Cell Metabolism
Review

# The Endocannabinoid System in Energy Homeostasis and the Etiopathology of Metabolic Disorders

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Endocannabinoids and cannabinoid CB1 receptors are known to play a generalized role in energy homeostasis. However, clinical trials with the first generation of CB1 blockers, now discontinued due to psychiatric side effects, were originally designed to reduce food intake and body weight rather than the metabolic risk factors associated with obesity. In this review, we discuss how, in addition to promoting energy intake, endocannabinoids control lipid and glucose metabolism in several peripheral organs, particularly the liver and adipose tissue. Direct actions in skeletal muscle and pancreas are also emerging. This knowledge may help in the design of future therapies for the metabolic syndrome.

## An "Expanded" View of the Endocannabinoid System

In the nearly 50 years since the identification of the principal active component of *Cannabis sativa*, the cannabinoid $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) (Gaoni and Mechoulam, 1964), great strides have been made in identifying and understanding the endogenous mediators, i.e., the endocannanabinoids, that this natural product biologically mimics. The most-studied endocannabinoids, *N*-arachidonoylethanolamide (anandamide; AEA) and 2-arachidonoylglycerol (2-AG), members of the fatty acid amide (FAA) and monoacylglycerol (MAG) families of neutral lipids, respectively, are produced, often with their congeners, from cell membrane phospholipids after cell stimulation and are immediately released to target the same cannabinoid receptors (CB1 and CB2) as $\Delta^9$-THC (Di Marzo, 2008b). The endocannabinoid system (ECS) as a whole refers to endocannabinoids and the proteins that regulate their production and degradation, as well as to the receptors through which they signal.

ECS "tone" within a biological system is mostly the result of the regulation of endocannabinoid levels as modulated by different, often concurring, enzymatic cascades, which are nearly ubiquitously expressed. Several pathways have been proposed to mediate AEA formation, such as, for example, the hydrolysis of *N*-acylphosphatidylethanolamine (NAPE) by NAPE-selective phospholipase D or the combinatorial action of α,β-hydrolase domain-containing 4 and glycerophosphodiesterase 1 on NAPE precursors (Muccioli, 2010). The production of 2-AG results from the sequential hydrolysis of *sn*-2-arachidonic acid (AA)-containing diacylglycerol (DAG) membrane phospholipids by phospholipase C (PLC) and diacylglycerol lipase α (DAGLα) or β (DAGLβ) (Muccioli, 2010). Degradation of AEA and 2-AG proceeds through their hydrolysis primarily by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively, resulting in the release of ethanolamine or glycerol, respectively, along with AA (Muccioli, 2010), which, when produced from 2-AG in the brain, may act as a precursor of prostanoids (Nomura et al., 2011). Alternatively, both AEA and 2-AG are subject to oxidative metabolism mediated by prostaglandin-endoperoxide synthase 2/cyclooxygenase 2 (PTGS2/COX-2) resulting in the eventual formation of various biologically active prostaglandin-ethanolamides and prostaglandin-glycerol esters, respectively (Kozak et al., 2004).

The canonical receptors for AEA and 2-AG are the mostly $G_{i/o}$-protein-coupled CB1 and CB2 receptors. Their activation by AEA and 2-AG results in a host of biochemical responses that are often cell-type specific, including the inhibition of various voltage-gated $Ca^{2+}$ channels and adenylate cyclase activity resulting in lower cAMP levels and the activation of $K^+$ channels, phospholipases, and mitogen-activated protein kinase (MAPK) pathways. CB1 is highly expressed throughout the central nervous system in neurons that regulate feeding, energy expenditure, and reward, as well as in peripheral organs that are critical for metabolic homeostasis. While there is some evidence that CB2 is also expressed neuronally, this receptor is mainly found within cells of the immune system, in line with its role as a major modulator of immune function (Howlett, 1995; Pertwee and Ross, 2002).

AEA and 2-AG also target noncannabinoid receptors. AEA activation of the transient receptor potential vanilloid 1 (TRPV1) channel, responsible for the sensation of heat produced by spicy peppers, increases intracellular $Ca^{2+}$ levels and produces several biological effects that sometimes oppose those produced by CB1 and CB2 activation (Di Marzo and De Petrocellis, 2010). At higher concentrations, AEA is also able to act within the nucleus by binding to the peroxisome proliferator-activated receptorγ (PPARγ) and activating transcription (Bouaboula et al., 2005; Gasperi et al., 2007; Karaliota et al., 2009), although there is still little evidence for such mechanism to occur in vivo. Our understanding of yet other "endocannabinoid receptors" proposed so far is limited, and in some cases controversial, requiring further investigation. On the other hand, it is now clear that AEA and 2-AG congeners, i.e., several FAAs and MAGs, respectively, are biosynthesized and/or degraded by the same enzymes as the two endocannabinoids but interact with non-CB1, non-CB2 receptors. The best established examples are *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA), which activate both PPARα and TRPV1, and 2-oleoylglycerol, a potent agonist at the orphan receptor GPR119, the activation of which in the small intestine stimulates the release

of the incretin, glucagon-like peptide-1 (GLP-1) (Lan et al., 2012; Hansen et al., 2012). The "promiscuity" of endocannabinoids, the "redundancy" of their metabolic pathways, and their being produced or degraded together with, or their giving rise to, other bioactive lipid mediators expands and complicates the interpretation of their role in metabolic control, posing new exciting challenges and opportunities for the development of endocannabinoid-based therapies against metabolic disorders.

## Control of Metabolism by the Central/Neuronal Endocannabinoid System and Its Dependence on the Nutritional Status and Caloric and Hedonic Value of Food

Within the central nervous system, endocannabinoids function, in general, in a retrograde manner, being produced by postsynaptic cells and acting on CB1 in presynaptic terminals to inhibit excitatory or inhibitory neurotransmitter release (Ohno-Shosaku et al., 2012). Endocannabinoid release occurs immediately after biosynthesis from postsynaptic phospholipids, with no intermediate storage in vesicles like for other neuromodulators. Thus, endocannabinoids are ideal mediators for responding in real-time to the ever-changing feeding state of an organism. They regulate appetite and food intake in a local manner by modulating, via activation of CB1 receptors, the activity of hypothalamic neurons and, subsequently, the release of orexigenic and anorexigenic neuropeptides, as well as the function of mesolimbic and brainstem neurons, by translating input information from the periphery to these neurons, thereby participating in both the homeostatic (i.e., based on energy balance) and hedonic (i.e., based on the incentive value of food) aspects of food intake (Broberger, 2005; Di Marzo et al., 2009b).

It is now well accepted that CB1 activation by $\Delta^9$-THC or synthetic CB1 agonists stimulates feeding. Injection of AEA or 2-AG in the hypothalamus and nucleus accumbens elicits increased feeding also in satiated rodents via CB1 activation (Jamshidi and Taylor, 2001; Kirkham et al., 2002). On the other hand, endocannabinoid levels increase significantly within the hypothalamus and accumbens in response to fasting, returning to normal after refeeding, without changing in brain areas not involved in feeding (Kirkham et al., 2002). This regulation is mediated, at least in part, through the action of feeding-regulated hormones, in particular, anorexigenic leptin and orexigenic ghrelin or glucocorticoids, which decrease and increase endocannabinoid levels within the hypothalamus, respectively, and become deregulated during obesity, thus resulting in elevated hypothalamic endocannabinoid tone (Di Marzo et al., 2001; Malcher-Lopes et al., 2006; Kola et al., 2008). Activation of the ECS also affects the reward and reinforcement circuits in the mesolimbic system, especially the nucleus accumbens and ventral tegmental area, where the ECS is highly expressed and interacts with both dopaminergic and opioidergic pathways, resulting in a preference for highly palatable food (Melis et al., 2007; Verty et al., 2004). For example, $\Delta^9$-THC increases sucrose-induced hedonic activity and dopamine release into the nucleus accumbens (De Luca et al., 2012), whereas CB1 antagonism reduces the increase of extracellular dopamine release induced in this nucleus by a novel highly palatable food (Melis et al., 2007). Furthermore, CB1 and μ-opioid blockade synergize at reducing food intake and body weight in rodents (Lockie et al., 2011; Tallett et al., 2009) (Figure 1).

Indeed, both pharmacological and genetic inhibition of CB1 results in hypophagia (Colombo et al., 1998; Di Marzo et al., 2001). In a recent study, virally mediated knockdown of CB1 within the mouse hypothalamus by 60% had no effects on basal food consumption, although the animals were less responsive to the hypophagic activity of the CB1 inverse agonist rimonabant, and insensitive to the anorexigenic actions of leptin, suggesting that this hormone relies entirely on its inhibition of CB1 tone to produce its effects in this brain area (Cardinal et al., 2012). Importantly, the orexigenic effect of ghrelin is also lost in rodents in which CB1 receptors are pharmacologically or genetically inactivated (Kola et al., 2008). It is not known, however, whether the effects of leptin and ghrelin on the hedonic neural correlates of food intake at the level of the mesolimbic nuclei are also exerted via modulation of ECS signaling. Interestingly, in healthy volunteers, consumption of favorite food, as compared to normal food, is accompanied by elevated plasma 2-AG levels which correlate positively with plasma ghrelin levels (Monteleone et al., 2012). It is not known whether this phenomenon reflects a direct or indirect stimulatory action by palatable-food-enhanced ghrelin signaling on endocannabinoid levels in the brain or peripheral tissues or, rather, the change in small intestine endocannabinoid levels after mouth exposure to fat, as shown in a recent study in rats (DiPatrizio et al., 2011). The latter seems less likely, considering that elevation of plasma 2-AG levels was observed also in anticipation of the favorite food, and not only after its consumption.

Perhaps surprisingly, CB1 activation can also produce hypophagic actions, depending on the type of axon terminals, i.e., excitatory versus inhibitory, where it occurs. In an elegant study using selective *CB1* deletion in either glutamatergic or GABAergic forebrain neurons, exogenous $\Delta^9$-THC, as well as endocannabinoid level elevation induced by fasting or exposure to palatable food, produced hyperphagic or hypophagic effects depending on the restriction of their action to CB1 on glutamatergic or GABAergic terminals, respectively (Bellocchio et al., 2010). In the lateral hypothalamus, however, CB1 activation results in retrograde inhibition of GABA or glutamate release from inputs onto melanin-concentrating hormone (MCH) or orexin-1 releasing neurons, thereby resulting in disinhibition or inhibition of stimulation, respectively, of two orexigenic signals and, potentially, in orexigenic or anorexic effects (Figure 1). On the other hand, presynaptic CB1 activation also inhibits glutamate release to parvocellular neurons, resulting in the inhibition of the release into the paraventricular nucleus of the anorectic corticotropin-releasing hormone (CRH) (Jo et al., 2005; Kola et al., 2008; Malcher-Lopes et al., 2006). Since global and conditional glutamatergic *CB1*$^{-/-}$ mice exhibit the same hypophagic phenotype after fasting or exposure to palatable foods (Bellocchio et al., 2010), one must surmise that the effect of CB1 inhibition of glutamatergic signaling overall predominates over that of GABAergic signaling when one needs to explain the hyperphagic effects of $\Delta^9$-THC and the hypophagic effects of CB1 inverse agonists in wild-type mice. However, it should be remembered that the hypothalamus can be rapidly "rewired," in terms of what and how many neurons are regulated by excitatory versus inhibitory inputs, in fasted versus ad-lib-fed or in lean versus obese animals, often as a consequence of changes in leptin or glucocorticoid signaling (Crosby et al., 2011; Pinto et al., 2004).

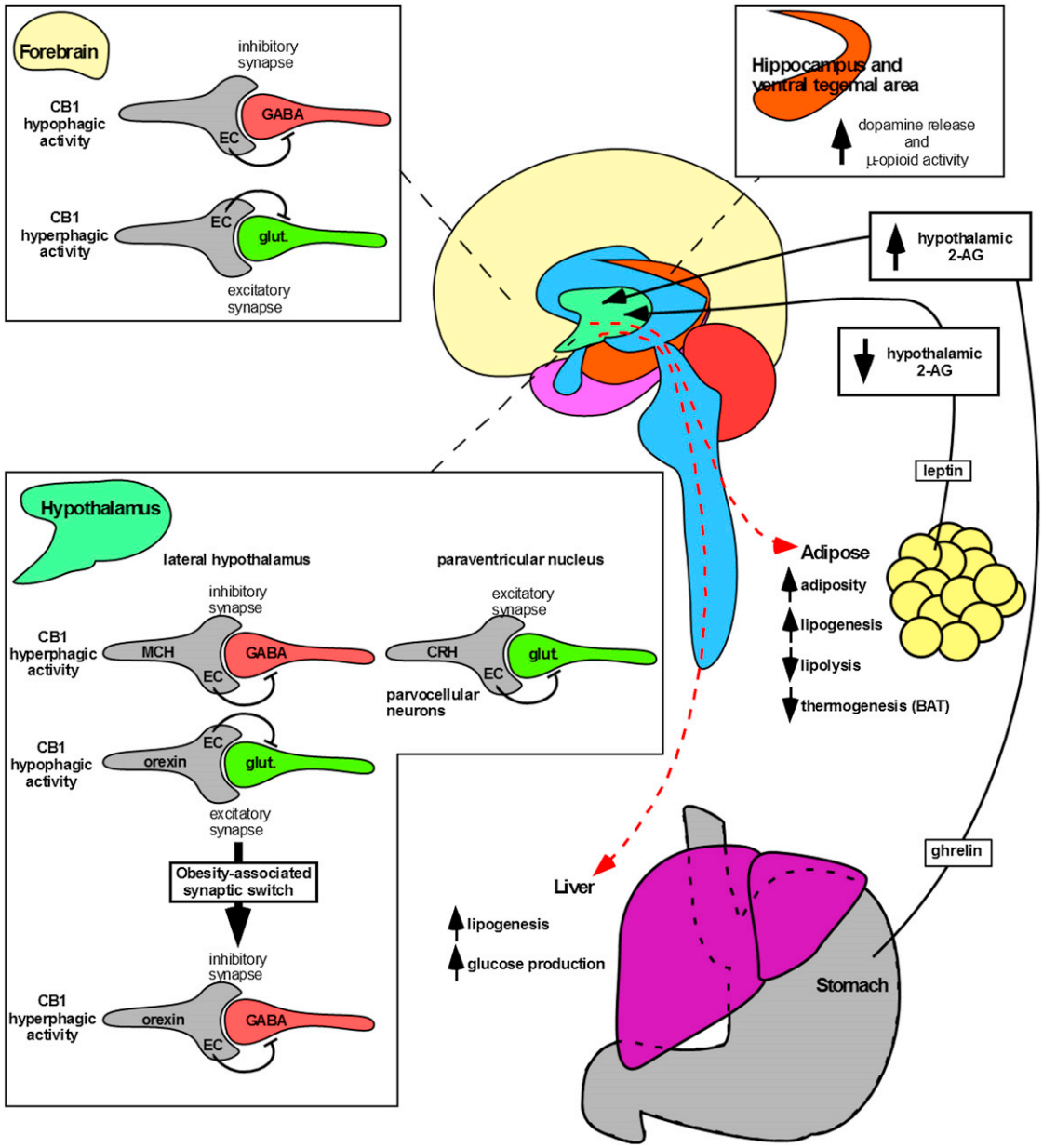


**Figure 1. Endocannabinoid- and CB1-Mediated Control of Central Functions Affecting Food Intake and Metabolism**
2-AG, 2-arachidonoylglycerol; BAT, brown adipose tissue; CRH, corticotrophin-releasing hormone; EC, endocannabinoid; glut., glutamate; MCH, melanin-concentrating hormone.

Thus, in orexin-A neurons of the lateral hypothalamus from leptin-deficient or leptin-resistant obese mice, CB1-expressing presynaptic inputs change from predominantly excitatory to inhibitory, resulting in elevated retrograde disinhibition, rather than inhibition of activation, and increased orexin-A release in target areas, which might contribute to hyperphagia in obesity (L. Cristino and V.D., unpublished data) (Figure 1).

Under conditions of diet-induced obesity (DIO) in mice, endocannabinoid levels are upregulated within the hippocampus, which is an important substrate of hedonic eating, indicating that highly palatable foods might be more satisfying under these conditions, resulting in a vicious circle leading to obesity (Massa et al., 2010). Also in the hypothalamus, 2-AG is transiently or permanently upregulated after acute or prolonged consumption, respectively, of a high-fat diet (HFD) and seems to determine, via CB1 receptors, the preference for such diet over a normal chow (Bisogno et al., 2012; Higuchi et al., 2011). Hypothalamic 2-AG level elevation, which accompanies impaired leptin signaling (Di Marzo et al., 2001), might also participate in peripheral metabolic dysfunctions, such as excess white adipose tissue (WAT) accumulation and hepatic glucose production, by causing insulin resistance in the mediobasal hypothalamus, as suggested by studies in rats either treated with intracerebroventricular infusion of a CB1 agonist or given

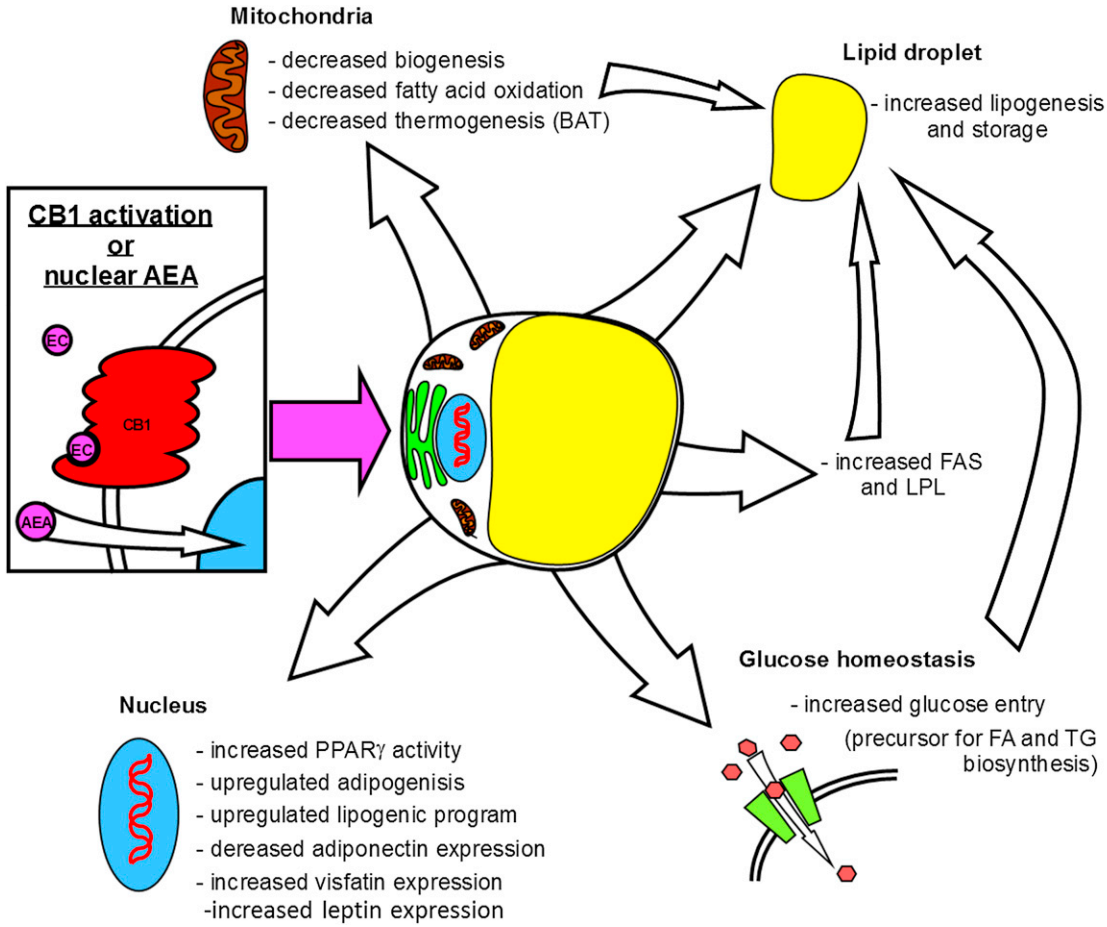


**Figure 2. Endocannabinoid Function in the Adipose Tissue**
AEA, anandamide; BAT, brown adipose tissue; EC, endocannabinoid; FA, fatty acid; FAS, fatty acid synthase; LPL, lipoprotein lipase; PPARγ, peroxisome proliferator-activated receptor-γ; TG, triglyceride.

a HFD for 3 days (O'Hare et al., 2011; Scherer et al., 2012). Accordingly, selective deletion of *CB1* receptors in central neurons confers resistance to HFD-induced obesity (Pang et al., 2011), whereas *CB1* deletion in both central and sympathetic neurons results in increased thermogenesis (Quarta et al., 2010). These data emphasize the key role of central-neuronal CB1 receptors in the control of both central and peripheral energy homeostasis. However, since central mechanisms of metabolic control can be both up- and downstream to peripheral ones, these findings in conditional *CB1* knockout mice do not rule out a role for peripheral, nonneuronal CB1 receptors in the metabolic syndrome (Figure 1).

## Control of Metabolism by the Peripheral and Nonneuronal Endocannabinoid System: Well-Established Mechanisms

### *White and Brown Adipocyte Endocannabinoids: Lipolysis, Lipogenesis, Inflammation, and Thermogenesis*

The WAT is the main energy repository organ of the human body through its capability of accumulating triglycerides. The WAT must be able to respond to the energetic status of the organism relatively quickly, by expanding in size not only through the accumulation of lipid stores but also via the recruitment of new adipocytes or by mobilizing lipid stores through increased metabolic flexibility (i.e., enhanced mitochondrial function and biogenesis) (Ahmadian et al., 2010). This ability results in the marked differences observed in the amount of WAT between individuals, which is not observed for other organ systems. In addition, the WAT is an endocrine organ that releases adipokines, like leptin and adiponectin, which are per se able to modulate energy homeostasis.

The presence of CB1 in mature white adipocytes, but not in preadipocytes, was demonstrated in both human primary cells and rodent primary cells and cell lines (Cota et al., 2003; Bensaid et al., 2003; Roche et al., 2006). These cells also express enzymes for the production and degradation of endocannabinoids (Blüher et al., 2006; Matias et al., 2006). Several studies have highlighted the function of CB1 receptors for 2-AG and AEA in the regulation of adipogenesis and lipogenesis. The biological actions reported thus far for CB1 activation in white adipocytes in vitro are all in the direction of maximizing fatty acid (FA) de novo biosynthesis and triglyceride (TG) accumulation and minimizing lipolysis (Figure 2). They include (1) activation

of glucose uptake, fatty acid synthase (FAS), and lipoprotein lipase (necessary for de novo FA biosynthesis and TG accumulation, respectively); (2) inhibition of cAMP release, adenosine monophosphate-activated protein kinase (AMPK), and mitochondrial biogenesis (and, as a consequence, of lipolysis and FA oxidation); (3) stimulation of PPARγ expression and adipogenesis; and (4) inhibition of adiponectin production in hypertrophic adipocytes (Di Marzo, 2008a; Silvestri et al., 2011; Vettor and Pagano, 2009). Opposite effects are, instead, usually observed after treatment of cultured adipocytes with CB1 receptor inverse agonists, which also reduce proinflammatory markers (Ge et al., 2012) in endocannabinoid-overproducing hypertrophic adipocytes. In fact, there is evidence that endocannabinoid production in white adipocytes is under the negative control of insulin (via stimulation of *Faah* expression) (D'Eon et al., 2008), PPARγ, and leptin (Matias et al., 2006), whereas CB1 expression is downregulated by PPARδ, after exercise in rats (Yan et al., 2007), and by PPARγ (Pagano et al., 2007). Importantly, leptin also inhibits WAT endocannabinoid levels through its action in the mediobasal hypothalamus (Buettner et al., 2008). These mechanisms may act as negative feedback to control endocannabinoid tone but become deranged after conditions leading to obesity, such as prolonged HFD and lack of exercise, thus subsequently contributing to further fat accumulation and macrophage infiltration in the WAT via excess CB1 activity. On the other hand, endocannabinoid/CB1 tone stimulates leptin signaling, since blockade of CB1 receptors with JD5037, a peripherally restricted CB1 inverse agonist, reverses hyperleptinemia in DIO mice by decreasing leptin expression and secretion by adipocytes (via both prejunctional and postjunctional mechanisms) and increasing leptin clearance by the kidney. This accounts for the unexpected reduction by JD5037 of food intake in fed animals, but not for its delay of meal initiation in fasted animals (Tam et al., 2012), which are expected to have low levels of leptin.

Although the picture seems to be quite clear in vitro, data obtained in vivo mostly with CB1 agonists or inverse agonists have provided contrasting results as to the actual contribution of adipocyte CB1 receptors to the control of energy metabolism. Thus, controversy exists as to whether these receptors control WAT lipogenesis and/or lipolysis only at the prejunctional (Mølhøj et al., 2010) or also at the postjunctional (Jourdan et al., 2010) level. Initial results with conditional and adipocyte-specific *CB1*$^{-/-}$ mice (Mancini et al., 2010) indicate that CB1 receptors in these cells do mediate at least part of the WAT accumulation and insulin resistance due to a prolonged HFD. Furthermore, recent evidence obtained in baboons through the use of radiolabeled FAs and TGs confirms the importance of the WAT in weight-loss-independent lipogenic and insulin-sensitizing actions of rimonabant (Vaidyanathan et al., 2012).

Functional CB1 receptors have been found also in brown adipocytes (Perwitz et al., 2006; Starowicz et al., 2008). However, most of the available data, obtained through the use of pharmacological and genetic tools inactivating CB1 receptors, point to inhibition of sympathetic inputs onto the brown adipose tissue (BAT) and decreased thermogenesis as one of the most important mechanisms through which CB1 receptor activation reduces energy expenditure by BAT and causes WAT accumulation in DIO mice (Bajzer et al., 2011; Quarta et al., 2010). The relevance of this to human obesity is yet unexplored.

### *Plasma Endocannabinoid Levels as Biomarkers of WAT Distribution and Insulin Resistance in Obesity*

Whatever the role of white adipocyte CB1 receptors, WAT AEA and 2-AG levels are usually deregulated in animal and human obesity. In particular, (1) in the visceral (i.e., intra-abdominal) WAT, the levels of both endocannabinoids and 2-AG are higher in DIO mice and in obese human subjects, respectively (D'Eon et al., 2008; Matias et al., 2006); (2) in the subcutaneous WAT, 2-AG and/or AEA levels are reduced in obese rodents (Izzo et al., 2009; Starowicz et al., 2008) and 2-AG levels are reduced in obese/T2D subjects and increased by weight loss (Annuzzi et al., 2010; Bennetzen et al., 2011); and (3) the amount of visceral, but not subcutaneous, WAT of obese human subjects is directly correlated with the plasma levels of 2-AG (Blüher et al., 2006; Côté et al., 2007). Thus, it is tempting to speculate that, when present, high circulating 2-AG levels in obese subjects reflect, in part, the upregulation of this endocannabinoid in visceral, but not subcutaneous, WAT. Accordingly, long-term weight loss and waist circumference reduction in intra-abdominally obese men was accompanied by a strong decrease in the plasma concentrations of 2-AG, directly correlated with decreased visceral adiposity, plasma TG levels, and insulin resistance (Di Marzo et al., 2009a). Given the very different metabolic roles of the two major types of WAT depots and the aforementioned prolipogenic action of CB1, deregulation of peripheral 2-AG levels might not only be the consequence but also one of the causes of increased visceral, at the expense of subcutaneous, WAT, and hence of insulin resistance. On the other hand, the circulating levels of AEA might reflect its concentrations in the subcutaneous WAT, particularly under conditions of strongly impaired insulin signaling, since overweight/obese type 2 diabetes (T2D) patients exhibit increased plasma AEA and 2-AG levels (Matias et al., 2006) but increased concentrations of only AEA in the subcutaneous WAT (Annuzzi et al., 2010). These data suggest that alterations of endocannabinoid levels in human obesity might occur in both a gender- and WAT-depot-specific and an insulin-dependent manner. Indeed, a recent study showed higher plasma 2-AG concentrations in men and AEA levels being correlated with adiposity and metabolic parameters in women (Fanelli et al., 2012). Genetic factors also play a role since the 385 C→A (P129T) mutation that causes FAAH to be less stable to degradation is associated with a high body mass index (BMI) and increased AEA levels, even when the latter were corrected for BMI (Sipe et al., 2010), and with a better initial percentage of excess weight loss 9 and 12 months after biliopancreatic diversion (de Luis et al., 2010).

Theoretically, stimulation of *FAAH* expression by insulin, observed in the subcutaneous tissue of lean but not obese patients (Murdolo et al., 2007), should control AEA, but not 2-AG, levels. It might thus explain not only the finding of decreased AEA but not 2-AG plasma levels in normoglycemic, and much less so in T2D, subjects after hyperinsulinemic/euglycemic clamps (Di Marzo et al., 2009c) and the postprandial decrease in AEA but not 2-AG plasma levels in normoweight but not obese subjects (Gatta-Cherifi et al., 2012; Matias et al., 2006), but also the observation of increased AEA but not 2-AG levels in the subcutaneous WAT of obese T2D subjects (Annuzzi

et al., 2010). Accordingly, increased plasma AEA levels in obese women are negatively correlated with *FAAH* messenger RNA (mRNA) expression in subcutaneous WAT (Engeli et al., 2005). Interestingly, it was recently shown that FAAH, but not MAGL, activity is increased in isolated adipocytes from non-insulin-resistant obese individuals in a way directly correlated with BMI (Cable et al., 2011), thus confirming the important role of insulin sensitivity, rather than BMI, in controlling WAT and circulating AEA levels via the upregulation of this enzyme. However, *FAAH* deregulation might also affect 2-AG levels, since increased plasma 2-AG levels correlate with decreased *FAAH* mRNA in visceral WAT of obese patients (Blüher et al., 2006).

In summary, although this possibility needs to be confirmed by studies employing cohorts of patients larger than those analyzed thus far, and via standardized methods dealing with the variability of and establishing reference levels for plasma endocannabinoids, the latter might in the future be considered as biomarkers of intra-abdominal versus subcutaneous WAT accumulation, in the case of 2-AG, and insulin-resistance in subcutaneous WAT, in the case of AEA. Prospective studies in which longitudinal analyses of multiple parameters of intra-abdominal obesity and their response to treatment are still required to assess whether plasma 2-AG levels might be used as predictive of responsiveness to therapeutic agents that counteract CB1 activity or as efficacy indicators of lifestyle or other pharmacological interventions aimed at specifically reducing this typical sign of the metabolic syndrome.

### *Hepatocyte Endocannabinoids: Glucose and Lipid Metabolism and Hepatic Insulin Resistance*

The liver plays key roles in regulating total body energy homeostasis, and its ability to do so is greatly affected by the occurrence of pathological conditions such as alcoholic or nonalcoholic fatty liver disease (NAFLD), which contribute to hepatic insulin resistance and end-stage liver disease-related mortality (Parekh and Anania, 2007). TG accumulation in hepatocytes results from the incorporation of plasma free fatty acids and de novo fat synthesis (Postic and Girard, 2008b). It is now well accepted that the hepatocytes express CB1 receptors and produce endocannabinoids (Kunos and Tam, 2011). During conditions that induce hepatosteatosis, be it diets rich in fat or alcohol, the liver expression of *CB1* and the levels of 2-AG and/or AEA are increased significantly in rodents (Jeong et al., 2008; Jourdan et al., 2010; Osei-Hyiaman et al., 2005), whereas hepatic CB1 is upregulated in patients with hepatosteatosis (Liu et al., 2012; Mendez-Sanchez et al., 2007). This increased ECS tone is due in part to the activation of a feed-forward loop, as *CB1* upregulation by both high fat and alcohol is CB1 dependent (Jourdan et al., 2010; Mukhopadhyay et al., 2010) and AEA increases *CB1* expression in organotypic liver slices, whereas rimonabant decreases it (Jourdan et al., 2012). Additionally, hepatic *CB2* mRNA is induced in obese mice, and CB2 agonism increases HFD-induced hepatosteatosis (Deveaux et al., 2009). CB1 and CB2 agonists increase the degree of steatosis of oleic acid-treated immortalized human hepatocytes, and CB2 agonism increases CB1 receptor expression (De Gottardi et al., 2010). Thus, CB2 receptors might also participate in the deregulation of hepatic function.

The hepatic ECS might contribute to fatty liver through disruption of hepatic lipogenic and lipolytic pathways and insulin signaling (Gary-Bobo et al., 2007; Jeong et al., 2008; Jourdan et al., 2010; Osei-Hyiaman et al., 2005; Osei-Hyiaman et al., 2008) (Figure 3A). The key lipogenic transcription factor sterol regulatory element binding transcription factor 1 (SREBF1) is upregulated by CB1, resulting in the enhanced expression of acetyl-Coenzyme A (CoA) carboxylase-$\alpha$ (*Acaca*, aka *Acc1*) and *Fas*, key enzymes in the regulation of lipogenesis. CB1 blockade causes downregulation of these enzymes (Jeong et al., 2008; Osei-Hyiaman et al., 2005; Tam et al., 2010). Conversely, AEA stimulates lipogenic genes in liver slices, in a manner reversed by rimonabant (Jourdan et al., 2012). In mice, acute pharmacological upregulation of 2-AG levels via a single dose of an inhibitor of 2-AG hydrolysis results in a significant increase in hepatic triglyceride levels, as well as insulin resistance, and a microarray cluster analysis identified the functional category of lipid, fatty acid, and steroid metabolism genes, including many SREBF1 targets, as being regulated in a CB1-dependent manner (Ruby et al., 2011).

AMPK is a key metabolic regulator, and in the liver it controls the expression and activity of several lipogenic factors, including SREBF1 (Viollet et al., 2009). CB1 activation by $\Delta^9$-THC in rats, and ACEA in mice on a HFD, decreases liver AMPK activity (Kola et al., 2005; Tedesco et al., 2010), whereas CB1 antagonism increases it in organotypic liver slices (Jourdan et al., 2012). CB1 blockade by rimonabant decreases lipogenesis through AMPK via the cAMP-dependent protein kinase A (PKA)-liver kinase B1 (LKB1) axis and downstream of $G\alpha_{i/o}$ inhibition. AMPK in turn inactivates the liver X receptor $\alpha$ (LXR$\alpha$), which is responsible for *Srebf1* expression (Wu et al., 2011). Presumably, activation of CB1 and $G\alpha_{i/o}$ works through the same pathway but with the opposite effects. AMPK also increases mitochondrial $\beta$-oxidation of fatty acids as a result of reduced malonyl CoA levels, leading to carnitine palmitoyltransferase 1a (Cpt1a) activation and fatty acid shuttling into mitochondria (Viollet et al., 2009). Accordingly, rimonabant decreases malonyl CoA in the liver of mice on a high-sugar and high-fat diet (Jourdan et al., 2010) and increases liver mitochondrial oxygen consumption and lipid $\beta$-oxidation in mice and liver slices (Flamment et al., 2009; Jourdan et al., 2012), whereas CB1 agonism decreases Cpt1a activity to increase liver lipogenesis (Osei-Hyiaman et al., 2008). Additionally, global or liver-specific CB1 knockout results in increased hepatic AMPK activation and Cpt1a levels and activity (Jeong et al., 2008; Osei-Hyiaman et al., 2008). CB1-mediated AMPK inhibition not only inhibits mitochondrial activity but also leads to decreased mitochondrial biogenesis in the liver of mice on a HFD (Tedesco et al., 2010). The ECS might impinge upon AMPK also indirectly via inhibition of adiponectin production (Kim et al., 2012a), since adiponectin stimulates hepatic AMPK activation and fatty acid entry into mitochondrial $\beta$-oxidation (Yamauchi et al., 2002) (Figure 3A).

Fatty liver caused by HFD is also associated with insulin resistance, characterized by the elevation of hepatic glucose production, plasma hyperglycemia, and hyperinsulinemia (Parekh and Anania, 2007; Postic and Girard, 2008a). Given the effects of the ECS on hepatosteatosis, it is therefore not surprising that CB1 activation in humans and rats is associated with decreased glucose tolerance (Bermúdez-Siva et al., 2006). Therefore, the amelioration of liver steatosis observed with CB1 antagonism or knockout is similarly associated with an improvement in

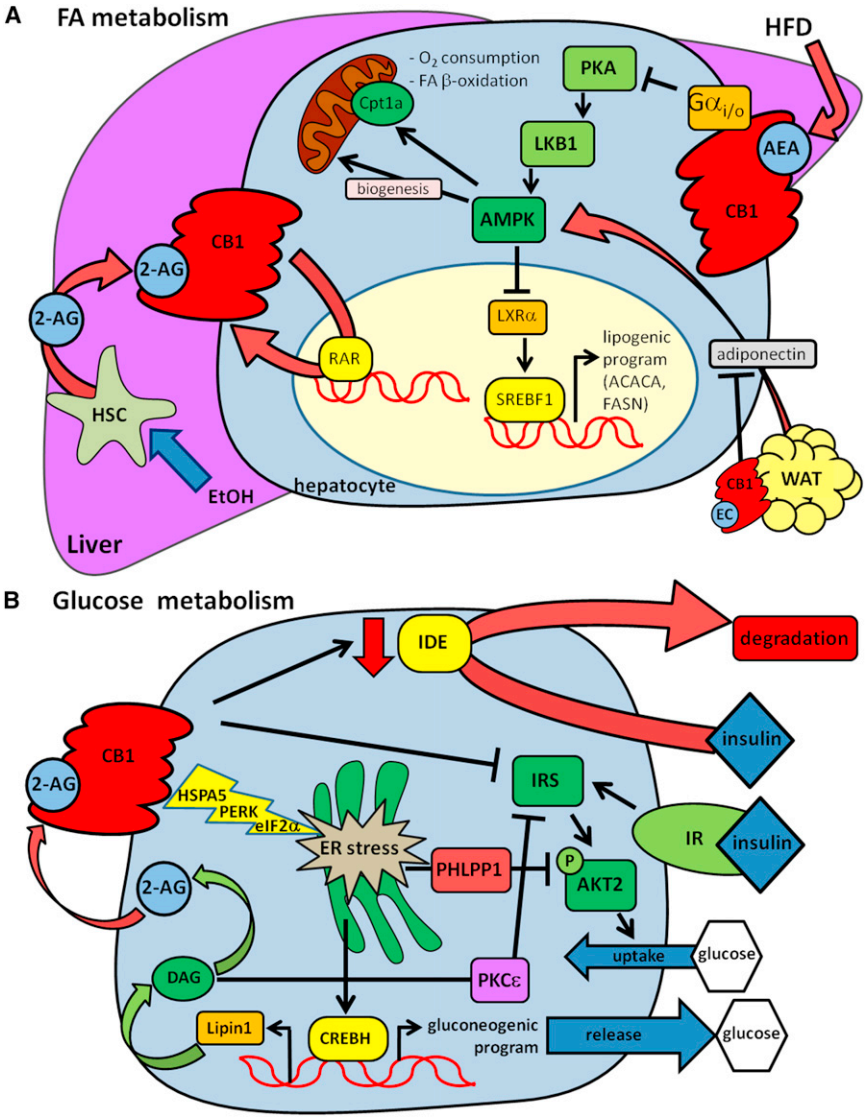


**Figure 3. Endocannabinoid Role in Lipid and Glucose Metabolism in the Liver**

Endocannabinoid control of hepatocyte (A) triglyceride levels and (B) insulin sensitivity and glucose production. In (B), note how hepatocyte CB1 activation has been proposed to inhibit insulin signaling by two mechanisms, i.e., upregulation of inhibitory phosphorylation of insulin receptor substrate (IRS) and stimulation of inhibitory dephosphorylation of insulin-activated protein kinase b (AKT2) via the upregulation of the S/T phosphatase PH domain and leucine-rich repeat protein phosphatase 1 (PHLPP11) downstream of a pathway dependent on heat shock protein 5 (HSPA5), PRKR-like endoplasmic reticulum kinase (PERK) and eukaryotic translation initiation factor 2 subunit 1 alpha (eIF2α) and subsequent endoplasmic reticulum (ER) stress (Liu et al., 2012). CB1-activated ER stress is also implicated in the upregulation of the liver-specific transcription factor cAMP-responsive element-binding protein H (CREBH), which increases gluconeogenic gene expression and glucose production via Lipin1 and potential feed-forward onto endocannabinoid (EC) biosynthesis (Chanda et al., 2011). ACACA, acetyl-Coenzyme A carboxylase-α; CPT1a, carnitine palmitoyltransferase 1a; CREBH, cAMP-responsive element-binding protein H; DAG, diacyl glycerol; FA, fatty acid; FAS, fatty acid synthase; HFD, high-fat diet; HSC, hepatic stellate cell; IDE, insulin-degrading enzyme; IR, insulin receptor; LKB1, liver kinase B1; LXRa, liver X receptor a; PKA, cAMP-dependent protein kinase A; PKCε, protein kinase Cε; RAR, retinoic acid receptor; SREBF1, sterol regulatory element binding factor 1.

insulin and glucose sensitivity (Gary-Bobo et al., 2007; Liu et al., 2012; Osei-Hyiaman et al., 2008; Ravinet Trillou et al., 2004). Mice with hepatocyte-specific knockout of *CB1* are not protected from increased weight and total adiposity in response to DIO but are resistant to the development of hepatosteatosis and insulin insensitivity (Liu et al., 2012; Osei-Hyiaman et al., 2008). However, acute upregulation of 2-AG levels results in decreased glucose tolerance after only a few hours (Ruby et al., 2011), and more-recent studies have highlighted direct (i.e., fatty-liver-independent) effects of hepatic CB1 receptors on insulin sensitivity in obesity. Mice in which *CB1* expression was reintroduced on a CB1 global knockout at levels similar to those induced by DIO (denoted as *htgCB1*$^{-/-}$ [hepatocyte transgenic *CB1*$^{-/-}$] mice) provided important insights into the molecular mechanism involved in this latter phenomenon (Liu et al., 2012). The *htgCB1*$^{-/-}$ mice exhibit much higher basal circulating glucose and insulin levels, and while they remain lean when maintained on a HFD, exactly like *CB1*$^{-/-}$ mice, they develop strong hepatic and systemic insulin resistance, opposite to mice with hepatocyte-specific *CB1* knockout, which become obese but remain insulin-sensitive, supporting the notion that hepatic CB1 reduces insulin sensitivity without affecting body weight (Liu et al., 2012; Osei-Hyiaman et al., 2008). Increased insulin levels in *htgCB1*$^{-/-}$ mice are associated with lower insulin-degrading enzyme (IDE) expression in the liver, which is also observed in wild-type mice with DIO, showing that hepatic CB1 is involved in regulating insulin clearance. Further, the study indicated that hepatic CB1 inhibits insulin signaling by upregulation of inhibitory phosphorylation of insulin receptor substrate (IRS) and stimulation of inhibitory dephosphorylation of insulin-activated protein kinase b (PKBβ/AKT2) via an endoplasmic reticulum (ER) stress-dependent pathway (Liu et al., 2012) (Figure 3B). The *htgCB1*$^{-/-}$ mice also exhibited increased glycogen phosphorylase-a activity, indicating that the increased hepatic glucose production observed was due to an increase in glycogenolysis. Interestingly, ER stress seems to also be implicated in the upregulation of gluconeogenic gene expression and glucose production by 2-AG in primary human hepatocytes, which occurs via CB1-mediated upregulation of the ER stress liver-specific transcription factor cAMP-responsive element binding protein 3-like 3 (*Crebh*) (Chanda et al., 2011). Thus, CB1 causes hepatocyte ER stress during obesity, perhaps also through AMPK (Dong et al., 2010), and this effect is critical to

its modulation of insulin signaling. Treatment of hepatocytes with 2-AG also induces, via CREBH activation, the expression of *Lipin1*, a phosphatidic acid phosphatase, and the subsequent release of DAG and phosphorylation of protein kinase C-ε, with inhibition of hepatic insulin receptor signaling. Knockdown of CREBH or CB1 antagonism attenuates 2-AG-mediated induction of *Lipin1* gene expression, decreases DAG production in mouse liver, and restores insulin receptor signaling (Chanda et al., 2011). Given the role of DAG in the biosynthesis of 2-AG, this mechanism might generate an ECS-mediated feed-forward loop leading to hepatic insulin resistance (Figure 3B).

## Control of Metabolism by the Peripheral and Nonneuronal Endocannabinoid System: Emerging Mechanisms

### *Muscle Endocannabinoids: Insulin Sensitivity and Beyond*

Muscle is a key tissue in the regulation of energy homeostasis, not only during periods of high activity, requiring large amounts of energy, but also during rest or basal activity, making it a major site of fatty acid β-oxidation and sink for glucose and thus a principal site of insulin-induced glucose mobilization (Abdul-Ghani and DeFronzo, 2010). It is now well accepted that muscle cells produce endocannabinoids and express a large number of the components of the ECS, including its synthesizing and degrading enzymes and its receptors (Cavuoto et al., 2007a, 2007b; Crespillo et al., 2011; Eckardt et al., 2009; Esposito et al., 2008; Lipina et al., 2010). There exist, however, discrepancies within the literature as to whether the muscle ECS becomes deregulated under conditions of obesity and/or insulin resistance. In primary skeletal muscle myotubes of lean and obese subjects, *CB1* gene expression remains unchanged (Cavuoto et al., 2007a). In rats fed a HFD, abdominal wall skeletal muscle gene expression of *CB2* and *Magl* is decreased and increased, respectively (Crespillo et al., 2011). In genetically obese Zucker rats, *CB1* mRNA levels in soleus are decreased (Lindborg et al., 2010), whereas AEA levels are increased (V.D., unpublished data). However, *CB1* expression in soleus muscle from mice on a HFD is increased (Pagotto et al., 2006). These data make it difficult to predict the possible effects of obesity on overall endocannabinoid tone in muscle and indicate that ECS response to the metabolic state of the organism might have muscle-subtype- or genetic-specific differences. In the soleus muscle from mice on a HFD for 14 weeks, 2-AG, but not AEA, levels were significantly elevated at the beginning of the study and near the end, when the animals had obesity and hyperglycaemia, but not in the middle (Matias et al., 2008b). This suggests that muscle endocannabinoid production is modulated by HFD and that compensatory mechanisms might overcome these changes initially but not once the organism has become obese and insulin resistant.

Pharmacological inhibition of CB1 activity in obese humans or in genetically or diet-induced obese rodents results in increased energy expenditure/oxygen consumption, which was not explainable by increased physical activity but rather associated with elevated FA oxidation (Addy et al., 2008; Herling et al., 2008; Kunz et al., 2008; Liu et al., 2005). Accordingly, studies on isolated myotubes indicate that the ECS has a negative impact on muscle oxidative pathways. AMPKα1 is a positive modulator of glucose and fatty acid oxidation and mitochondrial biogenesis, and while in myotubes from lean and obese donors AEA only produces a small increase in its levels, CB1 inhibition by AM251 significantly increases *AMPKα1* (similar to results obtained in liver) (Cavuoto et al., 2007a; Jourdan et al., 2012). In "lean" myotubes, however, AEA does increase the expression of pyruvate dehydrogenase kinase isoenzyme 4 (*PDK4*), an inhibitor of the pyruvate dehydrogenase complex that links glycolysis to the citric acid cycle and thus a negative regulator of mitochondrial glucose oxidative metabolism that is downregulated by insulin (Majer et al., 1998), whereas CB1 antagonism decreased the expression of this enzyme (Cavuoto et al., 2007a). These data, taken together with CB1 reduction of muscle mitochondrial biogenesis (Tedesco et al., 2010), imply that overactivity of the ECS in this tissue might drive defective skeletal muscle oxidative metabolism through impaired mitochondrial oxidative phosphorylation (Abdul-Ghani and DeFronzo, 2010).

Chronic treatment of *ob/ob* mice and lean or obese Zucker rats with rimonabant resulted in increased glucose uptake in the soleus muscle (Lindborg et al., 2011; Liu et al., 2005). Conversely, systemic (intravenous), but not central (intracerebroventricular), administration of a single dose of the CB1/CB2 agonist, HU210, resulted in the CB1-dependent reduction of whole-body glucose clearance and glucose transport into muscle but not adipose tissue (Song et al., 2011), indicating that HU210 may act directly on muscle CB1 receptors. Indeed, in soleus muscle, explant cultures from lean and insulin-resistant obese Zucker rats, AEA and rimonabant significantly decreased and increased both basal and insulin-dependent glucose import, respectively (Lindborg et al., 2010). In this study, as in subsequent work (Lindborg et al., 2011), no effects were observed on canonical insulin-independent or -dependent glucose transport pathways. Yet other studies have reported that, instead, CB1 impinges on muscle insulin signaling. In vivo, acute treatment of mice with HU210 resulted in decreased insulin-dependent AKT phosphorylation in hind leg muscle (Song et al., 2011). These results are consistent with in vitro data in L6 rat myotubes in which rimonabant or CB1 knockdown increased glucose uptake, with the former being accompanied by increased expression of phosphatidylinositol 3-kinase (PI3K) catalytic and regulatory subunits and subsequently by AKT and mitochondrial pyruvate dehydrogenase lipoamide kinase isozyme 1 (PDK1) and protein kinase cζ (PKCζ) activation (Esposito et al., 2008). Further, in an in vitro study that points to an endogenous role for ECS-mediated regulation of insulin sensitivity in mice, it was found that adipocyte-conditioned media (24 hr treatment) or AEA (24 or 1 hr treatment) inhibited insulin-dependent glucose uptake and AKT activation in skeletal muscle cells in a CB1-dependent manner (Eckardt et al., 2009). Conversely, in another study still in L6 myotubes, while 24 hr treatment with ACEA had no effect on insulin-dependent AKT activation, rimonabant sensitized AKT to insulin-dependent activation and increased phosphorylation of its targets, forkhead box O3A (Foxo3a) and glycogen synthase kinase 3 α/β (GSK3α/β) (Lipina et al., 2010). Despite some inconsistencies, altogether these data indicate that the ECS negatively regulates the P13K-AKT and P13K-PDK-PKCζ pathways downstream of insulin, which are required for glucose transporter 1/4 (GLUT1/4) translocation and glucose uptake. However, and surprisingly,

in none of the studies above was GLUT1/4 expression found to be altered, indicating that the ECS may posttranslationally regulate these proteins.

It should be noted that, in addition to the above actions, the ECS modifies other insulin signaling effectors as well. For instance, high AEA levels caused inhibitory insulin receptor substrate 1 (IRS1) phosphorylation (Eckardt et al., 2009). Further, the ECS modulates the MAPK arm of the insulin pathway that regulates gene expression and cell proliferation, as rimonabant stimulated, and the CB1 agonist ACEA inhibited, insulin-dependent extracellular signal-regulated kinase 1/2 (ERK1/2) activation (Lipina et al., 2010). Finally, at high concentrations, AEA activated p38 (in a sustained manner) and ERK1/2 (transiently) in primary human skeletal muscle cells (Eckardt et al., 2009), an effect that might be due to activation of non-CB1 receptors and underlie some of the beneficial effects of intensive exercise, given the stimulatory effect of the latter on plasma AEA levels (for a review, see Heyman et al., 2012).

### *Pancreatic Endocannabinoids: Direct Effects on Insulin Secretion or β Cell Health?*

The understanding of the role of the endocannabinoid system in β cells has been hindered by controversy regarding the presence of CB1 and CB2 receptors in these cells. However, most researchers agree that β cells do express the former receptor, although it is not clear yet whether CB1 activation controls only insulin signaling (and hence β cell health), as in hepatocytes and myotubes, or also insulin release. Furthermore, as in the case of adipocytes, the contribution of β cell CB1 receptors to the control of energy homeostasis has found so far support only from in vitro studies. Current data suggest that, although counterintuitive given the reduction of glucose tolerance observed after acute or chronic systemic administration of CB1 agonists (Bermúdez-Siva et al., 2006; Liu et al., 2012), in vitro stimulation of these receptors in β cells enhances, rather than reduces, either basal or glucose-stimulated (or both) insulin release (Bermúdez-Silva et al., 2008; Li et al., 2010; Matias et al., 2006). Accordingly, when using pancreatic Langerhan's islet preparations from lean and Zucker diabetic fatty rats, the latter of which likely exhibit elevated endocannabinoid levels (Izzo et al., 2009) much in the same way those from DIO mice do (Starowicz et al., 2008), Getty-Kaushik and colleagues (2009) found that CB1 blockade with rimonabant decreases basal insulin hypersecretion (Getty-Kaushik et al., 2009). Rimonabant also decreased nonstimulated insulin hypersecretion in islets from lean rats that had been treated with high glucose for 24 hr, a condition similar to that found to enhance basal endocannabinoid levels in a rat β cell line (Matias et al., 2006). On the other hand, rimonabant did not affect glucose-stimulated insulin secretion by islets from obese rats or glucose-treated islets from lean rats, whereas it did in untreated islets from lean rats. Thus, CB1 antagonism in islets reduces insulin secretion only when this is elevated above normal levels by diet or obesity, possibly because of higher endocannabinoid tone during these conditions. Especially if it also contributes to insulin resistance in the liver or skeletal muscle (see above), endocannabinoid overactivation of CB1 receptors in β cells during obesity, and the subsequent hypersecretion of insulin, might first represent an attempt at compensation for insulin resistance and, later, in the continued presence of insulin signaling impairment, contribute to β cell stress and damage. This might explain why another CB1 receptor inverse agonist, ibipinabant, attenuated β cell loss in Zucker diabetic fatty rats independently of its effects on body weight (Rohrbach et al., 2012).

Recently, another mechanism has been put forward that might explain why CB1 receptor blockade ameliorates β cell damage in obesity. It was shown that CB1 activation inhibits insulin signaling in β cells by preventing insulin-stimulated insulin receptor (IR) autophosphorylation in a $G_{\alpha i}$-dependent manner, whereas pharmacologic blockade of CB1 results in enhanced IR signaling through the AKT2 pathway in β cells and leads to increased β cell proliferation and mass both in vitro and in vivo in diabetic *db/db* mice (Kim et al., 2011). From the molecular point of view, the mechanism by which CB1 inhibits IR signaling seems to be quite novel. In fact, the same authors reported in a subsequent study (Kim et al., 2012b) that CB1 forms a heteromeric complex with the IR and $G_{\alpha i}$, leading to inhibition of the kinase activity of the IR by directly binding to the activation loop in the tyrosine kinase domain of this protein. IR impairment then leads to reduced phosphorylation of the proapoptotic protein *Bad* and subsequent stimulation of its apoptotic activity, thereby causing β cell death. Therefore, pharmacological blockade of CB1 receptors might represent a therapeutic opportunity for diabetes, independent of its other actions on body weight, WAT inflammation, and hepatic and muscle insulin resistance.

## Endocannabinoid Congeners and Metabolites and Their Emerging Role in Metabolic Control

As mentioned above, both AEA and 2-AG are biosynthesized in tissues together with congeners, the *N*-acyl-ethanolamines, and MAGs, which may stimulate metabolically active non-CB1, non-CB2 receptors, and are inactivated by FAAH and MAGL, respectively. PEA and, particularly, OEA activation of PPARα, unlike CB1, may lead to food-intake inhibition, lipolysis in the liver and adipose tissue, and induction of satiety via small intestine-mediated mechanisms (Schwartz et al., 2008). Also, activation of TRPV1, a potential target for OEA, PEA, and AEA (Di Marzo and De Petrocellis, 2010), stimulates lipolysis and improves mitochondrial activity in the skeletal muscle (Luo et al., 2012), two effects that are again the opposite of those exerted by CB1 activation (Tedesco et al., 2010). On the other hand, MAGs such as 2-oleoyl- and 2-linoleoyl-glycerol activate GPR119 in the small intestine, with subsequent stimulation of GLP-1 release and inhibitory and stimulatory effects of food intake and insulin secretion, respectively (Lan et al., 2012; Hansen et al., 2012). Although the role of endocannabinoid congeners in energy homeostasis and its pathological perturbations has not yet been fully investigated, stimuli leading to endocannabinoid biosynthesis and inactivation may also cause alterations in the tissue levels of these other mediators, the action of which might reinforce or, more likely, oppose that of CB1 receptor activation. Furthermore, given the important impact of the diet on the FA composition of phospholipid classes acting as ultimate biosynthetic precursors for endocannabinoids and their congeners, it's intriguing to speculate that the role of this "endocannabinoidome" in energy homeostasis, as well as in the etiopathology of metabolic disorders, might also depend on dietary factors, which, however, might or might not affect in

the same way lipid signaling pathways leading to positive (i.e., endocannabinoids, which are ultimately derived from AA and hence linoleic acid) and negative (i.e., palmitic-, oleic-, or linoleic-acid-containing *N*-acylethanolamines and MAGs) energy balance. In this sense, it is interesting to observe how a diet rich in linoleic acid was recently suggested to lead to obesity in mice in part by engendering excess peripheral endocannabinoid levels (Alvheim et al., 2012).

The co-occurrence in tissues of lipid mediators with similar biosynthetic and inactivating pathways but opposing metabolic actions may complicate the correct interpretation of data obtained in genetically modified mice in which such pathways have been inactivated. An example of this situation is the recently described phenotype of mice in which *Magl* was overexpressed in the small intestine (Chon et al., 2012), where activation of CB1 inhibits satiety. These mice are characterized by reduced energy expenditure and propensity to obesity after a HFD, a behavior opposite of what would be expected from a chronic reduction of 2-AG levels and also of that observed when the enzyme is overexpressed in forebrain neurons (Jung et al., 2012). However, if one remembers that MAGL overexpression in the small intestine may also lead to reduced activation of GPR119 in enterocrine L-cells by MAGs other than 2-AG, and subsequently to reduced release of GLP-1, one may well expect obesity in these mice. Likewise, it is possible that *Magl*-null mice do not exhibit the expected propensity to obesity and, in fact, show attenuated diet-induced insulin resistance (Taschler et al., 2011) because the action of elevated MAGs in metabolically active peripheral tissues expressing GPR119 receptors predominates on CB1 activation by upregulated 2-AG. However, as expected, *Faah*-null mice on a HFD exhibit stronger WAT accumulation, high plasma TG levels, and glucose intolerance as compared to wild-type mice (Touriño et al., 2010), despite the fact that they contain higher levels not only of the orexigenic and lipogenic mediator, AEA, but also of the anorexigenic and lipolytic mediator, OEA. Thus, one may speculate that concomitant activation of PPAR$\alpha$ and TRPV1 by OEA (and PEA) in these mice is not sufficient to override the effect of CB1 overactivation by AEA.

Recently, we have also investigated the potential role in WAT biology of another proposed route of endocannabinoid catabolism, i.e., that catalyzed by COX-2 and leading to prostaglandin analogs of AEA and 2-AG (Kozak et al., 2002). We found that the former metabolites, also known as prostamides, and in particular prostamide $F_{2\alpha}$, are produced in preadipocytes at the expense of AEA and exert antiadipogenic actions in these cells by activating a non-FP (prostaglandin F receptor), non-CB1 receptor (C.S., N. Poloso, D. Woodward, and V.D., unpublished data). We proposed that preadipocyte prostamide signaling represents a negative paracrine feedback mechanism to switch from an adipogenic (i.e., AEA) to an antiadipogenic pathway, which would be inhibited under conditions requiring the presence of new adipocytes, such as after a short period of HFD. However, during obesity, which is accompanied by COX-2 (Hsieh et al., 2010) and AEA level upregulation, FAAH downregulation, and inflammation in the WAT, the formation of prostamide $F_{2\alpha}$ might be enhanced, thus increasing the number of hypertrophic adipocytes at the expense of new small adipocytes and further favoring WAT inflammation.

## New Therapies for Correction of Endocannabinoid Dysregulation in Metabolic Disorders

To date, the majority of efforts to deal with upregulated ECS tone under conditions of excessive adiposity and metabolic deregulation have dealt with pharmacological antagonism of the CB1 receptor, exemplified by the development of the systemically penetrant CB1 inverse agonist, antiobesity drug rimonabant. However, the potential consequences associated with this drug's central side effects have resulted in the abandonment of pursuits to produce similar compounds. The development of neutral antagonists and peripherally restricted inverse agonists at CB1 receptors is being actively pursued (for a review, see Silvestri and Di Marzo, 2012) and has already provided very promising results at the preclinical level, particularly in terms of their reversal of insulin and leptin resistance and also of unexpected differences in the appetitive and metabolic profile of one such class of compounds versus the other (Tam et al., 2010, 2012) (Table 1). Given that at least part of the increased pathological tone of the ECS can be attributed to increased endocannabinoid biosynthesis, the regulation of this process also presents a seemingly viable strategy to regulate the ECS. Evidence of the efficacy of this strategy has been provided by the manipulation of 2-AG levels in two different ways. Systemic administration of DAGL inhibitors decreased 2-AG biosynthesis and inhibited palatable or HFD intake (Bisogno et al., 2009; Bisogno et al., 2012), whereas the overexpression of *Magl* specifically within forebrain neurons in mice resulted in increased energy expenditure and decreased weight gain on a HFD (Jung et al., 2012).

It is now accepted that dietary intake of the n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) reduce the levels of AA-esterified phospholipids (Kinsella, 1990), which include the *sn*-2- and *sn*-1-AA-containing phospholipids acting as ultimate precursors for 2-AG and AEA, respectively (Di Marzo, 2008b). Treatment of mouse adipocytes with AA increases 2-AG levels, whereas DHA or EHA decrease AEA and 2-AG levels with concomitant reduction of AA-esterified phospholipids (Matias et al., 2008a). Thus, dietary n-3 PUFAs may be able to correct an overactive ECS by modulating endocannabinoid precursor levels (Di Marzo, 2008b; Kinsella, 1990; Piscitelli et al., 2011). In fact, a linoleic-acid-enriched diet elevated peripheral AEA and 2-AG levels along with inducing obesity, unless high levels of DHA and EPA were included in the diet (Alvheim et al., 2012). Several in vivo studies with dietary n-3-PUFA-rich fish or krill oils have shown that such treatments reduce peripheral endocannabinoid levels without weight loss in rodents and humans, and in rats this effect occurs concomitantly to reduced ectopic fat in the liver and heart (Banni et al., 2011; Batetta et al., 2009; Di Marzo et al., 2010; Piscitelli et al., 2011). More recently, it was shown that dietary phospholipids enriched in EPA and DHA are superior to the corresponding TGs at ameliorating the metabolic profiles of obese mice, including significant reductions in hepatosteatosis, circulating insulin levels, and WAT hypertrophy (Rossmeisl et al., 2012). However, although generally less effective at modulating brain endocannabinoid tone in adult mice, changes in dietary n-3 PUFA must be considered with caution in newborns as it can cause deep and long-lasting alterations in brain phospholipid composition and function (Lafourcade

**Table 1. Therapeutic Strategies against Obesity and the Metabolic Syndrome that Are Based on or Affect Endocannabinoid Deregulation**

| Therapy | Inhibitory Effect on EC Tone | Reduction of Food Intake | Reduction of Body Weight | Reduction of Glucose Intolerance and Insulin Resistance[a] | Reduction of Plasma Triglycerides | Reduction of Visceral Adipose Tissue[b] | Reduction of Liver Fat | Central Side Effects | References |
|---|---|---|---|---|---|---|---|---|---|
| CB1 inverse agonists | In all tissues where EC levels are higher and/or CB1 is constitutively coupled to G protein | Yes, transient | Yes | Yes | Yes | Yes | Yes | Yes | (Addy et al., 2008; Bajzer et al., 2011; Colombo et al., 1998; Gary-Bobo et al., 2007; Jourdan et al., 2010; Kim et al., 2012a; Mølhøj et al., 2010; Rohrbach et al., 2012, Vaidyanathan et al., 2012) |
| CB1 neutral antagonists | In all tissues where EC levels are higher | Yes | Yes | Yes | Yes | Not yet assessed | Not yet assessed | Not yet fully assessed | (Silvestri and Di Marzo, 2012) |
| Peripherally restricted CB1 blockers | In all peripheral tissues where EC levels are higher and/or CB1 is constitutively coupled to G protein | Yes, through resensitization to leptin, observed with a CB1 inverse agonists | Yes | Yes | Yes | Not yet assessed | Yes | No | (Silvestri and Di Marzo, 2012; Tam et al., 2010, 2012) |
| DAGL inhibitors | In all tissues where EC levels are higher | Yes | Yes | Not yet assessed | Not yet assessed | Not yet assessed | Not yet assessed | Not yet assessed | (Bisogno et al., 2009, 2012) |
| Dietary n-3 PUFAs | In all peripheral tissues where EC levels are higher | No | No | Yes | Yes | Not yet assessed | Yes | No | (Banni et al., 2011; Batetta et al., 2009; Di Marzo et al., 2010; Matias et al., 2008a, Piscitelli et al., 2011; Rossmeisl et al., 2012) |
| Lifestyle (exercise + caloric restriction) | Plasma EC levels | – | Yes | Yes | Yes | Yes | Yes | No | (Bennetzen et al., 2011; Di Marzo, et al., 2009a; Heyman et al., 2012) |

EC, endocannabinoid; n-3 PUFAs, n-3 polyunsaturated fatty acids (i.e., docosahexaenoic and eicosapentaenoic acids).
[a]Fasting glucose or insulin, oral glucose tolerance test.
[b]As assessed by waist circumference or computer tomography scans in humans.

et al., 2011; D'Asti et al., 2010). Furthermore, since dietary EPA and DHA may reduce not only endocannabinoid levels but also those of other AA-derived metabolites, while increasing the tissue concentrations of n-3-PUFA-containing ethanolamides and glycerol esters potentially acting at noncannabinoid receptors (Brown et al., 2013), one will need to evaluate also the distinct role of each of these metabolites in n-3-PUFA-induced metabolic benefits. At any rate, the use of EPA and DHA as negative regulators of peripheral endocannabinoid upregulation merits further study and the development of specific clinical trials as it may provide a more easily applied regime for the prevention and treatment of the metabolic syndrome.

## Concluding Remarks: The Dawn of a New Beginning?

Research in the last decade has considerably increased our knowledge of the complexities and peculiarities of the ECS and what is emerging as its crucial intermediary role between circulating hormones and locally acting neurotransmitters or neuropeptides in nearly all aspects of energy homeostasis control. We are now starting to gather that this function is not just played by the usual suspects, i.e., AEA and 2-AG, or CB1 receptors. Indeed, CB2 receptors, which were initially considered to be devoid of a metabolic role, are now emerging as potential players not only, as it could be expected from their high expression in immune cells, in the inflammatory aspects of obesity and T2D (Pacher and Mechoulam, 2011), but perhaps also in the physiological control of hepatic lipogenesis and glucose tolerance at both peripheral (Agudo et al., 2010; De Gottardi et al., 2010; Deveaux et al., 2009) and, possibly, central (Romero-Zerbo et al., 2012) levels. However, as compared to the great wealth of descriptive and mechanistic information available on the function and dysfunction of CB1 in metabolism, much work is still required to fully understand the exact role of CB2, or of PPARs and TRPV1, in endocannabinoid control of metabolism, or to conclude that the several endocannabinoid-related mediators identified to date, through their putative or established molecular targets, do contribute to this already variegated scenario. Also thanks to the precocious (and, for now, discontinued) clinical development of inverse agonists as drugs against obesity first and T2D later, we do know, however, that rodent data on the role of CB1 in metabolic control can be translated to humans. Furthermore, the deregulated peripheral ECS, of which plasma endocannabinoid levels are probably a reflection, might be not only a secondary biomarker but also a key causative factor of metabolic disorders. Despite the fact that the strong connections between brain and periphery in the control of body weight cannot always be teased out experimentally with tissue-specific CB1 knockout mice, there is sufficient evidence to suggest that the metabolic effects of drugs counteracting CB1 activity is not only secondary to weight loss. Clinical trials with peripherally restricted pharmacological tools or, maybe even better, yet-to-be-devised organ-selective drugs (e.g., prodrugs exploiting the relative abundance of certain enzymes in the liver) must confirm this hypothesis and tell us whether it can be used to develop new and safe therapies against dyslipidemia, insulin resistance, and β cell damage. New endocannabinoid-based interventions are emerging, and, despite the disappointment caused by the failure of first-generation CB1 receptor blockers, optimism still exists regarding the future development of such therapies against the metabolic syndrome.

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