in infiltrating macrophages by endocannabinoids mediates beta cell loss in type 2 diabetes. *Nat Med* 19: 1132–1140, 2013. doi:10.1038/nm.3265.

62. Kim W, Lao Q, Shin Y-K, Carlson OD, Lee EK, Gorospe M, Kulkarni RN, Egan, JM. Cannabinoids induce pancreatic β-cell death by directly inhibiting insulin receptor activation. *Sci Signal* 5: ra23, 2012. doi:10.1126/scisignal.2002519.

63. Pacher P, Mechoulam, R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res* 50: 193–211, 2011. doi:10.1016/j.plipres.2011.01.001.

64. Buckley NE. The peripheral cannabinoid receptor knockout mice: an update. *Br J Pharmacol* 153: 309–318, 2008. doi:10.1038/sj.bjp.0707527.

65. Rossi F, Punzo F, Umano GR, Argenziano, MM. Del Giudice E. Role of cannabinoids in obesity. *Int J Mol Sci* 19, 2018. doi:10.3390/ijms19092690.

66. Romero-Zerbo SY, Garcia-Gutierrez MS, Suárez J, Rivera P, Ruz-Maldonado I, Vida M, Rodriguez de Fonseca F, Manzanares J, Bermúdez-Silva, FJ. Overexpression of cannabinoid CB2 receptor in the brain induces hyperglycaemia and a lean phenotype in adult mice. *J Neuroendocrinol* 24: 1106–1119, 2012. doi:10.1111/j.1365-2826.2012.02325.x.

67. Agudo J, Martin M, Roca C, Molas M, Bura AS, Zimmer A, Bosch F, Maldonado, R. Deficiency of CB2 cannabinoid receptor in mice improves insulin sensitivity but increases food intake and obesity with age. *Diabetologia* 53: 2629–2640, 2010. doi:10.1007/s00125-010-1894-6.

68. Alshaarawy O, Kurjan E, Truong N, Olson, LK. Diet-induced obesity in cannabinoid-2 receptor knockout mice and cannabinoid receptor 1/2 double-knockout mice. *Obesity (Silver Spring)* 27: 454–461, 2019. doi:10.1002/oby.22403.

69. Verty ANA, Stefanidis A, McAinch AJ, Hryciw DH, Oldfield, B. Anti-obesity effect of the CB2 receptor agonist JWH-015 in diet-induced obese mice. *PLoS One* 10: e0140592, 2015. doi:10.1371/journal.pone.0140592.

70. Rossi F, Bellini G, Luongo L, Manzo I, Tolone S, Tortora C, Bernardo ME, Grandone A, Conforti A, Docimo L, Nobili B, Perrone L, Locatelli F, Maione S, Del Giudice, EM. Cannabinoid receptor 2 as antiobesity target: inflammation, fat storage, and browning modulation. *J Clin Endocrinol Metab* 101: 3469–3478, 2016. doi:10.1210/jc.2015-4381.

71. Deveaux V, Cadoudal T, Ichigotani Y, Teixeira-Clerc F, Louvet A, Manin S, Nhieu JT-V, Belot MP, Zimmer A, Even P, Cani PD, Knauf C, Burcelin R, Bertola A, Le Marchand-Brustel Y, Gual P, Mallat A, Lotersztajn, S. Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. *PLoS One* 4: e5844, 2009. doi:10.1371/journal.pone.0005844.

72. González-Muniesa P, Bing C, Trayhurn, P. Upregulation of the expression of inflammatory and angiogenic markers in human adipocytes by a synthetic cannabinoid, JTE-907. *Horm Metab Res* 42: 710–717, 2010. doi:10.1055/s-0030-1255119.

73. Hoareau L, Buyse M, Festy F, Ravanan P, Gonthier M-P, Matias I, Petrosino S, Tallet F, d'Hellencourt CL, Cesari M, Di Marzo V, Roche, R. Anti-inflammatory effect of palmitoylethanolamide on human adipocytes. *Obesity (Silver Spring* 17: 431–438, 2009. doi:10.1038/oby.2008.591.

74. Pacher P, Gao, B. Endocannabinoids and liver disease. III. Endocannabinoid effects on immune cells: implications for inflammatory liver diseases. *Am J Physiol Gastrointest Liver Physiol* 294: G850–G854, 2008. doi:10.1152/ajpgi.00523.2007.

75. Julien B, Grenard P, Teixeira-Clerc F, van Nhieu JT, Li L, Karsak M, Zimmer A, Mallat A, Lotersztajn, S. Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology* 128: 742–755, 2005. doi:10.1053/j.gastro.2004.12.050.

76. Suijun W, Zhen Y, Ying G, Yanfang, W. A role for trans-caryophyllene in the moderation of insulin secretion. *Biochem Biophys Res Commun* 444: 451–454, 2014. doi:10.1016/j.bbrc.2013.11.136.

77. Li C, Bowe JE, Huang GC, Amiel SA, Jones PM, Persaud, SJ. Cannabinoid receptor agonists and antagonists stimulate insulin secretion from isolated human islets of Langerhans. *Diabetes, Obesity and Metabolism* 13: 903–910, 2011. doi:10.1111/j.1463-1326.2011.01422.x.

78. Zhang X, Gao S, Niu J, Li P, Deng J, Xu S, Wang Z, Wang W, Kong D, Li, C. Cannabinoid 2 receptor agonist improves systemic sensitivity to insulin in high-fat diet/streptozotocin-induced diabetic mice. *Cell Physiol Biochem* 40: 1175–1185, 2016. doi:10.1159/000453171.

79. Roche R, Hoareau L, Bes-Houtmann S, Gonthier M-P, Laborde C, Baron J-F, Haffaf Y, Cesari M, Festy, F. Presence of the cannabinoid receptors, CB1 and CB2, in human omental and subcutaneous adipocytes. *Histochem Cell Biol* 126: 177–187, 2006. doi:10.1007/s00418-005-0127-4.

80. van Eenige R, van der Stelt M, Rensen PCN, Kooijman, S. Regulation of adipose tissue metabolism by the endocannabinoid system. *Trends Endocrinol Metab* 29: 326–337, 2018. doi:10.1016/j.tem.2018.03.001.

81. Schmitz K, Mangels N, Häussler A, Ferreirós N, Fleming I, Tegeder, I. Pro-inflammatory obesity in aged cannabinoid-2 receptor-deficient mice. *Int J Obes* 40: 366–379, 2016. doi:10.1038/ijo.2015.169.

82. Mallat A, Teixeira-Clerc F, Deveaux V, Manin S, Lotersztajn, S. The endocannabinoid system as a key mediator during liver diseases: new insights and therapeutic openings. *Br J Pharmacol* 163: 1432–1440, 2011. doi:10.1111/j.1476-5381.2011.01397.x.

83. Mallat A, Teixeira-Clerc F, Lotersztajn, S. Cannabinoid signaling and liver therapeutics. *J Hepatol* 59: 891–896, 2013. doi:10.1016/j.jhep.2013.03.032.

84. Teixeira-Clerc F, Belot M-P, Manin S, Deveaux V, Cadoudal T, Chobert M-N, Louvet A, Zimmer A, Tordjmann T, Mallat A, Lotersztajn, S. Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. *Hepatology* 52: 1046–1059, 2010. doi:10.1002/hep.23779.

85. Mendez-Sanchez N, Zamora-Valdes D, Pichardo-Bahena R, Barredo-Prieto B, Ponciano-Rodriguez G, Bermejo-Martínez L, Chavez-Tapia NC, Baptista-González HA, Uribe, M. Endocannabinoid receptor CB2 in nonalcoholic fatty liver disease. *Liver Int* 27: 215–219, 2007. doi:10.1111/j.1478-3231.2006.01401.x.

86. Zamora-Valdés D, Ponciano-Rodríguez G, Chávez-Tapia NC, Méndez-Sánchez, N. The endocannabinoid system in chronic liver disease. *Annals of hepatology* 4: 248–254, 2005. doi:10.1016/S1665-2681(19)32047-2.

87. Denaës T, Lodder J, Chobert M-N, Ruiz I, Pawlotsky J-M, Lotersztajn S, Teixeira-Clerc, F. The cannabinoid receptor 2 protects against alcoholic liver disease via a macrophage autophagy-dependent pathway. *Sci Rep* 6: 28806, 2016. doi:10.1038/srep28806.

88. de G, Spahr L, Ravier-Dall'Antonia F, Hadengue, A. Cannabinoid receptor 1 and 2 agonists increase lipid accumulation in hepatocytes. *Liver Int* 30: 1482–1489, 2010. doi:10.1111/j.1478-3231.2010.02298.x.

89. Yu T-S, Cheng Z-H, Li L-Q, Zhao R, Fan Y-Y, Du Y, Ma W-X, Guan, D-W. The cannabinoid receptor type 2 is time-dependently expressed during skeletal muscle wound healing in rats. *Int J Legal Med* 124: 397–404, 2010. doi:10.1007/s00414-010-0465-1.

90. Jiang P, Wang L, Zhang M, Zhang M, Wang C, Zhao R, Guan, D. Cannabinoid type 2 receptor manipulates skeletal muscle regeneration partly by regulating macrophage M1/M2 polarization in IR injury in mice. *Life Sciences* 256: 117989, 2020. doi:10.1016/j.lfs.2020.117989.

91. Bermúdez-Silva FJ, Suárez J, Baixeras E, Cobo N, Bautista D, Cuesta-Muñoz AL, Fuentes E, Juan-Pico P, Castro MJ, Milman G, Mechoulam R, Nadal A, Rodríguez de Fonseca, F. Presence of functional cannabinoid receptors in human endocrine pancreas. *Diabetologia* 51: 476–487, 2008. doi:10.1007/s00125-007-0890-y.

92. Nakata M, Yada, T. Cannabinoids inhibit insulin secretion and cytosolic Ca^{2+} oscillation in islet β-cells via CB_1 receptors. *Regulatory peptides* 145: 49–53, 2008. doi:10.1016/j.regpep.2007.08.009.

93. Kumawat VS, Kaur, G. Therapeutic potential of cannabinoid receptor 2 in the treatment of diabetes mellitus and its complications. *Eur J Pharmacol* 862: 172628, 2019. doi:10.1016/j.ejphar.2019.172628.

94. Choi I-Y, Ju C, Anthony Jalin AMA, Lee DI, Prather PL, Kim, W-K. Activation of cannabinoid CB2 receptor-mediated AMPK/CREB pathway reduces cerebral ischemic injury. *Am J Pathol* 182: 928–939, 2013. doi:10.1016/j.ajpath.2012.11.024.

95. Juan-Picó P, Fuentes E, Bermúdez-Silva FJ, Javier Díaz-Molina F, Ripoll C, Rodríguez de Fonseca F, Nadal, A. Cannabinoid receptors regulate Ca^{2+} signals and insulin secretion in pancreatic β-cell. *Cell Calcium* 39: 155–162, 2006. doi:10.1016/j.ceca.2005.10.005.

96. TM Pharma. 7TM Pharma successfully conducts clinical phase I trial of its first in class peripheral CB1 receptor antagonist TM38837 demonstrating restriction from the human CNS. *BioSpace* 2010. https://www.biospace.com/article/releases/7tm-pharma-successfully-conducts-clinical-phase-i-trial-of-its-first-in-class-peripheral-cb1-receptor-antagonist-tm38837-demonstrating-restriction-fro/ [2020 Jun 5].

97. Chorvat RJ. Peripherally restricted CB1 receptor blockers. *Bioorg Med Chem Lett* 23: 4751–4760, 2013. doi:10.1016/j.bmcl.2013.06.066.

98. Klumpers LE, Fridberg M, Kam ML, de Little PB, Jensen NO, Kleinloog HD, Elling CE, van Gerven, JMA. Peripheral selectivity of the novel cannabinoid receptor antagonist TM38837 in healthy subjects. *Br J Clin Pharmacol* 76: 846–857, 2013. doi:10.1111/bcp.12141.

99. Micale V, Drago F, Noerregaard PK, Elling CE, Wotjak, CT. The cannabinoid CB1 antagonist TM38837 with limited penetrance to the brain shows reduced fear-promoting effects in mice. *Front Pharmacol* 10: 207, 2019. doi:10.3389/fphar.2019.00207.

100. Crunkhorn S. Metabolic disorders: safe cannabinoid receptor modulators in sight? *Nat Rev Drug Discov* 11: 749–749, 2012. doi:10.1038/nrd3851.

101. Kale VP, Gibbs S, Taylor JA, Zmarowski A, Novak J, Patton K, Sparrow B, Gorospe J, Anand S, Cinar R, Kunos G, Chorvat RJ, Terse, PS. Preclinical toxicity evaluation of JD5037, a peripherally restricted CB1 receptor inverse agonist, in rats and dogs for treatment of nonalcoholic steatohepatitis. *Regul Toxicol Pharmacol* 109: 104483, 2019. doi:10.1016/j.yrtph.2019.104483.

102. Iyer MR, Cinar R, Coffey NJ, Kunos, G. Synthesis of 13 C6 -labeled, dual-target inhibitor of cannabinoid-1 receptor (CB1 R) and inducible nitric oxide synthase (iNOS). *J Labelled Comp Radiopharm*, 2018. doi:10.1002/jlcr.3639.

103. Maccarrone M, Bab I, Bíró T, Cabral GA, Dey SK, Di Marzo V, Konje JC, Kunos G, Mechoulam R, Pacher P, Sharkey KA, Zimmer, A. Endocannabinoid signaling at the periphery: 50 years after THC. *Trends Pharmacol Sci* 36: 277–296, 2015. doi:10.1016/j.tips.2015.02.008.

104. Cinar R, Iyer MR, Liu Z, Cao Z, Jourdan T, Erdelyi K, Godlewski G, Szanda G, Liu J, Park JK, Mukhopadhyay B, Rosenberg AZ, Liow J-S, Lorenz RG, Pacher P, Innis RB, Kunos, G. Hybrid inhibitor of peripheral cannabinoid-1 receptors and inducible nitric oxide synthase mitigates liver fibrosis. *JCI Insight* 1, 2016. doi:10.1172/jci.insight.87336.

105. Udi S, Hinden L, Ahmad M, Drori A, Iyer MR, Cinar R, Herman-Edelstein M, Tam, J. Dual inhibition of cannabinoid CB_1 receptor and inducible NOS attenuates obesity-induced chronic kidney disease. *Br J Pharmacol* 177: 110–127, 2020. doi:10.1111/bph.14849.

106. Macedonio G, Stefanucci A, Maccallini C, Mirzaie S, Novellino E, Mollica, A. Hemopressin Peptides as modulators of the endocannabinoid system and their potential applications as therapeutic tools. *Protein Pept Lett* 23: 1045–1051, 2016. doi:10.2174/0929866523666161007152435.

107. Bauer M, Chicca A, Tamborrini M, Eisen D, Lerner R, Lutz B, Poetz O, Pluschke G, Gertsch, J. Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB_1 receptors. *J Biol Chem* 287: 36,944–36,967, 2012. doi:10.1074/jbc.M112.382481.

108. Reckziegel P, Festuccia WT, Britto LRG, Jang KLL, Romão CM, Heimann JC, Fogaça MV, Rodrigues NS, Silva NR, Guimarães FS, Eichler RAS, Gupta A, Gomes I, Devi LA, Heimann AS, Ferro, ES. A novel peptide that improves metabolic parameters without adverse central nervous system effects. *Sci Rep* 7: 14781, 2017. doi:10.1038/s41598-017-13690-9.

109. Chung H, Fierro A, Pessoa-Mahana, CD. Cannabidiol binding and negative allosteric modulation at the cannabinoid type 1 receptor in the presence of delta-9-tetrahydrocannabinol: An In Silico study. *PLoS One* 14: e0220025, 2019. doi:10.1371/journal.pone.0220025.

110. Laprairie RB, Bagher AM, Kelly MEM, Denovan-Wright, EM. Cannabidiol is a negative allosteric modulator of the cannabinoid CB_1 receptor. *Br J Pharmacol* 172: 4790–4805, 2015. doi:10.1111/bph.13250.

111. Lu D, Immadi SS, Wu Z, Kendall, DA. Translational potential of allosteric modulators targeting the cannabinoid CB1 receptor. *Acta Pharmacol Sin* 40: 324–335, 2019. doi:10.1038/s41401-018-0164-x.

112. Bermudez-Silva FJ, Sanchez-Vera I, Suárez J, Serrano A, Fuentes E, Juan-Pico P, Nadal A, Rodríguez de Fonseca, F. Role of cannabinoid CB2 receptors in glucose homeostasis in rats. *Eur J Pharmacol* 565: 207–211, 2007. doi:10.1016/j.ejphar.2007.02.066.

113. Basha RH, Sankaranarayanan, C. β-Caryophyllene, a natural sesquiterpene lactone attenuates hyperglycemia mediated oxidative and inflammatory stress in experimental diabetic rats. *Chem Biol Interact* 245: 50–58, 2016. doi:10.1016/j.cbi.2015.12.019.

114. Barutta F, Grimaldi S, Gambino R, Vemuri K, Makriyannis A, Annaratone L, Di Marzo V, Bruno G, Gruden, G. Dual therapy targeting the endocannabinoid system prevents experimental diabetic nephropathy. *Nephrol Dial Transplant* 32: 1655–1665, 2017. doi:10.1093/ndt/gfx010.

115. LoVerme J, Duranti A, Tontini A, Spadoni G, Mor M, Rivara S, Stella N, Xu C, Tarzia G, Piomelli, D. Synthesis and characterization of a peripherally restricted CB1 cannabinoid antagonist, URB447, that reduces feeding and body-weight gain in mice. *Bioorg Med Chem Lett* 19: 639–643, 2009. doi:10.1016/j.bmcl.2008.12.059.

116. Dhopeshwarkar A, Murataeva N, Makriyannis A, Straiker A, Mackie, K. Two Janus cannabinoids that are both CB_2 agonists and CB_1 antagonists. *J Pharmacol Exp Ther* 360: 300–311, 2017. doi:10.1124/jpet.116.236539.

117. Petrucci V, Chicca A, Glasmacher S, Paloczi J, Cao Z, Pacher P, Gertsch, J. Pepcan-12 (RVD-hemopressin) is a CB2 receptor positive allosteric modulator constitutively secreted by adrenals and in liver upon tissue damage. *Sci Rep* 7: 9560, 2017. doi:10.1038/s41598-017-09808-8.

118. Priestley RS, Nickolls SA, Alexander SPH, Kendall, DA. A potential role for cannabinoid receptors in the therapeutic action of fenofibrate. *FASEB J* 29: 1446–1455, 2015. doi:10.1096/fj.14-263053.

119. Fattore L, Fratta, W. How important are sex differences in cannabinoid action? *Br J Pharmacol* 160: 544–548, 2010. doi:10.1111/j.1476-5381.2010.00776.x.

120. Wagner EJ. Sex differences in cannabinoid-regulated biology: a focus on energy homeostasis. *Front Neuroendocrinol* 40: 101–109, 2016. doi:10.1016/j.yfrne.2016.01.003.

121. Kalyani RR, Corriere M, Ferrucci, L. Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. *Lancet Diabetes Endocrinol* 2: 819–829, 2014. doi:10.1016/S2213-8587(14)70034-8.

122. Tezze C, Romanello V, Sandri, M. FGF21 as modulator of metabolism in health and disease. *Front Physiol* 10: 419, 2019. doi:10.3389/fphys.2019.00419.

123. Yadav MR, Murumkar, PR. Advances in patented CB1 receptor antagonists for obesity. *Pharm Pat Anal* 7: 169–173, 2018. doi:10.4155/ppa-2018-0020.

124. Amato G, Khan NS, Maitra, R. A patent update on cannabinoid receptor 1 antagonists (2015–2018). *Expert Opin Ther Pat* 29: 261–269, 2019. doi:10.1080/13543776.2019.1597851.