Feature Review

The Endocannabinoid System: Pivotal Orchestrator of Obesity and Metabolic Disease

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The endocannabinoid system (ECS) functions to adjust behavior and metabolism according to environmental changes in food availability. Its actions range from the regulation of sensory responses to the development of preference for the consumption of calorically-rich food and control of its metabolic handling. ECS activity is beneficial when access to food is scarce or unpredictable. However, when food is plentiful, the ECS favors obesity and metabolic disease. We review recent advances in understanding the roles of the ECS in energy balance, and discuss newly identified mechanisms of action that, after the withdrawal of first generation cannabinoid type 1 (CB1) receptor antagonists for the treatment of obesity, have made the ECS once again an attractive target for therapy.

From the ‘Munchies’ to the ECS

The ECS exerts regulatory control essentially on every aspect related to search, intake, metabolism, and storage of calories, and it has been thus recognized to have a crucial role in the regulation of energy balance. This system, which is particularly well preserved across species [1], seems to have been selected by evolution to maximize intake and conservation of energy, likely to increase survival in times of scarcity [2,3]. Accordingly, activation of the ECS promotes consumption of palatable food, stimulates fat mass expansion and calorie preservation, while inhibiting energy expenditure and thermogenesis (see Glossary). However, in modern society where food is plentiful, excessive ECS activity is a landmark feature of obesity and metabolic disorders [4,5].

The first reports of increased appetite induced by cannabis (also known as marijuana) in humans were documented in AD 300 [6]. However, understanding the biological mechanisms underlying the ‘munchies’ started only after the discovery of specific G-protein-coupled cannabinoid receptors, CB1 and CB2, followed by the identification of endogenous lipid-derived ligands, termed endocannabinoids, and elucidation of their biosynthesis and degradation pathways [7,8] (Box 1). The best-characterized endocannabinoids are N-arachidonylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG). Both AEA and 2-AG increase food intake, usually through the activation of CB1 receptors (CB1Rs). These receptors are abundantly expressed throughout the central nervous system (CNS) in areas controlling food intake and energy expenditure (hypothalamus, brainstem) and reward-related responses (nucleus accumbens), as well as in the peripheral nervous system, and in organs affecting metabolic homeostasis, such as the gastrointestinal tract, adipose tissue, liver, and muscle. Central and peripheral inhibition of CB1-R activity, and more generally of the ECS, is beneficial for the treatment of obesity and metabolic disorders [4,5], Rimonabant, an anorectic drug and

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Box 1. Endocannabinoid Synthesis and Degradation

Within the CNS, endocannabinoids are synthesized and released on demand, and classically act on CB₁Rs that are mainly located presynaptically to inhibit neurotransmitter release, and are thereafter immediately metabolized [7,31,124] (Figure 1).

AEA synthesis takes place through the hydrolysis of the membrane phospholipid precursor N-arachidonylethanolamine (NAPE) by NAPE-selective phospholipase D (NAPE-PLD) [7] (Figure 1). The production of 2-AG depends on the activation of phospholipase C (PLC), which generates 1,2-arachidonoylglycerol that is then cleaved by diacylglycerol lipase (DAGL) α or β to produce 2-AG [7]. Endocannabinoid degradation requires cellular reuptake and enzymatic hydrolysis, which is under the control of a fatty acid amidase hydrolase (FAAH) for AEA, and a monoacylglycerol lipase (MAGL) for 2-AG, resulting in the release of ethanolamine or glycerol, together with arachidonic acid [7]. The transport of AEA from the extracellular space to the intracellular space may be facilitated by a FAAH-like anandamide transporter (FLAT) [125], although recent studies have questioned the possible role of FLAT as an AEA intracellular carrier [126]. In addition to AEA and 2-AG, other putative endocannabinoids have been identified [127], but their physiological function remains largely unknown. The canonical receptors for AEA and 2-AG are CB₁Rs and CB₂Rs, which classically act through Gₛ q, proteins to inhibit adenyl cyclase and various voltage-gated Ca²⁺ channels, leading to lower cAMP levels and activation of some types of K⁺ channels, as well as mitogen-activated protein kinase (MAPK) and phospholipase pathways [128]. In the CNS, apart from their localization on the neuronal cell membrane, CB₁Rs have been also identified on astrocytes [129,130] and intracellularly on mitochondria [41]. AEA and 2-AG can also bind to non-CB₁Rs, such as the transient receptor potential vanilloid 1 (TRPV1), whose activation sometimes opposes the effects of CB₁R or CB₂R activation [122]. In addition, AEA can bind to the peroxisome proliferator-activated receptor γ (PPARγ), thus inducing adipocyte differentiation through this mechanism [121]. Finally, endocannabinoid-related compounds, such as OEA, PEA, and 2-arachidonoylglycerol, are synthesized and degraded through the same enzymatic steps illustrated above for AEA and 2-AG, but do not bind to CB₁Rs or CB₂Rs and often have actions opposite to those of endocannabinoids.

Figure 1. Endocannabinoid Signaling at the Synapse and their Synthesis and Degradation within the Cell. (A) Retrograde endocannabinoid (eCB) signaling at the synapse, eCBs are mobilized from postsynaptic neurons and target presynaptic CB₁Rs to suppress neurotransmitter release. (B) Main enzymatic steps involved in the formation and hydrolysis of AEA and 2-AG within the cell. Abbreviations: mAChR, muscarinic acetylcholine receptor; mGluR, metabotropic glutamate receptor; PL, phospholipids.

systemic CB₁R inverse agonist developed by Sanofi-Aventis, was approved as anti-obesity therapy in Europe, but in late 2008 it was withdrawn because of its psychiatric side effects. This event profoundly affected further drug development efforts by the pharmaceutical industry, causing the termination of all clinical programs involving rimonabant-like CB₁R antagonists in development. Nevertheless, studies published during the past 5 years have not only provided information on new physiological roles played by the ECS in the context of energy balance but have also identified novel mechanisms of action that make the ECS once more a very attractive target for therapy. We therefore believe it is timely to review these recent advances, which clearly designate the ECS as a ‘chef d’orchestre’, strategically positioned to regulate every step affecting the intake and use of calories, while discussing evidence that brings this system back at the center stage in the treatment of obesity and metabolic diseases.

Glossary

Alosteric modulator: a compound that binds to a receptor at a site distinct from the active (or orthosteric) site and induces a conformational change in the receptor, thereby increasing or reducing the affinity of the receptor for its ligands.

Cannabinoid receptors (CBRs): a class of G-protein-coupled receptors activated by endogenous or exogenous cannabinoids. Two CBR subtypes have been cloned and characterized so far: the CB₁R and the CB₂R.

Cannabis: a preparation of the Cannabis sativa plant used as a psychoactive drug or medicine. The main psychoactive component of cannabis is tetrahydrocannabinol (THC).

Cephalic-phase responses: anticipatory responses elicited by the autonomic nervous system to enhance digestion and metabolism of a meal. Cephalic-phase responses can arise from cognitive or sensory stimuli regarding food.

Endocannabinoids: endogenous lipid-derived agonists for G-protein-coupled CBRs. They include anandamide (AEA) and 2-arachidonoylglycerol (2-AG).

Hemopressin: a biactive peptide fragment derived from the hemoglobin α₁ chain which has been proposed to work as an endogenous allosteric modulator of CB₁R.

Inverse agonist: a compound that has effects similar to those of an antagonist, but also causes a distinct set of downstream biological responses. Inverse agonists not only block the effects of binding agonists like a classical antagonist, but also inhibit the intrinsic or basal activity of the receptor.

Leptin: a hormone produced mainly by adipocytes that regulates energy balance, metabolism, and immune and reproductive functions.

Melanocortin system: a system crucially involved in the regulation of energy balance that in the CNS includes POMC and AgRP neurons of the hypothalamic arcuate nucleus, which respectively produce melanocortin agonists and antagonists acting on melanocortin receptors in target brain areas.

Munchies: a typical craving for palatable food after consuming marijuana-containing products.
The ECS as a Modulator of the Senses: Olfaction and Taste

A plethora of studies have shown that cannabinoids or endocannabinoids, and CB1R activation, favor the intake of food, particularly if palatable [4,5]. It is therefore not surprising that the ECS plays a role in the modulation of both smell and taste, two senses that, together with sight, guide the organism towards food intake or food rejection [9].

Malfunctioning of the olfactory system has been found in obesity in different organisms [10,11]. In larvae of Xanopus laevis, 2-AG controls odor sensitivity through a CB1R-dependent mechanism, such that hunger stimulates 2-AG synthesis in the olfactory epithelium and increases the sensitivity of olfactory neurons [12]. Thus, modulation of endocannabinoid-dependent signaling in the nose may substantially influence food-seeking behavior. Accordingly, Soriano-Gomez and colleagues have shown that olfactory neuronal circuits in the mouse are regulated by endocannabinoid signaling [13]. More specifically, food deprivation induces an increase in endocannabinoid levels in the olfactory bulb, activating CB1 receptors on olfactory cortex axon terminals, and consequently reducing the excitation of granular cells in the olfactory bulb, leading to an increase in odor detection and food intake once animals are re-exposed to food [13].

When food is introduced into the mouth, it is sensed by the taste buds on the papillae of the tongue. Gustatory neurotransmission from the oral cavity is carried by cranial nerves VII, IX, and X to the nucleus of the solitary tract (NTS) in the brainstem, which in turn sends projections to and receives information from the forebrain and peripheral organs [14]. CB1Rs colocalize in mouse taste cells with the sweet receptor component Tr13, and CB1R-dependent endocannabinoid signaling specifically enhances neural responses to sweet taste [15]. This effect may be magnified in obesity, likely through the production of endocannabinoids directly in taste tissue [16]. 2-AG, AEA, and related N-acylthanolamines, such as oleoylethanolamide (OEA) and palmitolethanolamide (PEA), which are produced together with endocannabinoids, are also quantifiable in human saliva, and their levels are significantly higher in obese patients than in normal weight subjects [17]. However, what would be the function of salivary endocannabinoids be? A possibility is that they might modulate taste perception and orosensory information. In particular, because fat intake affects endocannabinoids and N-acyluralonamines levels in tissues [18], it is likely that the presence of fat in the oral cavity might regulate salivary endocannabinoids and N-acylthanolamine pools, which in turn might modulate taste signaling. Although there is no evidence of a direct link between CB1R activation in the oral cavity and neural responses to fat, recent studies demonstrate that fat in the oral cavity induces production of endocannabinoids in the gastrointestinal tract through efferent vagal signaling [19,20]. This phenomenon, as will be detailed in the following section, further favors fat intake [19,20], implying that the ECS plays a positive role in the consumption of this nutrient.

Gastrointestinal Endocannabinoids Control Fat Intake

First evidence that endocannabinoids produced in the gut are able to affect food intake and work as hunger signals came from studies demonstrating their increased and decreased production in the small intestine, respectively, in response to fasting and satiety [21]. More recent investigations have instead detailed the relationship between these lipid molecules and the intake of specific nutrients, proving the key role played by gut endocannabinoids in fat preference and consumption.

When food is introduced into the mouth, cephalic-phase responses are initiated to enhance digestion and metabolism of the meal [22]. These responses can be studied in rodents using the sham-feeding model in which the physiological effects of the orosensory properties of food can be separated from its post-ingestive qualities. Using this experimental model it has been shown that gut-derived endocannabinoids regulate the intake of food and particular fat, based on its orosensory properties [19,20]. Sham-feeding a high-fat liquid meal in rats increases AEA and...
2-AG levels specifically in the jejunum, but not in other portions of the gastrointestinal tract nor in the brain [19,20]. Changes in endocannabinoid levels are observed in response to fat intake and to a nutritionally-complete meal, but not in response to proteins or carbohydrate ingestion [19]. Increases in gut endocannabinoids in turn food-forward food consumption because pharmacological blockade of CB1-Rs in the small intestine immediately before sham-feeding inhibits fat intake [19]. Fatty acid composition affects per se gut endocannabinoid production, suggesting that specific sensing mechanisms are at play in the oral cavity, and that mobilization of endocannabinoids in the gut may be required for fat preference [20,23]. Importantly, the sham-feeding effect on gastrointestinal endocannabinoids is lost after transection of the vagus, implying that signals originating in the oral cavity are transmitted to the brainstem, and then through the vagal efferents to the intestine, where they induce the production of endocannabinoids [19]. However, it is currently unclear how gut endocannabinoids communicate with the brain to regulate food intake. A possibility is that they may act through CB1-Rs on vagal terminals [24], on sympathetic nerve terminals [25], or indirectly through the modulation of gastrointestinal hormones. In particular, activation of CB1-Rs in gastric cells induces secretion of ghrelin [26,27], an orexigenic hormone able to increase fat-taste perception [28] and the rewarding value of high-fat diets [29].

The ECS and the Neuronal Control of Food Intake and Fuel Partitioning

That cannabinoids and endocannabinoids can act at several brain regions to stimulate food intake is well established [4]. However, recent studies have now shown that the net effect of endocannabinoids on food intake depends on the neuronal type and possibly on the subcellular location of CB1-Rs.

Endocannabinoids control both excitatory and inhibitory neurotransmission by classically acting as retrograde signals on presynaptic CB1-Rs, and inhibit neurotransmitter release [30,31] (Box 1). Experiments with genetically modified mice have demonstrated that, although the well-known orexigenic effect of endocannabinoids depends on actions at CB1-Rs located at the terminals of cortical glutamatergic neurons, CB1-R signalling in GABAergic neurons actually suppresses food intake [32]. In particular, high levels of CB1-R activation reduce feeding through inhibition of GABAergic transmission within the ventral striatum, a brain area crucially involved in reward [32]. On the other hand, fasting increases endocannabinoid levels in the nucleus accumbens (NAc) [33], which in turn may drive the liking for and the motivation to consume palatable food by acting in this brain region to induce dopamine release [34]. This effect might depend upon activation of CB1-Rs on glutamatergic terminals which, by inhibiting glutamate release, would inhibit GABAergic neurons that project from the NAc to the ventral tegmental area (VTA), and consequently disinhibit VTA dopaminergic neurons [35]. Modulation of endocannabinoid signalling in the VTA may be also relevant for the actions of hormones on VTA dopaminergic neurons. A recent study by Labouèbe and colleagues demonstrated that the appetite-suppressant effects of insulin may be related to the ability of the hormone to depress glutamatergic synaptic transmission onto VTA dopaminergic neurons in an endocannabinoid-dependent manner [36]. Insulin-induced long-term depression (LTD) requires postsynaptic activation of insulin receptor signaling, endocannabinoid synthesis, and presynaptic CB1-R-mediated inhibition of glutamate release [36]. This is in turn associated with inhibition of anticipatory activity and preference for food-related cues [36]. These findings therefore suggest, somewhat surprisingly, that insulin may recruit endocannabinoids to inhibit feeding by reducing the rewarding impact of food.

Within the hypothalamus, different neuronal populations are known to exert distinct functions in the context of energy-balance regulation [37,38]. Recent data have however expanded this notion by demonstrating that cannabinoids can subvert an appetite-inhibitory circuit to become orexigenic [39]. When given in doses intended to simulate the effects of marijuana, CB1-R agonists unexpectedly activate hypothalamic proopiomelanocortin (POMC) neurons, which
are part of the melanocortin system and classically play a central role in inhibiting food intake [39,40]. CB₁R activation stimulates the release of the orexigenic neuropeptide β-endorphin from POMC neurons, while preventing release of the appetite-suppressing peptide α-melanocyte-stimulating hormone (α-MSH) produced by these same neurons [39]. Interestingly, these processes seem to depend upon mitochondrial CB₁R [41] activation and consequent increase in reactive oxygen species (ROS) and mitochondrial adaptations in POMC neurons that, when blocked, abolish CB₁R-induced cellular responses and feeding [39]. However, somewhat in contrast with these findings, recent in vitro data obtained from hypothalamic cell lines and primary neuronal cultures instead suggest that activation of CB₁R decreases ROS formation [42]. Thus, although further studies will be necessary to clarify the molecular underpinnings of CB₁R activation, the fact that (en)ocannabinoids may act upon POMC neurons to increase food intake is particularly noteworthy because this neuronal population, together with Agouti-related protein (AgRP) neurons of the hypothalamic arcuate nucleus, are under the tight control of mesolimbic pathways and match the energy needs of the organism to contextual information [43]. Because of their inherent characteristics (local, on-demand production) endocannabinoids are ideal integrators of the rapid changes in the needs of the organisms and, as such, they are the target of the actions of hormones in the hypothalamus [4]. The relation between endocannabinoids and leptin in particular has been the focus of intense research. Leptin decreases hypothalamic endocannabinoids levels [44], and interferes with ECS signaling for instance in the lateral hypothalamus, where it prevents CB₁R-dependent suppression of inhibition of orexigenic melanin-concentrating hormone (MCH) neurons [45], and in the paraventricular nucleus (PVN), where it inhibits glucocorticoid-mediated endocannabinoid biosynthesis and consequent suppression of excitatory neurotransmission of neurosecretory neurons [46]. Thus, in fasting, when circulating leptin levels are low, endocannabinoids may rise and cause changes in neurotransmission that would promote food intake. Similarly, in conditions of resistance to the actions of leptin, as observed in obesity, synaptic remodeling occurs on appetite-stimulating neurons, such as orexin neurons, and CB₁R-expressing presynaptic inputs change from predominantly excitatory to inhibitory, overall driving neuronal disinhibition and consequent hyperphagia [47].

Finally, for the brain to exert its regulatory control on energy intake, storage, and use, bidirectional communication with peripheral tissues and organs must be continuously assured. As mentioned earlier, CB₁Rs are expressed on peripheral terminals of sensory neurons [24] as well as in peripheral parasympathetic [48] and sympathetic terminals [25]. While CB₁Rs on both vagal afferents and efferents regulate gastrointestinal motility [49], on sensory nerve terminals they may specifically affect food intake because the acute appetite-suppressant effect of rimonabant is abolished by capsaicin-induced sensory deafferentation [21,50]. However, the rapid hypophagic effect of rimonabant also requires glutamatergic signaling in the NTS and peripheral β-adrenergic transmission [50]. Thus, CB₁R-dependent regulation of food intake likely involves a neural loop, constituted by sensory visceral afferents impinging on the NTS, that in turn modulates sympathetic nervous system (SNS) activity. Recent evidence suggests that the ECS favors weight gain by toning-down processes under the control of the SNS, such as energy expenditure, brown adipose tissue (BAT) thermogenesis, and white adipose tissue (WAT) lipolysis [51–54]. Mice lacking CB₁Rs in the forebrain and sympathetic ganglia are resistant to diet-induced obesity because they display increased lipid oxidation and thermogenesis as a consequence of an enhanced sympathetic tone associated with a decrease in energy absorption [51]. Similarly, genetic induction of 2-AG hydrolysis in the forebrain, by overexpressing the 2-AG hydrolyzing enzyme monoaclglycerol lipase (MAGL), causes resistance to diet-induced obesity through increased SNS-dependent BAT thermogenesis and mitochondrial activity [55]. CB₁Rs in hypothalamic circuits play a major role in the regulation of these processes. If CB₁Rs are deleted from single-minded homolog 1 (Sim1)-expressing neurons, which constitute the majority of glutamatergic neurons of the PVN [56], mice are protected from diet-induced obesity as a result of an SNS-dependent increase in BAT thermogenesis and energy expenditure [57].
Similarly, deletion of CB1Rs from steroidogenic factor 1 (SF1)-expressing neurons of the ventromedial hypothalamus protects chow-fed mice from body-weight gain by inducing increased BAT metabolism and WAT lipolysis through heightened SNS activity [54]. These effects further depend upon the diet because, although SF1-CB1-KO mice (mice carrying a deletion of the CB1 receptor gene in SF1-positive neurons) on a chow diet are leaner than their wild-type littersmates, on a high-fat diet they gain more weight than the wild type owing, at least in part, to decreased SNS-driven lipolysis [54]. Thus, there are strong links between the ECS and the SNS in the regulation of energy balance and use of fuel substrates. Whether this is relevant for human obesity remains to be established.

Endocannabinoids and the Non-Neuronal Regulation of Energy Storage and Utilization

Evidence from studies investigating the function of the ECS in peripheral tissues further highlights how this system orchestrates the transport, metabolism, and use of nutrients as energy substrate (Figure 1). For instance, the ECS regulates the transport of lipids in the bloodstream because systemic administration of an irreversible inhibitor of endocannabinoid degradation in mice causes hypertriglyceridemia with reduced plasma triglyceride clearance and the accumulation of apolipoprotein E-depleted triglyceride-rich lipoproteins [58]. Furthermore, several in vitro studies have demonstrated that CB1R activation in white adipocytes promotes adipogenesis and build-up of triglyceride-rich lipid droplets, favoring storage of energy through mechanisms ranging from the induction of adipocyte differentiation to the inhibition of mitochondrial biogenesis [5]. Inhibition of CB1s induces fatty acid oxidation, mitochondrial biogenesis, and even transdifferentiation of white adipocytes into mitochondria-rich, thermogenic brown fat cells [59,60]. Whether these responses are relevant in vivo is, however, unclear because WAT and BAT function can be affected by CB1-R signaling originating from the CNS and involving the SNS (see previous section). Nevertheless, preliminary data obtained using adipocyte-specific CB1 knockout mice do suggest that adipocyte CB1-Rs regulate WAT expansion and the development of obesity and insulin resistance [61]. Endocannabinoid production in white adipocytes is negatively regulated by insulin and leptin, with the latter acting through both local and distant, hypothalamus-dependent, mechanisms [62–64]. Consequently, development of insulin and leptin resistance could lead to ECS overactivity in the WAT, favoring fat accumulation. Increased ECS tone in obese animals and humans (see below) is also observed in the liver, and this is probably due to a feed-forward loop in which CB1-R upregulation by high-fat diet is CB1-R-dependent [65–67]. Activation of CB1-Rs in hepatocytes enhances the expression of key lipogenesis markers, such as sterol regulatory element binding transcription factor 1 (SREBP1), acetyl coenzyme-A carboxylase-1 (ACO1), and fatty acid synthase (FAS), likely through inhibition of the activity of 5’-AMP-activated protein kinase (AMPK), causing lipid accumulation and hepatic steatosis [68–70]. Activation of hepatic CB1-Rs also inhibits insulin clearance and insulin-induced phosphorylation of the Akt (protein kinase B) kinase via the inhibitory phosphorylation of insulin receptor substrate-1 (IRS1). The latter is due to activation of an endoplasmic reticulum stress-dependent pathway and increased ceramide synthesis, resulting in augmented glycogenolysis [66,71]. The impact of hepatic CB1-Rs on lipid and glucose metabolism is important in vivo because mice with specific overexpression of CB1-Rs in hepatocytes, and lacking the receptor in other organs, develop strong hepatic and systemic insulin resistance when exposed to a high-fat diet while remaining lean [66]. Conversely, mice with specific deletion of CB1-Rs in hepatocytes gain body weight when consuming a high-fat diet, but are protected against hepatic steatosis, hyperglycemia, dyslipidemia, and insulin resistance [68]. Accordingly, the beneficial effects of administering a peripherally restricted CB1-R antagonist on liver steatosis and insulin resistance are due to the effect of the compound on hepatocyte CB1-Rs [69,72]. Peripheral blockade of CB1-Rs also diminishes food intake [72]. In particular, appetite and weight reduction induced by the peripherally restricted CB1-R inverse agonist JD5037 are due to the re-sensitization of diet-induced obese mice to endogenous leptin. This is accomplished through
**Key Figure**

A Schematic Illustrating the Multi-Organ Action of the Endocannabinoid System (ECS)

**Figure 1.** The ECS favors the intake and conservation of energy at the organism level by exerting key actions on target tissues. In particular, by acting on the brain and the autonomous nervous system, the ECS favors food intake while decreasing energy expenditure and diminishing the use of lipids as energy substrates in the periphery. The ECS also affects feeding by modulating olfaction and taste, and by specifically inducing fat preference and intake through its action in the gastrointestinal tract. The ECS exerts a multi-organ control of glucose metabolism by modulating liver and skeletal muscle responsiveness to insulin, hepatic insulin clearance, and pancreatic insulin secretion. Finally, the ECS regulates lipid metabolism by increasing storage capacity in the adipose tissue and by favoring fat accumulation and eventually steatosis in the liver. Abbreviations: BAT, brown adipose tissue; EE, energy expenditure; FI, food intake; SNS, sympathetic nervous system; VAT, white adipose tissue.
reversing the hyperleptinemia by decreasing leptin expression and secretion by adipocytes and increasing leptin clearance via the kidney [72]. Peripheral CB1 receptor signaling seems also relevant in determining glucocorticoid-dependent metabolic responses. An increase in circulating glucocorticoids is believed to be a contributing factor to obesity and metabolic syndrome [73]. Using a mouse model of excess corticosterone exposure, it was found that the ability of glucocorticoids to induce obesity, hepatic steatosis, and dyslipidemia was reduced or reversed in CB1-knockout mice as well as in mice treated with the global CB1 receptor antagonist AM251 [74]. Similarly, the peripherally-restricted CB1R neutral antagonist AM6545 attenuated the glucocorticoid-induced metabolic changes, suggesting a peripheral mechanism for these effects. In fact, chronic excess glucocorticoid exposure increased hepatic and circulating AEA levels, whereas no effect was observed in the hypothalamus [74]. Of note, hepatic CB1 receptor signaling is particularly important in determining glucocorticoid-dependent changes in lipid metabolism because mice with specific deletion of CB1Rs in hepatocytes are protected against glucocorticoid-induced dyslipidemia, while developing obesity [74].

The ECS exerts a multi-organ control of glucose metabolism by modulating liver and skeletal muscle responsiveness to insulin [75], as well as insulin secretion from the pancreas. In particular, activation of CB1 receptors in β cells recruits focal adhesion kinases (FAK), causing exocytosis of secretory insulin vesicles through cytoskeletal reorganization [76]. This mechanism could therefore participate to the hyperinsulinemia typically observed in type 2 diabetes [76]. In addition, CB1 receptors may regulate β cell health, either indirectly, through the induction of proinflammatory macrophages in the pancreas [77], or directly by stimulating apoptotic activity and β cell death [78]. Conversely, CB1 receptor blockade increases β cell proliferation and mass [79].

Thus, independently of its actions at neuronal level, the ECS exerts diverse effects on peripheral tissues, which, by being devoted to promoting an anabolic tone, may easily contribute to metabolic disease.

The ECS and Human Obesity

Endocannabinoids can be detected in the circulation, and measurement of circulating endocannabinoids has been a preferred strategy for the study of the ECS in humans. Plasma endocannabinoids correlate positively with markers of obesity and metabolic disorder, such as body mass index, waist circumference, visceral fat mass, and insulin resistance [80–85]. However, it is not known whether circulating endocannabinoids simply reflect spillover from sites of production, or participate in signaling events that might in turn affect feeding and metabolism. In support of the latter, plasma endocannabinoid levels change in healthy humans and in relation to food intake [84,86]. Both normal-weight and obese subjects have a significant preprandial peak in AEA, which decreases after the meal in normal-weight subjects, but not in obese subjects [84]. No changes are observed for 2-AG [84]. Thus, AEA may work as a physiological meal initiator in humans, whose dysregulation in obese patients favors excessive caloric intake [84]. Conversely, when motivation to eat is generated by the availability of highly-palatable food, and not by food deprivation, an increase in plasma 2-AG levels is observed [87]. Such increase is missing in pathologies characterized by dysregulated reward processes, such as anorexia nervosa [88]. Hence, AEA and 2-AG may play different roles in the regulation of eating behavior, with the first essentially acting to start the intake of calories, and the second to maintain intake beyond the physiological needs. As to from where the observed changes in AEA and 2-AG plasma levels originate, the gastrointestinal tract is a likely candidate because this organ is directly exposed to the nutrients introduced through the diet and produces endocannabinoids in response to both fasting [21] and to the ingestion of fatty food [19].

Moreover, genetic studies have been carried out to link polymorphisms in CNR1 (coding for CB1-R) and the AEA-degrading enzyme fatty acid amide hydrolase (FAAH) with metabolic
alterations in humans. Despite some conflicting results, CNR1 variants have been associated with metabolic syndrome [89] and dyslipidemia [90], whereas a FAAH 385 A/A missense polymorphism was associated with overweight and obesity [91,92]. Carriers of this polymorphism have increased circulating AEA and related N-acylethanolamine levels [93], and show greater reward-related ventral striatal reactivity [94]. Likely, this genetic variation in humans may influence the propensity to develop obesity depending on the diet. Accordingly, mice lacking FAAH have normal body weight when consuming chow, but become obese when fed with a high-fat diet [95] and display altered hepatic lipid and glucose metabolism [96]. Thus, genetic analysis as well as assessment of circulating endocannabinoids might help to identify specific obese subpopulations that are expected to particularly benefit from pharmacological treatments targeting the ECS.

The ECS and Its Comeback as a Target for Treatment

Several studies over the past 12 years have shown that brain-penetrant CB₁R antagonists, such as rimonabant, reliably decrease body weight, fat mass, and associated cardiometabolic risks, while improving glucose homeostasis and insulin sensitivity in obese rodents and humans [97]. However, because of the psychiatric side effects and the overall benefits being lower than the risks, rimonabant was withdrawn from the market, and research programs pursuing the production of similar CB₁R antagonists were abandoned. But, 7 years after these events, novel evidence points again to the CB₁R as a promising target for therapy. Preclinical studies have demonstrated that new CB₁R antagonists, with limited or null ability to pass the blood–brain barrier, may efficaciously treat metabolic disease [97] (Table 1). Among these compounds, the peripherally restricted CB₁R inverse agonist JD5037 and the peripherally restricted CB₁R neutral antagonist AM6545 seem of particular interest because of the lack of central side effects while retaining ability to reduce obesity, reverse leptin resistance, and improve glucose homeostasis, hepatic steatosis, and plasma lipid profile in genetically and diet-induced obese mice [69,72,98]. Chronic administration of JD5037 is even more efficacious than AM6545 in reducing food intake, body weight, and adiposity in the absence of brain CB₁R occupancy [72], thus potentially representing an ideal anti-obesity treatment to reduce cardiometabolic risk. Other CB₁R neutral antagonists, such as AM4113 and AM6527, have also been developed [97]. Although still acting within the CNS, they do not induce nausea or malaise, while retaining ability to decrease food intake and body weight [99–101]. Another class of interesting compounds might be developed by studying recently identified endogenous allosteric modulators able to inhibit the activity of CB₁-Rs in the presence of their ligands. In particular, hemopressin, small peptide endocannabinoids called pepcans, and the neurosteroid pregnenolone have been identified as endogenous allosteric inhibitors of the CB₁-Rs [102–104]. Hemopressin has been shown to reduce food intake without causing any obvious adverse side effects, predominantly by modulating circuits of the mediobasal hypothalamus rather than reward-related areas [105,106]. However, further studies will be necessary to confirm that these effects depend upon the direct action of hemopressin on CB₁-R. Notably, chronic administration of pregnenolone limits body-weight gain and adiposity in diet-induced mice without causing anxiety [107].

Because human obesity is characterized by increased endocannabinoid tone (discussed above), other viable therapeutic strategies are to increase endocannabinoid degradation or interfere with endocannabinoid synthesis. Taking into account that endocannabinoids are mainly n-6 polyunsaturated fatty acids (PUFA) derivatives, an increase of n-3 PUFA should decrease the availability of endocannabinoid precursors by reducing the levels of arachidonic acid-esterified phospholipids [108,109]. Indeed, a dietary increase in the levels of n-3 PUFA, such as eicosapentaenoic and docosahexaenoic acids, decreases endocannabinoid levels in adipose tissue, liver, and heart, reducing ectopic fat and associated inflammation in obese Zucker rats [110]. In particular, reduced concentrations of AEA and 2-AG in tissues were associated with lower levels of arachidonic acid in membrane phospholipids, while no change in the activity of
Table 1. Novel Therapeutic Approaches for the Modulation of the ECS in Obesity and Metabolic Disease

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<th>Target</th>
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<tr>
<td>CB₁R</td>
<td>JD5037; AM6545</td>
<td>Diet-induced obese mice; ob/ob mice</td>
<td>Reduction of body weight; improvement of glucose metabolism; improvement of lipid metabolism; no central side effects (anxiety)</td>
<td>[69, 71, 72, 98]</td>
</tr>
<tr>
<td>Allosteric inhibitors</td>
<td>Hemopressin</td>
<td>Chow-fed rats and mice; ob/ob mice</td>
<td>Reduction of body weight and food intake; no obvious adverse effects</td>
<td>[105]</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td></td>
<td>Diet-induced obese mice</td>
<td>Reduction in body weight and fat mass; no central side effects (anxiety)</td>
<td>[107]</td>
</tr>
<tr>
<td>PSN CBAM-1</td>
<td></td>
<td>Diet-induced obese mice chow-fed rats</td>
<td>Reduction of body weight and food intake</td>
<td>[131]</td>
</tr>
<tr>
<td>CB₁R neutral antagonists</td>
<td>AM4113, AM6527, compound 2c, VChISRs1, BPR0432, C-2050</td>
<td>Rats and mice</td>
<td>Reduction of body weight and food intake</td>
<td>[97]</td>
</tr>
<tr>
<td>Combinatorial therapy</td>
<td>Rimonabant/SNAP9484/47 (MCH1-R² antagonist)</td>
<td>Diet-induced obese mice</td>
<td>Reduction of body weight, fat mass, and food intake; no central side effects (depression)</td>
<td>[117]</td>
</tr>
<tr>
<td></td>
<td>Rimonabant/peptide YY₆₋₁₆ or rimonabant/oxymotomodulin</td>
<td>Chow-fed mice</td>
<td>Reduction of food intake</td>
<td>[118]</td>
</tr>
<tr>
<td>eCBs b</td>
<td>Diet enriched in n3-PUFA (human studies)</td>
<td>90 g/day of control or n3-PUFA-enriched cheese for 3 weeks</td>
<td>Forty-two adult volunteers (19 males and 23 females) with diagnosed mild hypercholesterolemia</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 week dietary supplementation with krill powder (4 g/day per os)</td>
<td>Eleven obese men</td>
<td>[111]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Significant decrease in AEA; reduction of LDL cholesterol</td>
<td></td>
</tr>
<tr>
<td>Inhibitors of DAGL-α</td>
<td>O-7460</td>
<td>Mice</td>
<td>Reduced 2-AG levels; inhibition of high-fat diet intake and decrease in body weight</td>
<td>[114]</td>
</tr>
</tbody>
</table>

*Melanin-concentrating hormone type 1 receptor.

**Entocannabinoids.

endocannabinoid-degrading enzymes was observed. Consequently, the reduction of membrane substrates for inflammatory molecules and endocannabinoids may dampen inflammation and favor appropriate body fat deposition [110].

These dietary strategies may be effective in humans because consumption of food enriched in n-3 PUFA decreases plasma endocannabinoid levels and improves the lipid profile in obese or hypercholesterolemic subjects [111–113]. Thus, higher dietary consumption of n-3 PUFA could be a relatively easy way to reduce endocannabinoid levels in a manner that helps to prevent or
treat metabolic disorders. As mentioned earlier, reduction of endocannabinoid levels can be also achieved by pharmacologically blocking endocannabinoid synthesis. Interestingly, it has been shown that the compound O-7460, an inhibitor of the DAG lipase (DAGL)-α, the enzyme regulating the synthesis of 2-AG, acutely reduces high-fat diet intake and body weight in mice [114]. In agreement with these data, mice lacking DAGL-α are hypophagic and lean, and phenotypically and metabolically resemble CB1 knockout mice [115,116]. Finally, the ECS could be targeted in combination with other systems that are also potential targets for therapy, such as the gastrointestinal hormones peptide YY (PYY) and oxyntomodulin, the MCH system [117,118], or other systems. This combinatorial approach would have the advantage of using lower doses of the compounds of interest, limiting possible side effects, while targeting different biological systems that participate in the regulation of energy balance, possibly having greater therapeutic efficacy.

Concluding Remarks and Future Perspectives
What we eat affects ECS activity, which in turn modulates eating behavior and metabolism. ECS actions range from the regulation of sensory responses, to the development of preference for the consumption of particular nutrients, and also influence their metabolic handling (see Outstanding Questions). The ECS is therefore positioned to adjust behavior and metabolism in response to environmental changes in food availability. Its activity is advantageous when food access is limited or cannot be predicted, but becomes harmful when food is abundant, favoring the development of obesity and metabolic syndrome (Figure 1, Kay Figure).

Most of the available information concerning the role of the ECS in energy balance is related to the endocannabinoids AEA and 2-AG and to the CB1R. The latter remains an attractive target for therapy because recent discoveries have demonstrated that peripheral antagonism of the receptor might be a viable treatment strategy for metabolic disease. In addition, novel therapeutic venues have been opened by the possibility of modulating, in a signal-specific manner, the activity of CB1R through allosteric inhibitors. Testing in larger animals and clinical trials will clearly be necessary to assess both the efficacy and the unwanted effects of these strategies. Notwithstanding these challenges, there is once again strong optimism that such therapeutic approaches might successfully help in the battle against obesity, dyslipidemia, and diabetes. However, while CB1R continues to gain attention as a therapeutic target, endocannabinoids affect metabolism also through the activation of CB2R [119,120], peroxisome proliferator-activated receptor γ (PPARγ) [121] and transient receptor potential vanilloid 1 (TRPV1) [122]; endocannabinoid-related compounds such as OEA oppose CB2R-dependent metabolic effects by acting through PPARα [123]. Thus, researchers face a complicated scenario, which will require substantial additional investigation to fully decipher the roles played by the ECS and its close partners in energy homeostasis and metabolic disease.

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Resources

References
2. Mattia, L. et al. (2006) Endogenous cannabinoids in the brain and peripheral tissues: regulation of their levels and control of food intake. Int. J. Obes. (Lond.) 30 (Suppl. 1), S7–S12

Outstanding Questions
What is the function of mitochondrial CB2R in energy balance? CB2Rs are located on mitochondrial membranes and regulate cellular energy metabolism as well as neuronal function in the mouse brain. In addition, endocannabinoids may modulate the activity of POMC neurons through mitochondrial CB2Rs. Although more definitive support for this proposed mechanism is needed, these data point to a novel, subcellular role of the ECS in energy balance.

Is there a role for astroglial CB2Rs in energy balance? Astrocytes have important functions in the regulation of energy balance. CB2Rs located on astrocytes interfere with leptin signaling and its ability to regulate astroglial glycogen storage, representing a novel mechanism for regulating brain energy storage and neuronal functions. How do such mechanisms impact on whole-body energy-balance regulation?

Is the ability of the ECS to regulate adult neurogenesis relevant in the context of energy-balance regulation? The ECS modulates adult neurogenesis, a process involved in the hypothalamic control of feeding behavior. Does the ECS alter food intake by modulating adult neurogenesis?

What is the link between gut microbiota and endocannabinoids? Gut microbiota might control intestinal ECS tone, which in turn modulates gut permeability. CB2R stimulation in the gut increases gut permeability and ECS tone in both gut and WAT, favoring body weight gain. However, WAT-specific deletion of NAPE-PLD (N-acylphosphatidylethanolamine-selective phospholipase D), a major synthesizing enzyme of endocannabinoids and related compounds, leads to obesity, altered adipose metabolism, and dysbiosis, intensifying metabolic dysregulation. What is the relationship between the gut microbiota and the ECS?

Can endocannabinoids be used as phenotype biomarker in humans? Plasma endocannabinoids could represent biomarkers of WAT distribution and insulin resistance, and salivary endocannabinoids may provide information on specific obese phenotypes. However, several issues need to be addressed, such as the requirement for standardized methods for the extraction and measurement of


10. Karthe, B. et al. (2011) Rat strains with different metabolic statues differ in food enteron-driven behavior. Behav. Brain Res. 270, 228–239


25. Ishac, E.J. et al. (1996) Inhibition of exocytotic neurotransmitter release by presynaptic cannabinoid CB1 receptors on parasympathetic nerves. J. Pharmacol. 118, 2232–2238


27. Serin, L.L. et al. (2013) The cannabinoid CB1 receptor modulates ghrelin production through the mTOR pathway to regulate food intake. PLoS ONE 8, e60339


42. Palmita, R. et al. (2010) Negative regulation of leptin-induced reactive oxygen species (ROS) formation by cannabinoid CB1 receptor activation in hypothalamic neurons. Mol. Biol. Chem. 290, 13666–13677


51. Quarta, G. et al. (2010) CB1 signaling in forebrain and sympa-thetic neurons is a key determinant of endocannabinoid actions on energy balance. Cell Metab. 12, 273–285


111. Benge, K. et al. (2013) Chronic treatment with ketoprofen reduces plasma triglyceride and anandamide levels in mildly obese men. Lipids Health Dis. 12, 78


115. Powell, D.R. et al. (2016) Diacylglycerol lipase alpha knockout mice demonstrate metabolic and behavioral phenotypes similar to those of cannabinoid receptor 1 knockout mice. Front. Endocrinol. (Lausanne) 6, 86


129. Han, J. et al. (2012) Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Curr. Opin. 109, 1039–1050

130. Bosley, B. et al. (2013) Antiregional CB1 cannabinoid receptors regulate learn signaling in mouse brain astrocytes. Mol. Metab. 2, 393–404