MECHANISMS IN ENDOCRINOLOGY

Endocannabinoids and metabolism: past, present and future

Vincent Simon and **Daniela Cota**

INSERM and University of Bordeaux, Neurocentre Magendie, Physiopathologie de la Plasticité Neuronale, U1215, Bordeaux, France

Correspondence
should be addressed
to D Cota
Email
daniela.cota@inserm.fr

Abstract

The endocannabinoid system (ECS), including cannabinoid type 1 and type 2 receptors (CB_1R and CB_2R), endogenous ligands called endocannabinoids and their related enzymatic machinery, is known to have a role in the regulation of energy balance. Past information generated on the ECS, mainly focused on the involvement of this system in the central nervous system regulation of food intake, while at the same time clinical studies pointed out the therapeutic efficacy of brain penetrant CB_1R antagonists like rimonabant for obesity and metabolic disorders. Rimonabant was removed from the market in 2009 and its obituary written due to its psychiatric side effects. However, in the meanwhile a number of investigations had started to highlight the roles of the peripheral ECS in the regulation of metabolism, bringing up new hope that the ECS might still represent target for treatment. Accordingly, peripherally restricted CB_1R antagonists or inverse agonists have shown to effectively reduce body weight, adiposity, insulin resistance and dyslipidemia in obese animal models. Very recent investigations have further expanded the possible toolbox for the modulation of the ECS, by demonstrating the existence of endogenous allosteric inhibitors of CB_1R, the characterization of the structure of the human CB_1R, and the likely involvement of CB_2R in metabolic disorders. Here we give an overview of these findings, discussing what the future may hold in the context of strategies targeting the ECS in metabolic disease.

Introduction

For centuries, marijuana (*Cannabis sativa*) has been known to stimulate food intake. However, understanding of the underlying biological mechanisms started only in the 60s, with the identification of Δ^9-tetrahydrocannabinol (THC, the main psychoactive component of marijuana) (1). Almost 30 years later, specific cannabinoid receptors (CBRs) were identified as the target of the action of THC (2, 3); a discovery that was followed soon afterwards by the characterization of endogenous ligands for CBRs, called endocannabinoids (4, 5), and of their

Invited Author's profile

Daniela Cota, MD is Team leader, Team "Energy Balance and Obesity", INSERM U1215 Neurocentre Magendie, Bordeaux, France. The main objective of Dr Cota's team is to understand the physiopathological mechanisms leading to obesity and diabetes, by focusing on the roles of circuits and different cell types located in the hypothalamus and on how these cells decode nutrients and nutrient-related signals in order to regulate metabolic responses. Dr Cota's work is renowned at International level, having critically contributed in determining the role of intercellular systems, like the endocannabinoid system, and of intracellular signaling mechanisms, like the mTORC1 pathway, in the regulation of energy balance and metabolism.

enzymatic machinery (reviewed in (6)). Since then, a great number of studies have investigated the functions of the endocannabinoid system (ECS) in the regulation of metabolic homeostasis. Strong evidence now clearly illustrates the pleiotropic roles of the ECS in energy balance, highlighting its involvement in every single aspect related to the search, intake and metabolic handling of calories. Consequently, the ECS has been identified as a target for the treatment of obesity and type 2 diabetes. Accordingly, the first in class CB_1R antagonist rimonabant was briefly approved for the treatment of obesity by the European Medicines Agency (EMA), but was later withdrawn from the marked due to its psychiatric side effects. This event negatively affected both the pharmaceutical industry and the academic research in the field, but it has also led to the investigation of novel approaches to target the ECS while favoring the quest for a more in depth knowledge of its physiological roles.

Here we will provide an overview of the past and present work on the functions of the ECS in metabolism and of what the future may hold for the therapeutic exploitation of this system against obesity and metabolic disease.

Components of the ECS

The ECS is an evolutionary well-preserved system (7), which includes the CBRs, the endocannabinoids and the pathways responsible for the synthesis and degradation of those ligands. Two CBRs types have been identified so far:

the cannabinoid type 1 receptor (CB_1R) and the cannabinoid type 2 receptor (CB_2R). CB_1R is widely distributed in the brain and also in peripheral tissues, whereas CB_2R is preferentially expressed in immune cells (8). As the role of CB_2R in energy homeostasis is still poorly known, we will mainly focus on the CB_1R, which is expressed in major brain regions (hypothalamus, limbic structures and hindbrain) and peripheral organs (gastrointestinal tract, liver, adipose tissue, muscle and pancreas) involved in the regulation of feeding and metabolism (9). Both CB_1R and CB_2R are G-protein coupled receptors whose activation modulates the adenylate cyclase and mitogen-activated protein kinase (MAPK) pathways via Gi/o type protein (8). Endocannabinoids are polyunsaturated fatty acids (PUFAs) produced on demand from membrane phospholipids to act on CBR in an autocrine or paracrine manner. Other endogenous ligands include allosteric inhibitors such as pepcans (peptide endocannabinoids) (10) and the neurosteroid pregnenolone (11). Here we will briefly mention the synthesis and degradation mechanisms of endocannabinoids and their action on CBR. The reader is also invited to refer to recent reviews that have extensively described these topics (12, 13).

Arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are the 2 best-known endocannabinoids. They both derive from membrane phospholipid precursors and arachidonic acid (6). For the synthesis of AEA, phosphatidylethanolamine and arachidonic acid are transformed into N-arachidonoyl phosphatidylethanolamine (NAPE) by the enzyme
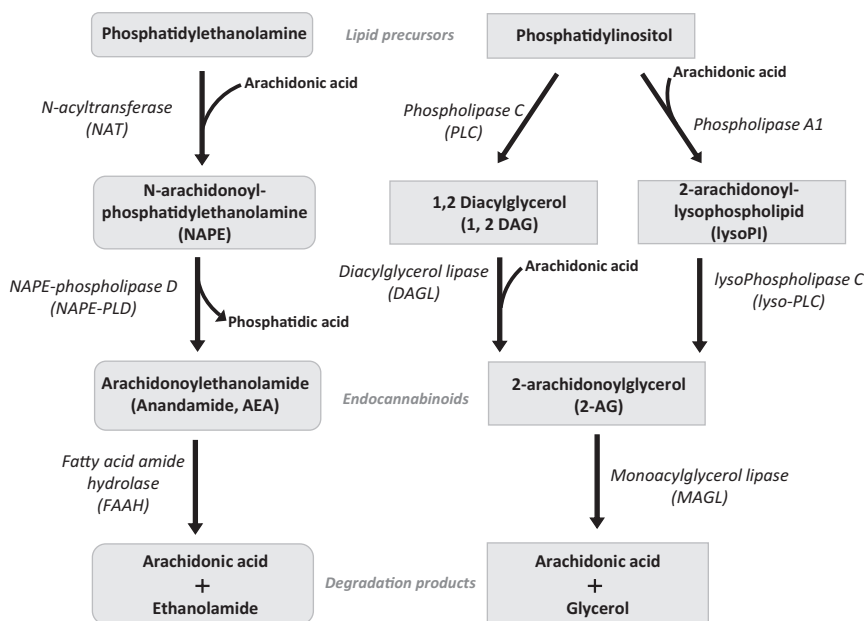
Figure 1

Different pathways involved in the biosynthesis and degradation of AEA and 2-AG.

N-acyltransferase (NAT) (6). Then, the phospholipase NAPE-PLD converts NAPE into AEA and phosphatidic acid (14) (Fig. 1). Instead, for 2-AG production, phosphatidylinositol is transformed in 1,2 diacylglycerol (1,2 DAG) by the phospholipase C (PLC), then in 2-AG by the diacylglycerol lipase (DAGL). An alternative pathway also exists for the synthesis of 2-AG and includes the transformation of phosphatidylinositol into 2-arachidonoyl-lysophospholipid (lyso-PI) by the phospholipase A1. Lyso-PI is then hydrolyzed by the lyso-phospholipase C (lyso-PLC) into 2-AG (6) (Fig. 1).

In the central nervous system (CNS), endocannabinoids act as retrograde inhibitors of neurotransmitter release through their binding and activation of presynaptic CB_1Rs (15). As AEA and 2-AG are lipids, they cannot be stored in lipid vesicles but are produced on demand. The stimulus triggering endocannabinoids synthesis from the post-synapse is likely to be an increase in intracellular Ca2+ due to metabotropic or ionotropic receptor activation (15).

During autocrine activation, endocannabinoids can activate CB_1R by lateral diffusion in the plasma membrane (15). However, it is still unclear how these ligands move through the extracellular space to reach their targets in a paracrine manner. In addition, the levels of endocannabinoids are tightly regulated by a 2-step degradation pathway. First, a passive re-uptake of released endocannabinoids occurs (6), followed by an intracellular enzymatic degradation. AEA is destroyed by the fatty acid amide hydrolase (FAAH) into ethanolamide and arachidonic acid (6), whereas 2-AG is inactivated by the monoacylglycerol lipase (MAGL) into glycerol and arachidonic acid (6) (Fig. 1).

Once its active form is stabilized by an endogenous or exogenous agonist, CB_1R can trigger multiple intracellular pathways such as a Gi/o-protein dependent inhibition of the adenylate cyclase or activation of the MAPK cascade (8). Of note, CB_1R can also couple Gq or Gs proteins in some cell types (8). Thus, the effects of CB_1R activation can be quite complex, spanning from ion channels modulation to intracellular kinases regulation. A typical short-term effect of neuronal CB_1R activation is the closure of N and P/Q types calcium channels and the opening of potassium channels. As a consequence, presynaptic neuronal CB_1R activation causes hyperpolarization, preventing further neurotransmitter release (6, 15). CB_1R activation exerts also long-term cellular effects by altering the expression of transcription factors through the modulation of various kinases, resulting in modification of a number of cellular mechanisms (i.e., protein synthesis, synaptic plasticity

and neurite remodeling) (16). CB_1R signaling can then be stopped by the internalization of the receptor mediated by β-Arrestin 2 (17). However, internalized CB_1Rs can still activate the MAPK pathway (18). Besides, CB_1Rs have been also found within neurons at mitochondrial membranes, where they regulate bioenergetic processes and mitochondrial respiration (19).

Apart from endogenous agonists, there are also endogenous molecules that can inhibit CB_1Rs. For instance, the neurosteroid pregnenolone is produced in the brain to prevent any neurotoxicity due to a huge load of exogenous CB_1R agonist (11). This allosteric inhibitor acts as a biased functional antagonist, preventing the ability of the agonist to activate the MAPK cascade (11).

The past: role of the ECS in the CNS regulation of food intake and the rise and fall of rimonabant

Cannabis as a bi-modulator of food intake in humans and rodents

The first documented use of cannabis dates back to 300 A.D. in India, where it was administered as a treatment for appetite loss. Interestingly, ancient Indian texts also report the use of potent preparations with high dose of cannabis by ascetics to overcome their hunger. Later, in the 1960s, the increase of recreational use of marijuana despite a federal prohibition in the USA (Marijuana Tax Act, 1937) raised public concern about the potential detrimental effects on human health. At the same time, the structure of THC, the main psychoactive component of marijuana, was described (1), initiating a series of scientific studies to understand the mechanisms of action of cannabis and its related compounds.

The first scientific study in humans was conducted in 1933 on 34 US soldiers stationed in Panama, who had access to marijuana. Apart from the sensation of feeling 'high' and 'happy,' these soldiers reported an increased appetite (20). Further studies confirmed that cannabis consumption induced hyperphagia (21), but these early investigations have to be reviewed with caution, as the doses of THC used were not standardized. In 1971, came the first study with controlled amount of oral THC consumption in young healthy subjects. A significant increase in food intake was observed after cannabis use when the subjects were already fed, with only a trend when they were in fasting (22). Later, in 1975, another study highlighted the fact that different doses of marijuana produce opposite outcomes on food intake: low doses

increased appetite whereas high doses suppressed it (23). Apart from effects caused by an acute administration of THC, investigators were also interested in assessing the changes in body weight and metabolism due to chronic marijuana consumption. The two first studies following human subjects daily smoking marijuana under laboratory conditions for 1 month described an increased body weight and food intake (24, 25). It was actually reported that the marijuana-induced hyperphagia was more prominent during periods of social interaction, suggesting that the context in which cannabis is consumed might affect its behavioral consequences. Another interesting observation was that marijuana specifically increased the consumption of sweets, suggesting that cannabinoids could not only enhance global caloric intake but also modulate food preference toward highly palatable foods. More data on the chronic consumption of cannabinoids came when THC (under the name of dronabinol) was approved by the Food and Drug Administration (FDA) in 1985 as a treatment for chemotherapy-induced nausea. The positive effects on appetite and body weight were observed in AIDS patients chronically treated with dronabinol (26, 27).

In order to further understand the mechanisms underlying these effects, investigators also studied cannabis administration in laboratory animals, especially in rodents. Again, as previously observed with marijuana consumption in humans (23), low doses of THC were associated with hyperphagia, whereas high doses had the opposite effect (21). Interestingly, a central effect of THC as a way to modulate food intake was proposed as far back as 1979, when a strong hyperphagia was observed in free-fed rats following THC injections in the ventromedial or latero-hypothalamic areas (LHA) (28).

## Beyond cannabis: the ECS as a central modulator of food intake

However, the understanding of cannabinoids action really flourished after the discovery of CB_1R in 1988 (2), followed soon afterward by the identification of AEA in 1992 (4). At this time, CB_1R was considered as the 'central' cannabinoid receptor, whereas CB_2R was the 'peripheral' receptor, found on immune cells. An important outcome at that time was also the development of the potent synthetic CB_1R inverse agonist rimonabant (SR141716A), a very useful pharmacological tool for the study of the physiological functions of the ECS (29).

The central effect of THC on feeding found in 1979 (28) was later confirmed in 1991, when THC facilitated food intake in rats during electrical stimulation of the LHA (30). Blockade of CB_1R during 14 days by daily intraperitoneal administration of rimonabant reduced body weight and food intake in non-obese, chow-fed rats in a dose-dependent manner (31). In addition, rimonabant reduced spontaneous or Neuropeptide Y (NPY)-elicited sucrose intake in rats (32), whereas CB_1R agonists, such as THC or CP 55 940, enhanced sucrose consumption (33, 34), emphasizing the role of the ECS in controlling hedonic properties of ingesta. In fact, both CB_1R and endocannabinoids are largely expressed in brain regions involved in the regulation of food intake and reward-related responses (reviewed in (9)). Consequently, several studies attempted to dissect the relationship between the ECS and the neuropeptidergic, dopaminergic and opioid systems known to have a role in the regulation of these processes.

Infusion of 2-AG in the nucleus accumbens (NAc) shell induced hyperphagia in free-fed rats in a CB_1R-dependent manner (35). The interaction with the opioid system was also demonstrated when a co-administration of rimonabant and naloxone (an opioid receptor antagonist) at doses that *per se* did not alter food intake resulted in a synergistic inhibition of food intake (36). Of note, subanorectic doses of naloxone and rimonabant were able to reduce palatable solution intake after CB_1R stimulation with THC (37). These studies therefore revealed some of the biological substrates for the modulation of food preference and the known ability of cannabinoids to increase consumption of palatable food (see also Fig. 2).

Investigations focusing on actions of the ECS on neuropeptidergic circuits demonstrated that a CB_1R agonist (WIN552122) inhibited orexin neurons and activated melanin-concentrating hormone neurons in the LHA, by inducing retrograde inhibition of presynaptic glutamate and GABA release respectively (38) (Fig. 2). Close links were also found between the ECS and hormones that affect energy balance regulation, like glucocorticoids, ghrelin and leptin (reviewed in (39)). In particular, it was shown that the orexigenic action of glucocorticoids in the paraventricular nucleus (PVN) of the hypothalamus relies on the ability of these steroid hormones to elicit endocannabinoid synthesis in parvocellular and magnocellular neurons. In turn, endocannabinoids would act presynaptically to suppress glutamatergic inputs onto the PVN, resulting in neuronal inhibition (40) (Fig. 2). Pharmacological blockade of CB_1R signaling with rimonabant can also prevent the orexigenic effect of intra-PVN injections of the hormone
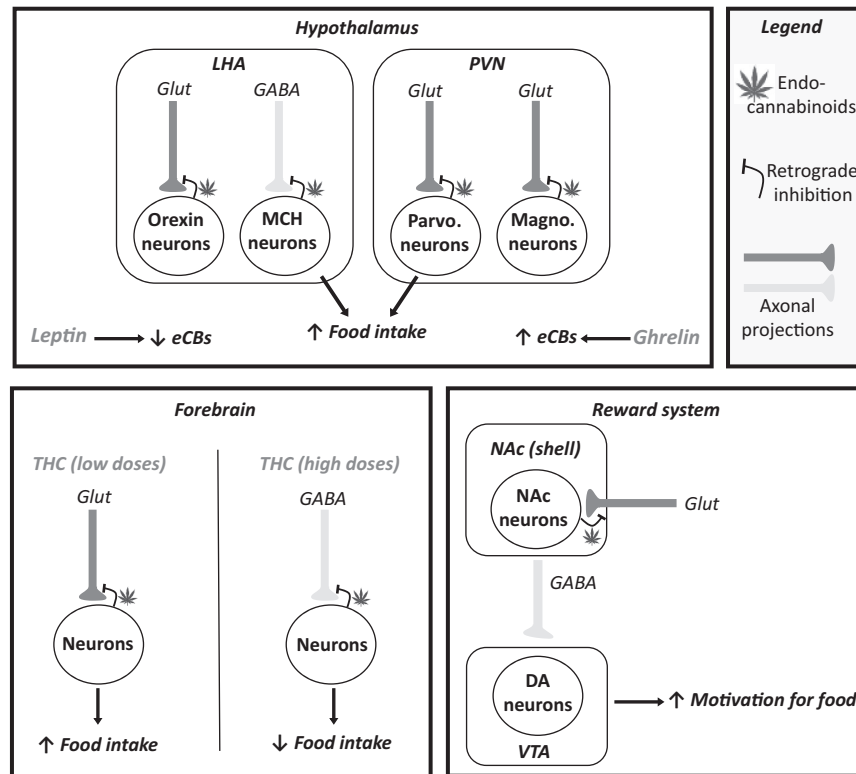
Figure 2

Schematic representation of the action of endocannabinoids on the CNS regulation of food intake. DA, dopamine; eCBs, endocannabinoids; Glut, glutamate; LHA, lateral hypothalamic area; MCH, melanin-concentrating-hormone; Magno., magnocellular; NAc, nucleus accumbens; Parvo., parvocellular; PVN, paraventricular nucleus; THC, Δ^9-tetrahydrocannabinol; VTA, ventral tegmental area.

ghrelin (41). Other studies then demonstrated that ghrelin increases hypothalamic endocannabinoid levels (42) and that systemic administration of rimonabant decreases while CB_1R agonists increase circulating ghrelin levels (43, 44). Thus, ECS stimulation facilitates ghrelin synthesis in the gut and ghrelin then modulates food intake by activating the ECS within hypothalamic circuits (reviewed in (39)).

A very close link was also found between the ECS and leptin. In 2001, Di Marzo *et al.* showed that genetically obese animals characterized by lack of leptin (i.e. *ob/ob* mice) or by defective leptin signaling had increased hypothalamic level of AEA and 2-AG (45) Conversely, acute leptin treatment in *ob/ob* mice or normal rats was able to decrease hypothalamic endocannabinoid levels, overall suggesting that endocannabinoids in the hypothalamus could act on CB_1Rs to maintain food intake and form part of the neural circuitry regulated by leptin (45). Soon afterward, it was shown that chronic administration of rimonabant had anti-obesity effects in diet-induced obese mice (46) and that animals genetically

lacking CB_1R (CB_1-KO) were hypophagic and lean (47). These studies therefore supported the idea that CB_1R antagonists could be effective therapeutic tools against obesity and metabolic disorders.

Rimona…Ban

Rimonabant was therefore tested in humans as anti-obesity drug. The Rimonabant In Obesity (RIO) phase III clinical trials started in August 2001 (48) and were supported by concomitant studies linking increased plasma levels of endocannabinoids with markers of obesity and metabolic syndrome in humans (see further below, section on human studies). Chronic administration of rimonabant in humans was successful at reducing body weight, fat mass and metabolic impairments related to obesity, such as dyslipidemia and diabetes (49). In 2006, the compound was approved by the EMA as an anti-obesity therapy under the name of Acomplia. In the US however, the concerns about severe psychiatric side effects halted its approval by the FDA. Shortly after Acomplia was released on the

European market, reports of increased anxiety, depression and even suicides raised serious concerns about its actual safety. Finally, in late 2008, the EMA suspended the use of Acomplia, based on the fact that its benefits no longer outweighed its risks, and also considering that patients at an elevated risk of developing psychiatric disorders could not be identified. In January 2009, the drug was finally withdrawn from the market. Because of this, regulatory authorities also terminated all ongoing clinical studies, including the Comprehensive Rimonabant Evaluation Study of Cardiovascular Endpoints and Outcomes (CRESCENDO) trial in which rimonabant was evaluated for the prevention of cardiovascular events. Four patients in the rimonabant group and one in the placebo group had committed suicide (50). As for the actual mechanism leading to the observed psychiatric side effects, it has been suggested that they might be due to the inverse agonist properties of rimonabant at constitutively active CB_1R in the ventral tegmental area (VTA) and amygdala (51).

The fall of rimonabant cast a shadow on future drug development targeting the ECS and caused profound controversy about the relevance of modulating the ECS in obesity and metabolic disorders.

The present: role of the ECS in the periphery and in brain–periphery interactions

In 2003, two independent studies unraveled the presence of functional CB_1Rs in white adipocytes (47, 52). This groundbreaking discovery paved the way for many other investigations exploring the presence and function of this receptor in peripheral non-neuronal tissues (adipose tissue, liver, gastrointestinal tract, pancreas and skeletal muscles) and helped the field to move forward after the fall of rimonabant.

The ECS in the adipose tissue

A complete ECS has been found in both murine and human adipocytes (53, 54). The first *in vitro* studies on white adipocytes showed that CB_1R activation increases the activity of the lipoprotein lipase (LPL), promoting the hydrolysis of triglycerides into non-esterified fatty acids and their subsequent uptake (47). In addition, CB_1R stimulation enhances fat storage within adipocytes through activation of lipogenetic enzymes and inhibition of the activity of the 5′-AMP-activated protein kinase (AMPK) (55). Apart from favoring lipogenesis, CB_1R also regulates adipogenesis by increasing the expression of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ), which promotes adipocyte differentiation (54). Interestingly, AEA can act as a PPARγ agonist, amplifying this ECS-induced adipogenesis (56, 57). Conversely, mitochondrial biogenesis is impaired by CB_1R activation, which favors the white adipocyte phenotype, while inhibiting the brown and 'beige' phenotype (reviewed in (9, 58)). Accordingly, the pharmacological inhibition of CB_1R induces fatty acid oxidation, mitochondrial biogenesis via increased expression of the endothelial nitric oxide synthase (59) and the transdifferentiation of white adipocytes into beige adipocytes (60). These cells, similar to brown adipocytes, are characterized by enriched mitochondria number, higher AMPK activity and increased uncoupling-protein 1 (UCP1) expression. Data obtained from genetically modified mice lacking CB_1R selectively on adipocytes indicate that these receptors regulate white adipose tissue (WAT) expansion, maintenance of white adipocyte phenotype and the development of obesity and insulin resistance (61). Thus, the results observed *in vitro* might be at least in part due to a direct peripheral action of endocannabinoids, although the sympathetic nervous system (SNS) is also involved in these responses (see further below). Of note, endocannabinoid levels in the WAT are negatively regulated by insulin (55) and leptin (62). This effect might be lost under insulin or leptin resistance, thus favoring ECS overactivity and fat accumulation. Interestingly, treatment of diet-induced obese mice with a peripherally restricted CB_1R inverse agonist (JD5037) decreases leptin production and release by adipocytes and increases leptin clearance. The consequent diminished leptinemia reverses leptin resistance, resulting in a decrease in body weight and food intake (63).

The ECS in the liver

Expression of CB_1R in hepatocytes can be induced by retinoic acid produced by the hepatic stellate cells (64), while the increase in the hepatic levels of AEA, typically observed during high-fat diet (HFD) exposure, is caused by a decrease in hepatic FAAH activity (65). FAAH is actually inhibited by monounsaturated fatty acids generated via the stearoyl CoA desaturase-1 (SCD-1), an enzyme whose expression in the liver is induced by HFD (65). In turn, activation of hepatic CB_1R by endocannabinoids induces the expression of sterol regulatory element binding

transcription factor 1 (SREBPF1), fatty acid synthase (FAS) and acetyl coenzyme-A carboxylase-1 (ACC1) resulting in fatty acid synthesis, which can lead to hepatic steatosis (66). Of note, serum levels of endocannabinoids have been found to be associated with nonalcoholic fatty liver disease, independent of obesity (67). Activation of hepatic CB_1R during HFD also causes insulin resistance through different molecular mechanisms, including inhibition of insulin signaling and clearance (68), and increased endoplasmic reticulum stress-dependent synthesis of long chain ceramides (69).

In contrast, mice with specific deletion of hepatic CB_1R develop obesity when fed a high-fat diet or after excessive glucocorticoid exposure, but they are protected against metabolic disorders such as liver steatosis, dyslipidemia, hyperglycemia and insulin resistance (70, 71). Accordingly, the beneficial effects of JD5037 on glucose and lipid metabolism in diet-induced obese mice rely on hepatic CB_1R (63), implying that hepatic CB_1R exerts a critical role in the regulation of lipid metabolism and insulin sensitivity.

The ECS in the gastrointestinal (GI) tract

The GI tract also contains all the elements of the ECS. At the beginning of the meal, when food is introduced into the mouth, cephalic-phase responses occur to anticipate and prepare proper digestion. This phenomenon can be studied using the sham-feeding model, in which animals eat and swallow ingesta, which will be re-routed out of their bodies, preventing digestion. Using this model, it has been shown that fat intake (but not proteins or carbohydrates) increases endocannabinoid levels in the rat jejunum. Interestingly, vagotomy prevents this sham-feeding effect on gut-derived endocannabinoids. This effect is also lost when rimonabant is given before sham feeding to locally block CB_1R in the small intestine (72, 73). Hence, the presence of fat in the oral cavity induces a cephalic-phase response resulting in jejunal production of endocannabinoids, which will further increase fat intake. The exact mechanisms behind this positive loop are not yet fully understood, but the orexigenic hormone ghrelin might play a role. Indeed, gastric CB_1R activation leads to ghrelin secretion, which increases fat-taste perception and promotes fat intake (74). Furthermore, the ECS in the gut may alter cholinergic transmission to the intestine, thereby reducing intestinal motility in rodents and humans (75). Together with this, the protective effects of CB_1Rs against intestinal

inflammation (75) make the ECS a putative enhancer of nutrient absorption in the GI tract. Interestingly, salivary endocannabinoids are measurable in human saliva and are found in higher proportion in obese patients compared to normal subjects (76). Although the function of salivary endocannabinoids is currently unknown, CB_1R is present on the mouse tongue and endocannabinoids are able to increase neural response specifically to sweet taste through a CB_1R-dependent mechanism (77). Thus, salivary endocannabinoids may modulate orosensory information and taste perception. This interpretation would agree with other data showing that the ECS can also modulate olfactory responses (78), pointing to a role for this system in the regulation of sensory information associated with food intake.

The ECS in the endocrine pancreas

Endocannabinoids play an important role in the regulation of cell proliferation and α/β cell sorting during pancreatic islets formation, which consequently has an impact on life-long programming of pancreatic glucagon and insulin secretion (79). CB_1R stimulation also induces exocytosis of insulin vesicles likely via the recruitment of focal adhesion kinases (FAK) allowing cytoskeletal reorganization (80). Consistently, pharmacological blockade of CB_1R in isolated pancreatic islets of lean mice inhibits glucose-mediated insulin release (81). While the activation of CB_1R on infiltrating macrophages in the pancreas increases the expression of interferon regulatory factor-5 (IRF5), a marker of M1 inflammatory macrophage polarization, causing inflammatory responses and β-cell death in type 2 diabetes (82, 83).

The ECS in the skeletal muscle

Finally, some evidence suggests that the ECS can affect glucose homeostasis by also acting onto the skeletal muscle, where CB_1R activation decreases basal and insulin-mediated glucose uptake, an effect blocked by pharmacological inhibition of CB_1R (84). As for the molecular mechanisms involved, it has been shown that the activation of CB_1R negatively impacts the responsiveness of skeletal muscle to insulin by acting on the PI 3-kinase/PKB and of the Raf-MEK1/2-ERK1/2 pathways (85). Besides, the activation of the ECS in muscle inhibits substrate oxidation and mitochondrial biogenesis, as similarly found at the level of the adipose tissue and liver (59).

Back to the brain

Despite intensive research focusing on peripheral ECS, recent investigations have also expanded our knowledge of the function of the ECS within the CNS.

In particular, it has been established in recent years that depending on the brain region and the location of CB_1Rs, the consequences of their activation can be completely different. Bellocchio *et al.* demonstrated in 2010 that the bi-modal control of food intake by the ECS depends on whether CB1Rs are located on glutamatergic or GABAergic terminals (Fig. 2). With low doses of THC, the suppression of glutamatergic transmission increases the appetite. However, when the doses are higher, GABAergic transmission is altered, especially at the level of the ventral striatum, resulting in hypophagia (86). This could therefore explain earlier reports in humans where biphasic effects of cannabis and/or THC were observed on food intake depending on the dose used. Besides, neurotransmitter release in reward-related brain regions linked to the mesolimbic dopaminergic pathway is crucial in the regulation of food intake. By acting on glutamatergic terminals, endocannabinoids reduce the activation of GABAergic NAc neurons projecting on the ventral tegmental area (VTA). Consequently, the dopamine-producing VTA neurons are relieved from their inhibition (87) and are allowed to release dopamine, likely driving the motivation for food. This same circuit can be 'rewired' by short exposure to palatable food, which can prime feeding behavior. This effect is mediated by the strengthening of excitatory synaptic transmission onto dopamine neurons that is offset by a short-term increase in the endocannabinoid tone (88). Recent studies further suggest that not just neuronal, but astroglial CB_1R might play a role in energy metabolism by modulating the action of leptin onto astrocytes (89) and that mitochondrial CB_1R might affect the function of hypothalamic circuits critically involved in the regulation of feeding (90).

Then, it is not surprising that the ECS regulates the bidirectional communication between the brain and the periphery, in the context of energy intake and storage. This is facilitated by the fact that CB_1Rs are present in the peripheral SNS and parasympathetic nervous system (91, 92). The alterations of CB_1R signaling in the forebrain can modify peripheral energy utilization via sympathetic outputs (93). Similarly, when 2-AG levels in the forebrain are decreased through the overexpression of its hydrolyzing enzyme MAGL, mice are protected against diet-induced obesity, thanks to increased β(3)-adrenergic-stimulated thermogenesis (94). Conversely, the deletion of the 2-AG

degrading enzyme ABHD6 hydrolase in the ventromedial hypothalamus (VMH) of mice causes an increase in VMH 2-AG content, which alters metabolic flexibility (95). Deletion of CB_1R in the VMH has also been shown to alter metabolic flexibility, which is associated with changes in SNS-dependent alterations in lypolysis/lipogenesis in the WAT (96). Additionally, genetic deletion of CB_1R in the PVN causes phenotypic changes due to increased SNS-driven energy expenditure and BAT thermogenesis (97). Finally, also the rapid hypophagic effect of rimonabant observed within 1-h from its administration relies on β-adrenergic transmission (98).

The ECS and metabolic disorders in humans

The past few years have seen an important increase in the number of human studies attempting to understand the role of the ECS in the regulation of eating behavior and metabolism. Endocannabinoids can be detected in the circulation and their assessment from blood samples is a simple strategy used for the study of the ECS. Notwithstanding the limitations of this approach related to differences in the handling, preparation or extraction of the sample among different laboratories and due to the lack of 'normal' range reference levels, several studies have shown positive association of plasma endocannabinoids with markers of obesity and metabolic disorder (53, 99, 100, 101, 102). Increased plasma endocannabinoids have been also found in obese subjects affected by Prader–Willi syndrome (103), in which treatment with rimonabant proved effective in reducing weight (104).

Levels of endocannabinoids in both plasma and cerebrospinal fluid may vary depending on the race (105). Circulating endocannabinoids also change across the 24-h sleep-wake cycle and sleep deprivation alters their levels, which is accompanied by increased hunger scores (106). Sleep disturbances are actually known to be a risk of obesity (107). Accordingly, plasma AEA is increased in patients suffering from obstructive sleep apnea, a condition that is often associated with obesity (108).

Several studies have then attempted to establish a functional link between circulating endocannabinoids and feeding behavior. We have reported that normal weight and obese subjects have a pre-prandial peak in plasma AEA, but not 2-AG, implying that AEA may act as a meal initiator signal in humans (109). However, when motivation for food is related to its palatability and not to hunger, others have observed an increase in plasma 2-AG in both healthy and obese subjects (110, 111) that is

Table 1 Evaluation of endocannabinoids and related compounds in human studies.

Measurements	Normal subjects	Obesity (vs normal weight)	T2D (vs healthy subjects)	Sleep apnea (vs non sleep apnea)	Coronary circulatory dysfunction
Plasma AEA	↑ after OGTT vs obese subjects (101)	↑ levels (53, 99, 116) Ø after OGTT – hyperinsulinemic subjects (101)	Ø (101) or ↑ (55) circulating levels	↑ in overweight (108) and obese subjects (144)	↑ in obese subjects (145)
	↓ after the meal vs obese subjects (109)	Ø after the meal – hyperinsulinemic subjects (109)			
	Positively associated with adiposity (133)				
Salivary AEA		↑ levels (76)			
Plasma 2-AG	↑ before consumption of favorite food, associated with ↑ levels of ghrelin (110)	Positive correlation with BMI and intraabdominal adiposity (100)			
		↑ levels in insulin-resistant obese women vs insulin-sensitive (102)			
	↓ after consumption of favorite food (110)	↑ after consumption of favorite food (111)	Ø (101) or ↑ (55) circulating levels	↑ in overweight (108) and obese subjects (144)	↑ in obese subjects (145)
	↑ after sleep restriction vs normal sleep (106)	↑ circulating levels (53) linked to ↓ olfactory capacity (110)			
Plasma OEA				↑ in normal weight (146) and obese subjects (144)	
OEA in CSF	↑ after 24h sleep deprivation vs sleep (147) Positively associated with EE (105)				

↑, increase; ↓, decrease; Ø, no difference; 2-AG, 2-Arachidonoylglycerol; AEA, N-arachidonoylethanolamide; CSF, cerebrospinal fluid; EE, energy expenditure; OEA, Oleoylethanolamide; OGTT, oral glucose tolerance test.

missing in anorexia nervosa (112). This implies that AEA and 2-AG may have different roles in the regulation of eating behavior, with the first acting to initiate the intake of calories, and the second to maintain the intake beyond satiety. Table 1 summarizes human studies, in which changes in endocannabinoids and endocannabinoid-related compounds have been investigated in the context of obesity and associated disorders.

Human genetic investigations further support a causative role of ECS overactivity in the pathogenesis of obesity. Metabolic syndrome and dyslipidemia have been associated with variants of the CNR1 gene (coding for CB_1R in humans) (113, 114). The 385 A/A missense polymorphism of FAAH, the major degrading enzyme of AEA, has been also associated with an obese phenotype characterized by cardiometabolic risk (115). Carriers of the 385 A/A FAAH polymorphism have increased the circulating AEA levels (115, 116) and show greater reward-related brain reactivity (117). Thus, assessment of circulating endocannabinoids and genetic analysis may

lead to the identification of obese subpopulations that should particularly benefit from treatments targeting the ECS.

The new findings discussed here clearly show that the ECS acts as a *chef d'orchestre* of metabolic homeostasis. Located both at a central and peripheral level, the ECS modulates the orders issued by different brain regions, it regulates the communication between the brain and the periphery and it fine-tunes the activity of every organ involved in lipid and glucose metabolism. Its overall action favors energy intake and storage. But when highly-caloric and palatable food is always available, the anabolic consequences of ECS overactivation likely promote obesity and metabolic disorders.

The future: focus on novel therapeutic approaches to modulate the ECS

Since the major side effects of drugs like rimonabant were CNS related, one opportunity to move forward could be

provided by CB_1R antagonists that are unable to pass the blood-brain barrier (118). Some of these drugs, such as the peripherally restricted CB_1R inverse agonist JD5037 and the CB_1R antagonist AM6545 have been shown to reduce obesity, reverse leptin resistance and improve hepatic steatosis, dyslipidemia and insulin resistance in genetically and diet-induced obese mice (63, 119, 120). These are quite important observations, because they imply that the blockade of CNS CB_1Rs is not required for the treatment of metabolic disease. As for the organs that are targeted by these peripherally restricted compounds in order to observe the beneficial effects, liver and adipose tissue likely play a key role.

JD5037 was shown to decrease hyperleptinemia in diet-induced obese mice by inhibiting leptin expression and secretion from adipocytes via both prejunctional and postjunctional mechanisms (63). This in turn seems to explain the decrease in food intake observed with the administration of JD5037 (63). Moreover, adipocyte CB_1R might be an interesting target for fat browning therapy, as suggested by both *in vitro* and *ex-vivo* data (reviewed in (58)). Considering that the browning process of the adipose tissue represents a promising venue for the development of new anti-obesity drugs (121), the action of peripherally restricted CB_1R antagonists or inverse agonists on the browning process and consequently on thermogenesis

should be further investigated. Alternatively, since some studies suggest that the psychiatric side effects of rimonabant were due to its inverse agonism on CB_1R (51); brain penetrant neutral CB_1R antagonists could eventually be used as therapeutic options. This class of compounds is able to decrease body weight in a manner comparable to rimonabant, while lacking anxiety/depression-like side effects (51, 122).

Combinatorial approaches could also be envisaged. This type of approach would benefit from the use of lower doses of the drugs, thus limiting possible side effects, while acting on different biological systems that participate in the regulation of energy balance, possibly leading to greater therapeutic success. For instance, a recent study by Kunos *et al*. has characterized the effects of a hybrid inhibitor of peripheral CB_1R and inducible nitric oxide synthase (iNOS) for the treatment of liver fibrosis (123, 124). This orally bioavailable compound accumulates in the liver where it releases an iNOS inhibitor, providing the possible advantage of an organ-targeted action. When administered to mice, the hybrid inhibitor was able to slow fibrosis progression and to attenuate established fibrosis (123).

Another way to modulate CB_1R activity is represented by compounds that could be developed by studying recently identified endogenous allosteric inhibitors
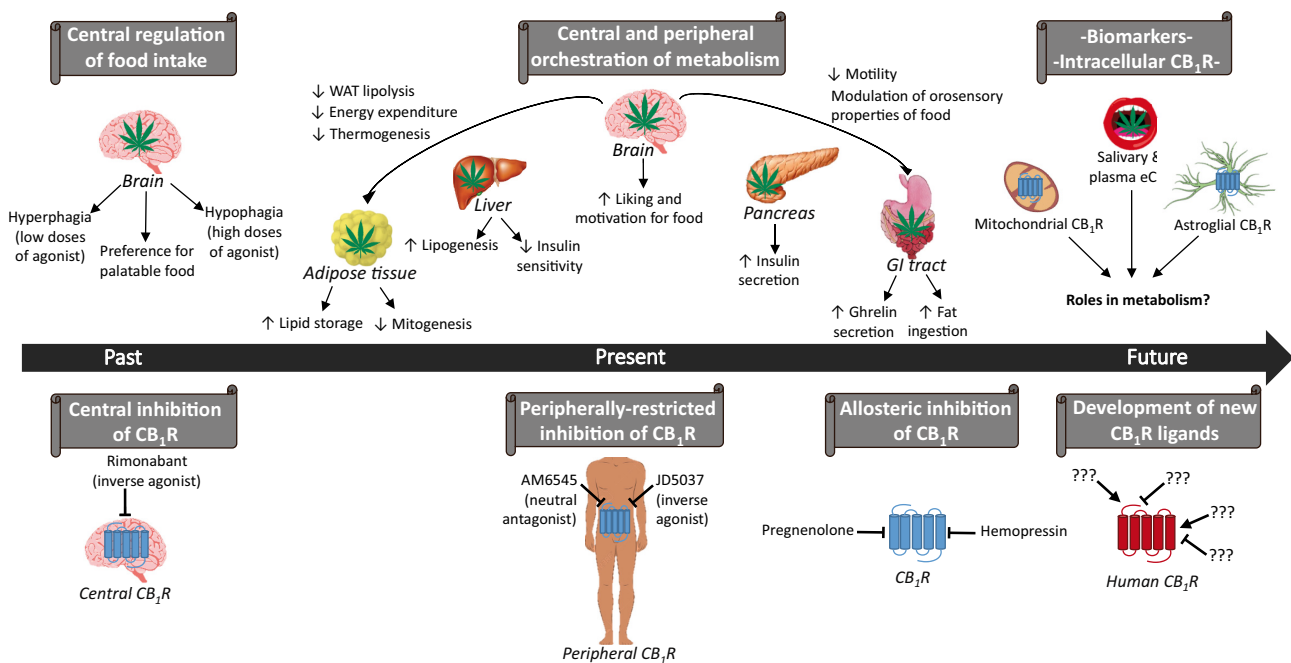
Figure 3

Major milestones through past and present studies, leading to possible future exploitation of the ECS for the treatment of metabolic disorders. Therapeutic targeting of CB_1Rs has been highlighted. eCBs, endocannabinoids; WAT, white adipose tissue.

of CB$_1$R. Hemopressin, pepcans and the neurosteroid pregnenolone have been identified as such (10, 11, 125). Hemopressin reduces food intake without causing any obvious adverse side effects, by mostly involving circuits of the mediobasal hypothalamus, rather than reward-related areas (126, 127). However, further studies are needed in order to confirm that these effects are due to the direct action of hemopressin on CB$_1$R (128). Pregnenolone is a signaling specific inhibitor of CB$_1$R (11, 129), whereas pregnenolone binding to CB$_1$R does not modify the binding of agonists, but selectively inhibits CB1R-mediated activation of the MAPK pathway, without affecting the inhibition of adenylate (adenylyl) cyclase (11, 129). Chronic administration of pregnenolone reduces body weight gain in diet-induced obese mice and it does not induce anxiety (129). Moreover, CB$_1$Rs are also localized on mitochondrial membranes, both in the brain (19, 90, 130) and in peripheral tissues (131). This evidence might open new perspectives for the development of novel CB$_1$R targeting drugs. Besides, the very recent description of the crystal structure of the human CB$_1$R will certainly help in designing and optimizing new CB$_1$R modulators with potential therapeutic use (132, 133).

A different approach then would be to modulate the levels of the ligands instead of directly targeting CB$_1$Rs. For instance, nutritional approaches aimed at limiting the presence of n-6 (PUFA) in the diet or at increasing n-3. PUFA should decrease the availability of endocannabinoid precursors by reducing the levels of arachidonic acid-esterified phospholipids (134, 135). Indeed, the consumption of food enriched in n-3 PUFA decreases plasma endocannabinoid levels and improves the lipid profile in obese or hypercholesterolemic subjects (136, 137). Thus, higher dietary consumption of n-3 PUFA might represent a simple, effective approach to reduce endocannabinoid levels to help prevent or treat metabolic disorders.

Conclusions

The ECS is involved in the development of preference for the consumption of certain foods, even in humans (138), it regulates taste and olfactory responses (77, 78) and controls metabolic changes associated with food intake. In turn, the type of diet consumed affects endocannabinoid levels and ECS activity. The ECS is therefore critically positioned to act at every level of the biological machinery aimed at modulating behavior and metabolism in response to changes in food availability (see also Fig. 3).

Here we have mostly reviewed information on the roles of anandamide, 2-AG and CB$_1$R in energy balance; however, it should be mentioned that some evidence suggests an involvement for CB$_2$R in energy balance (139, 140, 141) and that compounds structurally related to endocannabinoids but unable to bind to CB$_1$R, like oleoylethanolamide (OEA), actually oppose endocannabinoids effects on energy balance (142). Besides, endocannabinoids do not exclusively exert their biological functions through CBRs, however they can also bind transient receptor potential vanilloid 1 (TRPV1) (143) and the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (56). Nevertheless, and thanks to the recent advances in the field, the ECS and particularly the CB$_1$R are again interesting targets for therapy. Although it remains to be seen whether some of the new pharmacological approaches characterized in animals models will be equally efficient and safe in humans; this evidence provides renewed hope for the battle against obesity and metabolic disorders.

References

1   Gaoni Y & Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society* 1964 **86** 1646–1647. (doi:10.1021/ja01062a046)

2   Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS & Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology* 1988 **34** 605–613.

3   Munro S, Thomas KL & Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993 **365** 61–65. (doi:10.1038/365061a0)

4   Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A & Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992 **258** 1946–1949. (doi:10.1126/science.1470919)

5   Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR & Compton DR. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors.

European Journal of Endocrinology

Biochemical Pharmacology 1995 **50** 83–90. (doi:10.1016/0006-2952(95)00109-D)

6   Piomelli D. The molecular logic of endocannabinoid signalling. *Nature Reviews Neuroscience* 2003 **4** 873–884. (doi:10.1038/nrn1247)

7   McPartland JM, Matias I, Di Marzo V & Glass M. Evolutionary origins of the endocannabinoid system. *Gene* 2006 **370** 64–74. (doi:10.1016/j.gene.2005.11.004)

8   Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G & Mackie K. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacological Reviews* 2010 **62** 588–631. (doi:10.1124/pr.110.003004)

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

10 Bauer M, Chicca A, Tamborrini M, Eisen D, Lerner R, Lutz B, Poetz O, Pluschke G & Gertsch J. Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. *Journal of Biological Chemistry* 2012 **287** 36944–36967. (doi:10.1074/jbc.M112.382481)

11 Vallee M, Vitiello S, Bellocchio L, Hebert-Chatelain E, Monlezun S, Martin-Garcia E, Kasanetz F, Baillie GL, Panin F & Cathala A. Pregnenolone can protect the brain from cannabis intoxication. *Science* 2014 **343** 94–98. (doi:10.1126/science.1243985)

12 Ueda N, Tsuboi K & Uyama T. Metabolism of endocannabinoids and related N-acylethanolamines: canonical and alternative pathways. *FEBS Journal* 2013 **280** 1874–1894. (doi:10.1111/febs.12152)

13 Fezza F, Bari M, Florio R, Talamonti E, Feole M & Maccarrone M. Endocannabinoids, related compounds and their metabolic routes. *Molecules* 2014 **19** 17078–17106. (doi:10.3390/molecules191117078)

14 Okamoto Y, Morishita J, Tsuboi K, Tonai T & Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. *Journal of Biological Chemistry* 2004 **279** 5298–5305. (doi:10.1074/jbc.M306642200)

15 Castillo PE, Younts TJ, Chavez AE & Hashimotodani Y. Endocannabinoid signaling and synaptic function. *Neuron* 2012 **76** 70–81. (doi:10.1016/j.neuron.2012.09.020)

16 Basavarajappa BS. Neuropharmacology of the endocannabinoid signaling system-molecular mechanisms, biological actions and synaptic plasticity. *Current Neuropharmacology* 2007 **5** 81–97. (doi:10.2174/157015907780866910)

17 Turu G & Hunyady L. Signal transduction of the CB1 cannabinoid receptor. *Journal of Molecular Endocrinology* 2010 **44** 75–85. (doi:10.1677/JME-08-0190)

18 Rozenfeld R & Devi LA. Regulation of CB1 cannabinoid receptor trafficking by the adaptor protein AP-3. *FASEB Journal* 2008 **22** 2311–2322. (doi:10.1096/fj.07-102731)

19 Benard G, Massa F, Puente N, Lourenco J, Bellocchio L, Soria-Gomez E, Matias I, Delamarre A, Metna-Laurent M *et al*. Mitochondrial CB(1) receptors regulate neuronal energy metabolism. *Nature Neuroscience* 2012 **15** 558–564. (doi:10.1038/nn.3053)

20 Siler JF, Sheep WL, Bates LB, Clark GF, Cook GW & Smith WH. Marihuana smoking in Panama. *Military Surgeon* 1933 **73** 269–280.

21 Abel EL. Cannabis: effects on hunger and thirst. *Behavioral Biology* 1975 **15** 255–281. (doi:10.1016/S0091-6773(75)91684-3)

22 Hollister LE. Hunger and appetite after single doses of marihuana, alcohol, and dextroamphetamine. *Clinical Pharmacology and Therapeutics* 1971 **12** 44–49. (doi:10.1002/cpt197112144)

23 Gagnon MA & Elie R. Effects of marijuana and D-amphetamine on the appetite, food consumption and various cardio-respiratory variables in man. *L'union Medicale du Canada* 1975 **104** 914–921.

24 Greenberg I, Kuehnle J, Mendelson JH & Bernstein JG. Effects of marihuana use on body weight and caloric intake in humans. *Psychopharmacology* 1976 **49** 79–84.

25 Foltin RW, Fischman MW & Byrne MF. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite* 1988 **11** 1–14. (doi:10.1016/S0195-6663(88)80017-5)

26 Struwe M, Kaempfer SH, Geiger CJ, Pavia AT, Plasse TF, Shepard KV, Ries K & Evans TG. Effect of dronabinol on nutritional status in HIV infection. *Annals of Pharmacotherapy* 1993 **27** 827–831. (doi:10.1177/106002809302700701)

27 Beal JE, Olson R, Laubenstein L, Morales JO, Bellman P, Yangco B, Lefkowitz L, Plasse TF & Shepard KV. Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *Journal of Pain and Symptom Management* 1995 **10** 89–97. (doi:10.1016/0885-3924(94)00117-4)

28 Anderson-Baker WC, McLaughlin CL & Baile CA. Oral and hypothalamic injections of barbiturates, benzodiazepines and cannabinoids and food intake in rats. *Pharmacology Biochemistry and Behavior* 1979 **11** 487–491. (doi:10.1016/0091-3057(79)90030-3)

29 Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Néliat G & Caput D. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letters* 1994 **350** 240–244. (doi:10.1016/0014-5793(94)00773-X)

30 Trojniar W & Wise RA. Facilitory effect of delta 9-tetrahydrocannabinol on hypothalamically induced feeding. *Psychopharmacology* 1991 **103** 172–176. (doi:10.1007/BF02244199)

31 Colombo G, Agabio R, Diaz G, Lobina C, Reali R & Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Science* 1998 **63** PL113–117. (doi:10.1016/s0024-3205(98)00322-1)

32 Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P & Le Fur G. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology* 1997 **132** 104–106. (doi:10.1007/s002130050326)

33 Brown JE, Kassouny M & Cross JK. Kinetic studies of food intake and sucrose solution preference by rats treated with low doses of delta9-tetrahydrocannabinol. *Behavioral Biology* 1977 **20** 104–110. (doi:10.1016/S0091-6773(77)90606-X)

34 Gallate JE, Saharov T, Mallet PE & McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *European Journal of Pharmacology* 1999 **370** 233–240. (doi:10.1016/S0014-2999(99)00170-3)

35 Kirkham TC, Williams CM, Fezza F & Di Marzo V. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *British Journal of Clinical Pharmacology* 2002 **136** 550–557. (doi:10.1038/sj.bjp.0704767)

36 Rowland NE, Mukherjee M & Robertson K. Effects of the cannabinoid receptor antagonist SR 141716, alone and in combination with dexfenfluramine or naloxone, on food intake in rats. *Psychopharmacology* 2001 **159** 111–116. (doi:10.1007/s002130100910)

37 Williams CM & Kirkham TC. Reversal of delta 9-THC hyperphagia by SR141716 and naloxone but not dexfenfluramine. *Pharmacology Biochemistry and Behavior* 2002 **71** 333–340. (doi:10.1016/S0091-3057(01)00694-3)

38   Huang H, Acuna-Goycolea C, Li Y, Cheng HM, Obrietan K & van den Pol AN. Cannabinoids excite hypothalamic melanin-concentrating hormone but inhibit hypocretin/orexin neurons: implications for cannabinoid actions on food intake and cognitive arousal. *Journal of Neuroscience* 2007 **27** 4870–4881. (doi:10.1523/JNEUROSCI.0732-07.2007)

39   Bermudez-Silva FJ, Viveros MP, McPartland JM & Rodriguez de Fonseca F. The endocannabinoid system, eating behavior and energy homeostasis: the end or a new beginning? *Pharmacology Biochemistry and Behavior* 2010 **95** 375–382.

40   Tasker JG. Rapid glucocorticoid actions in the hypothalamus as a mechanism of homeostatic integration. *Obesity* 2006 **14** (Supplement 5) 259S–265S. (doi:10.1038/oby.2006.320)

41   Tucci SA, Rogers EK, Korbonits M & Kirkham TC. The cannabinoid CB1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin. *British Journal of Pharmacology* 2004 **143** 520–523. (doi:10.1038/sj.bjp.0705968)

42   Kola B, Farkas I, Christ-Crain M, Wittmann G, Lolli F, Amin F, Harvey-White J, Liposits Z, Kunos G & Grossman AB. The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS ONE* 2008 **3** e1797. (doi:10.1371/journal.pone.0001797)

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

44   Zbucki RL, Sawicki B, Hryniewicz A & Winnicka MM. Cannabinoids enhance gastric X/A-like cells activity. *Folia Histochemica et Cytobiologica* 2008 **46** 219–224.

45   Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, Fezza F, Miura GI, Palmiter RD & Sugiura T. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2001 **410** 822–825. (doi:10.1038/35071088)

46   Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP & Soubrie P. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *American Journal of Physiology* 2003 **284** R345–353. (doi:10.1152/ajpregu.00545.2002)

47   Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C & Ortmann S. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *Journal of Clinical Investigation* 2003 **112** 423–431. (doi:10.1172/JCI17725)

48   Fernandez JR & Allison DB. Rimonabant sanofi-synthelabo. *Current Opinion in Investigational Drugs* 2004 **5** 430–435.

49   Sam AH, Salem V & Ghatei MA. Rimonabant: from RIO to Ban. *Journal of Obesity* 2011 **2011** 432607. (doi:10.1155/2011/432607)

50   Topol EJ, Bousser MG, Fox KA, Creager MA, Despres JP, Easton JD, Hamm CW, Montalescot G, Steg PG & Pearson TA. Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial. *Lancet* 2010 **376** 517–523. (doi:10.1016/S0140-6736(10)60935-X)

51   Meye FJ, Trezza V, Vanderschuren LJ, Ramakers GM & Adan RA. Neutral antagonism at the cannabinoid 1 receptor: a safer treatment for obesity. *Molecular Psychiatry* 2013 **18** 1294–1301. (doi:10.1038/mp.2012.145)

52   Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F & Soubrie P. The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Molecular Pharmacology* 2003 **63** 908–914. (doi:10.1124/mol.63.4.908)

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

54   Matias I, Bisogno T & Di Marzo V. Endogenous cannabinoids in the brain and peripheral tissues: regulation of their levels and control of food intake. *International Journal of Obesity* 2006 **30** (Supplement 1) S7–S12. (doi:10.1038/sj.ijo.0803271)

55   Matias I, Gonthier MP, Orlando P, Martiadis V, De Petrocellis L, Cervino C, Petrosino S, Hoareau L, Festy F & Pasquali R. Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 3171–3180. (doi:10.1210/jc.2005-2679)

56   Bouaboula M, Hilairet S, Marchand J, Fajas L, Le Fur G & Casellas P. Anandamide induced PPARgamma transcriptional activation and 3T3-L1 preadipocyte differentiation. *European Journal of Pharmacology* 2005 **517** 174–181. (doi:10.1016/j.ejphar.2005.05.032)

57   Karaliota S, Siafaka-Kapadai A, Gontinou C, Psarra K & Mavri-Vavayanni M. Anandamide increases the differentiation of rat adipocytes and causes PPARgamma and CB1 receptor upregulation. *Obesity* 2009 **17** 1830–1838. (doi:10.1038/oby.2009.177)

58   Matias I, Belluomo I & Cota D. The fat side of the endocannabinoid system: role of endocannabinoids in the adipocyte. *Cannabis and Cannabinoid Research* 2016 **1** 176–185. (doi:10.1089/can.2016.0014)

59   Tedesco L, Valerio A, Dossena M, Cardile A, Ragni M, Pagano C, Pagotto U, Carruba MO, Vettor R & Nisoli E. Cannabinoid receptor stimulation impairs mitochondrial biogenesis in mouse white adipose tissue, muscle, and liver: the role of eNOS, p38 MAPK, and AMPK pathways. *Diabetes* 2010 **59** 2826–2836. (doi:10.2337/db09-1881)

60   Perwitz N, Wenzel J, Wagner I, Buning J, Drenckhan M, Zarse K, Ristow M, Lilienthal W, Lehnert H & Klein J. Cannabinoid type 1 receptor blockade induces transdifferentiation towards a brown fat phenotype in white adipocytes. *Diabetes, Obesity and Metabolism* 2010 **12** 158–166. (doi:10.1111/j.1463-1326.2009.01133.x)

61   Mancini G, Quarta C, Srivastava RK, Klaus S, Pagotto U & Lutz B. Adipocye-specific CB1 conditional knock-out mice: new insights in the study of obesity and metabolic syndrome. In *Paper Presented at the 20th Annual Symposium of the International Cannabinoid Society (Lund, Sweden)*, 2010.

62   Buettner C, Muse ED, Cheng A, Chen L, Scherer T, Pocai A, Su K, Cheng B, Li X & Harvey-White J. Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. *Nature Medicine* 2008 **14** 667–675. (doi:10.1038/nm1775)

63   Tam J, Cinar R, Liu J, Godlewski G, Wesley D, Jourdan T, Szanda G, Mukhopadhyay B, Chedester L & Liow JS. Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metabolism* 2012 **16** 167–179. (doi:10.1016/j.cmet.2012.07.002)

64   Mukhopadhyay B, Liu J, Osei-Hyiaman D, Godlewski G, Mukhopadhyay P, Wang L, Jeong WI, Gao B, Duester G & Mackie K Transcriptional regulation of cannabinoid receptor-1 expression in the liver by retinoic acid acting via retinoic acid receptor-gamma. *Journal of Biological Chemistry* 2010 **285** 19002–19011. (doi:10.1074/jbc.M109.068460)

65   Liu J, Cinar R, Xiong K, Godlewski G, Jourdan T, Lin Y, Ntambi JM & Kunos G. Monounsaturated fatty acids generated via stearoyl CoA desaturase-1 are endogenous inhibitors of fatty acid amide hydrolase. *PNAS* 2013 **110** 18832–18837. (doi:10.1073/pnas.1309469110)

66   Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, Harvey-White J, Mackie K, Offertaler L & Wang L.

Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *Journal of Clinical Investigation* 2005 **115** 1298–1305. (doi:10.1172/JCI200523057)

67 Zelber-Sagi S, Azar S, Nemirovski A, Webb M, Halpern Z, Shibolet O & Tam J. Serum levels of endocannabinoids are independently associated with nonalcoholic fatty liver disease. *Obesity* 2017 **25** 94–101. (doi:10.1002/oby.21687)

68 Liu J, Zhou L, Xiong K, Godlewski G, Mukhopadhyay B, Tam J, Yin S, Gao P, Shan X & Pickel J. Hepatic cannabinoid receptor-1 mediates diet-induced insulin resistance via inhibition of insulin signaling and clearance in mice. *Gastroenterology* 2012 **142** 1218–1228 e1211. (doi:10.1053/j.gastro.2012.01.032)

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

70 Osei-Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey-White J, Jeong WI, Batkai S, Marsicano G, Lutz B & Buettner C. Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *Journal of Clinical Investigation* 2008 **118** 3160–3169. (doi:10.1172/JCI34827)

71 Bowles NP, Karatsoreos IN, Li X, Vemuri VK, Wood JA, Li Z, Tamashiro KL, Schwartz GJ, Makriyannis AM & Kunos G. A peripheral endocannabinoid mechanism contributes to glucocorticoid-mediated metabolic syndrome. *PNAS* 2015 **112** 285–290. (doi:10.1073/pnas.1421420112)

72 DiPatrizio NV, Astarita G, Schwartz G, Li X & Piomelli D. Endocannabinoid signal in the gut controls dietary fat intake. *PNAS* 2011 **108** 12904–12908. (doi:10.1073/pnas.1104675108)

73 DiPatrizio NV, Joslin A, Jung KM & Piomelli D. Endocannabinoid signaling in the gut mediates preference for dietary unsaturated fats. *FASEB Journal* 2013 **27** 2513–2520. (doi:10.1096/fj.13-227587)

74 Perello M, Sakata I, Birnbaum S, Chuang JC, Osborne-Lawrence S, Rovinsky SA, Woloszyn J, Yanagisawa M, Lutter M & Zigman JM. Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biological Psychiatry* 2010 **67** 880–886. (doi:10.1016/j.biopsych.2009.10.030)

75 Izzo AA & Sharkey KA. Cannabinoids and the gut: new developments and emerging concepts. *Pharmacology and Therapeutics* 2010 **126** 21–38. (doi:10.1016/j.pharmthera.2009.12.005)

76 Matias I, Gatta-Cherifi B, Tabarin A, Clark S, Leste-Lasserre T, Marsicano G, Piazza PV & Cota D. Endocannabinoids measurement in human saliva as potential biomarker of obesity. *PLoS ONE* 2012 **7** e42399. (doi:10.1371/journal.pone.0042399)

77 Yoshida R, Ohkuri T, Jyotaki M, Yasuo T, Horio N, Yasumatsu K, Sanematsu K, Shigemura N, Yamamoto T & Margolskee RF. Endocannabinoids selectively enhance sweet taste. *PNAS* 2010 **107** 935–939. (doi:10.1073/pnas.0912048107)

78 Soria-Gomez E, Bellocchio L, Reguero L, Lepousez G, Martin C, Bendahmane M, Ruehle S, Remmers F, Desprez T & Matias I. The endocannabinoid system controls food intake via olfactory processes. *Nature Neuroscience* 2014 **17** 407–415. (doi:10.1038/nn.3647)

79 Malenczyk K, Keimpema E, Piscitelli F, Calvigioni D, Bjorklund P, Mackie K, Di Marzo V, Hokfelt TG, Dobrzyn A & Harkany T. Fetal endocannabinoids orchestrate the organization of pancreatic islet microarchitecture. *PNAS* 2015 **112** E6185–E6194. (doi:10.1073/pnas.1519040112)

80 Malenczyk K, Jazurek M, Keimpema E, Silvestri C, Janikiewicz J, Mackie K, Di Marzo V, Redowicz MJ, Harkany T & Dobrzyn A. CB1 cannabinoid receptors couple to focal adhesion kinase to control insulin release. *Journal of Biological Chemistry* 2013 **288** 32685–32699. (doi:10.1074/jbc.M113.478354)

81 Bermudez-Silva FJ, Romero-Zerbo SY, Haissaguerre M, Ruz-Maldonado I, Lhamyani S, El Bekay R, Tabarin A, Marsicano G & Cota D. The cannabinoid CB1 receptor and mTORC1 signalling pathways interact to modulate glucose homeostasis in mice. *Disease Models and Mechanisms* 2016 **9** 51–61. (doi:10.1242/dmm.020750)

82 Jourdan T, Godlewski G, Cinar R, Bertola A, Szanda G, Liu J, Tam J, Han T, Mukhopadhyay B & Skarulis MC. Activation of the Nlrp3 inflammasome in infiltrating macrophages by endocannabinoids mediates beta cell loss in type 2 diabetes. *Nature Medicine* 2013 **19** 1132–1140. (doi:10.1038/nm.3265)

83 Jourdan T, Szanda G, Cinar R, Godlewski G, Holovac DJ, Park JK, Nicoloro S, Shen Y, Liu J & Rosenberg AZ. Developmental role of macrophage cannabinoid-1 receptor signaling in type-2 diabetes. *Diabetes* 2017. **66** 994–1007. (doi:10.2337/db16-1199)

84 Lindborg KA, Teachey MK, Jacob S & Henriksen EJ. Effects of in vitro antagonism of endocannabinoid-1 receptors on the glucose transport system in normal and insulin-resistant rat skeletal muscle. *Diabetes, Obesity and Metabolism* 2010 **12** 722–730. (doi:10.1111/j.1463-1326.2010.01227.x)

85 Lipina C, Stretton C, Hastings S, Hundal JS, Mackie K, Irving AJ & Hundal HS. Regulation of MAP kinase-directed mitogenic and protein kinase B-mediated signaling by cannabinoid receptor type 1 in skeletal muscle cells. *Diabetes* 2010 **59** 375–385. (doi:10.2337/db09-0979)

86 Bellocchio L, Lafenetre P, Cannich A, Cota D, Puente N, Grandes P, Chaouloff F, Piazza PV & Marsicano G. Bimodal control of stimulated food intake by the endocannabinoid system. *Nature Medicine* 2010 **13** 281–283. (doi:10.1038/nn.2494)

87 Maldonado R, Valverde O & Berrendero F. Involvement of the endocannabinoid system in drug addiction. *Trends in Neurosciences* 2006 **29** 225–232. (doi:10.1016/j.tins.2006.01.008)

88 Liu S, Globa AK, Mills F, Naef L, Qiao M, Bamji SX & Borgland SL. Consumption of palatable food primes food approach behavior by rapidly increasing synaptic density in the VTA. *PNAS* 2016 **113** 2520–2525. (doi:10.1073/pnas.1515724113)

89 Bosier B, Bellocchio L, Metna-Laurent M, Soria-Gomez E, Matias I, Hebert-Chatelain E, Cannich A, Maitre M, Leste-Lasserre T & Cardinal P. Astroglial CB1 cannabinoid receptors regulate leptin signaling in mouse brain astrocytes. *Molecular Metabolism* 2013 **2** 393–404. (doi:10.1016/j.molmet.2013.08.001)

90 Koch M, Varela L, Kim JG, Kim JD, Hernandez-Nuno F, Simonds SE, Castorena CM, Vianna CR, Elmquist JK & Morozov YM. Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature* 2015 **519** 45–50. (doi:10.1038/nature14260)

91 Ishac EJ, Jiang L, Lake KD, Varga K, Abood ME & Kunos G. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *British Journal of Pharmacology* 1996 **118** 2023–2028. (doi:10.1111/j.1476-5381.1996.tb15639.x)

92 Coutts AA & Pertwee RG. Inhibition by cannabinoid receptor agonists of acetylcholine release from the guinea-pig myenteric plexus. *British Journal of Pharmacology* 1997 **121** 1557–1566. (doi:10.1038/sj.bjp.0701301)

93 Quarta C, Bellocchio L, Mancini G, Mazza R, Cervino C, Braulke LJ, Fekete C, Latorre R, Nanni C & Bucci M. CB(1) signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. *Cell Metabolism* 2010 **11** 273–285. (doi:10.1016/j.cmet.2010.02.015)

94 Jung KM, Clapper JR, Fu J, D'Agostino G, Guijarro A, Thongkham D, Avanesian A, Astarita G, DiPatrizio NV & Frontini A. 2-arachidonoylglycerol signaling in forebrain regulates systemic energy metabolism. *Cell Metabolism* 2012 **15** 299–310. (doi:10.1016/j.cmet.2012.01.021)

95 Fisette A, Tobin S, Decarie-Spain L, Bouyakdan K, Peyot ML, Madiraju SR, Prentki M, Fulton S & Alquier T. alpha/beta-

Hydrolase domain 6 in the ventromedial hypothalamus controls energy metabolism flexibility. *Cell Reports* 2016 **17** 1217–1226. (doi:10.1016/j.celrep.2016.10.004)

96 Cardinal P, Andre C, Quarta C, Bellocchio L, Clark S, Elie M, Leste-Lasserre T, Maitre M, Gonzales D & Cannich A. CB1 cannabinoid receptor in SF1-expressing neurons of the ventromedial hypothalamus determines metabolic responses to diet and leptin. *Molecular Metabolism* 2014 **3** 705–716. (doi:10.1016/j.molmet.2014.07.004)

97 Cardinal P, Bellocchio L, Guzman-Quevedo O, Andre C, Clark S, Elie M, Leste-Lasserre T, Gonzales D, Cannich A & Marsicano G. Cannabinoid type 1 (CB1) receptors on Sim1-expressing neurons regulate energy expenditure in male mice. *Endocrinology* 2015 **156** 411–418. (doi:10.1210/en.2014-1437)

98 Bellocchio L, Soria-Gomez E, Quarta C, Metna-Laurent M, Cardinal P, Binder E, Cannich A, Delamarre A, Haring M & Martin-Fontecha M. Activation of the sympathetic nervous system mediates hypophagic and anxiety-like effects of CB(1) receptor blockade. *PNAS* 2013 **110** 4786–4791. (doi:10.1073/pnas.1218573110)

99 Bluher M, Engeli S, Kloting N, Berndt J, Fasshauer M, Batkai S, Pacher P, Schon MR, Jordan J & Stumvoll M. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 2006 **55** 3053–3060. (doi:10.2337/db06-0812)

100 Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres JP & Di Marzo V. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *International Journal of Obesity* 2007 **31** 692–699.

101 Di Marzo V, Verrijken A, Hakkarainen A, Petrosino S, Mertens I, Lundbom N, Piscitelli F, Westerbacka J, Soro-Paavonen A & Matias I. Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects. *European Journal of Endocrinology* 2009 **161** 715–722. (doi:10.1530/EJE-09-0643)

102 Abdulnour J, Yasari S, Rabasa-Lhoret R, Faraj M, Petrosino S, Piscitelli F, Prud' Homme D & Di Marzo V. Circulating endocannabinoids in insulin sensitive vs. insulin resistant obese postmenopausal women. A MONET group study. *Obesity* 2014 **22** 211–216. (doi:10.1002/oby.20498)

103 Knani I, Earley BJ, Udi S, Nemirovski A, Hadar R, Gammal A, Cinar R, Hirsch HJ, Pollak Y & Gross I. Targeting the endocannabinoid/CB1 receptor system for treating obesity in Prader-Willi syndrome. *Molecular Metabolism* 2016 **5** 1187–1199. (doi:10.1016/j.molmet.2016.10.004)

104 Motaghedi R, Lipman EG, Hogg JE, Christos PJ, Vogiatzi MG & Angulo MA. Psychiatric adverse effects of rimonobant in adults with Prader Willi syndrome. *European Journal of Medical Genetics* 2011 **54** 14–18. (doi:10.1016/j.ejmg.2010.09.015)

105 Jumpertz R, Guijarro A, Pratley RE, Piomelli D & Krakoff J. Central and peripheral endocannabinoids and cognate acylethanolamides in humans: association with race, adiposity, and energy expenditure. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 115–121. (doi:10.1210/jc.2010-1669)

106 Cedernaes J, Fanelli F, Fazzini A, Pagotto U, Broman JE, Vogel H, Dickson SL, Schioth HB & Benedict C. Sleep restriction alters plasma endocannabinoids concentrations before but not after exercise in humans. *Psychoneuroendocrinology* 2016 **74** 258–268. (doi:10.1016/j.psyneuen.2016.09.014)

107 Broussard JL & Van Cauter E. Disturbances of sleep and circadian rhythms: novel risk factors for obesity. *Current Opinion in Endocrinology, Diabetes and Obesity* 2016 **23** 353–359. (doi:10.1097/MED.0000000000000276)

108 Wang X, Yu Q, Yue H, Zhang J, Zeng S & Cui F. Circulating endocannabinoids and insulin resistance in patients with obstructive sleep apnea. *BioMed Research International* 2016 **2016** 9782031. (doi:10.1155/2016/9782031)

109 Gatta-Cherifi B, Matias I, Vallee M, Tabarin A, Marsicano G, Piazza PV & Cota D. Simultaneous postprandial deregulation of the orexigenic endocannabinoid anandamide and the anorexigenic peptide YY in obesity. *International Journal of Obesity* 2012 **36** 880–885. (doi:10.1038/ijo.2011.165)

110 Monteleone P, Piscitelli F, Scognamiglio P, Monteleone AM, Canestrelli B, Di Marzo V & Maj M. Hedonic eating is associated with increased peripheral levels of ghrelin and the endocannabinoid 2-arachidonoyl-glycerol in healthy humans: a pilot study. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E917–E924. (doi:10.1210/jc.2011-3018)

111 Monteleone AM, Di Marzo V, Monteleone P, Dalle Grave R, Aveta T, Ghoch ME, Piscitelli F, Volpe U, Calugi S & Maj M. Responses of peripheral endocannabinoids and endocannabinoid-related compounds to hedonic eating in obesity. *European Journal of Nutrition* 2016 **55** 1799–1805. (doi:10.1007/s00394-016-1153-9)

112 Monteleone AM, Di Marzo V, Aveta T, Piscitelli F, Dalle Grave R, Scognamiglio P, El Ghoch M, Calugi S, Monteleone P & Maj M. Deranged endocannabinoid responses to hedonic eating in underweight and recently weight-restored patients with anorexia nervosa. *American Journal of Clinical Nutrition* 2015 **101** 262–269. (doi:10.3945/ajcn.114.096164)

113 Bordicchia M, Battistoni I, Mancinelli L, Giannini E, Refi G, Minardi D, Muzzonigro G, Mazzucchelli R, Montironi R & Piscitelli F. Cannabinoid CB1 receptor expression in relation to visceral adipose depots, endocannabinoid levels, microvascular damage, and the presence of the Cnr1 A3813G variant in humans. *Metabolism* 2010 **59** 734–741. (doi:10.1016/j.metabol.2009.09.018)

114 Baye TM, Zhang Y, Smith E, Hillard CJ, Gunnell J, Myklebust J, James R, Kissebah AH, Olivier M & Wilke RA. Genetic variation in cannabinoid receptor 1 (CNR1) is associated with derangements in lipid homeostasis, independent of body mass index. *Pharmacogenomics* 2008 **9** 1647–1656. (doi:10.2217/14622416.9.11.1647)

115 Martins CJ, Genelhu V, Pimentel MM, Celoria BM, Mangia RF, Aveta T, Silvestri C, Di Marzo V & Francischetti EA. Circulating Endocannabinoids and the polymorphism 385C>A in fatty acid amide hydrolase (FAAH) gene may identify the obesity phenotype related to cardiometabolic risk: a study conducted in a Brazilian population of complex interethnic admixture. *PLoS ONE* 2015 **10** e0142728. (doi:10.1371/journal.pone.0142728)

116 Sipe JC, Scott TM, Murray S, Harismendy O, Simon GM, Cravatt BF & Waalen J. Biomarkers of endocannabinoid system activation in severe obesity. *PLoS ONE* 2010 **5** e8792. (doi:10.1371/journal.pone.0008792)

117 Hariri AR, Gorka A, Hyde LW, Kimak M, Halder I, Ducci F, Ferrell RE, Goldman D & Manuck SB. Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. *Biological Psychiatry* 2009 **66** 9–16. (doi:10.1016/j.biopsych.2008.10.047)

118 Silvestri C & Di Marzo V. Second generation CB1 receptor blockers and other inhibitors of peripheral endocannabinoid overactivity and the rationale of their use against metabolic disorders. *Expert Opinion on Investigational Drugs* 2012 **21** 1309–1322. (doi:10.1517/13543784.2012.704019)

119 Cluny NL, Vemuri VK, Chambers AP, Limebeer CL, Bedard H, Wood JT, Lutz B, Zimmer A, Parker LA & Makriyannis A. A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. *British Journal of Pharmacology* 2010 **161** 629–642. (doi:10.1111/j.1476-5381.2010.00908.x)

120 Tam J, Vemuri VK, Liu J, Batkai S, Mukhopadhyay B, Godlewski G, Osei-Hyiaman D, Ohnuma S, Ambudkar SV & Pickel J. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *Journal of Clinical Investigation* 2010 **120** 2953–2966. (doi:10.1172/JCI42551)

121 Giordano A, Frontini A & Cinti S. Convertible visceral fat as a therapeutic target to curb obesity. *Nature Reviews Drug Discovery* 2016 **15** 405–424. (doi:10.1038/nrd.2016.31)

122 Gueye AB, Pryslawsky Y, Trigo JM, Poulia N, Delis F, Antoniou K, Loureiro M, Laviolette SR, Vemuri K, Makriyannis A & Le Foll B. The CB1 neutral antagonist AM4113 retains the therapeutic efficacy of the inverse agonist rimonabant for nicotine dependence and weight loss with better psychiatric tolerability. *International Journal of Neuropsychopharmacology* 2016 **19** pyw068. (doi:10.1093/ijnp/pyw068)

123 Cinar R, Iyer MR, Liu Z, Cao Z, Jourdan T, Erdelyi K, Godlewski G, Szanda G, Liu J & Park JK. Hybrid inhibitor of peripheral cannabinoid-1 receptors and inducible nitric oxide synthase mitigates liver fibrosis. *JCI Insight* 2016 **1** e87336. (doi:10.1172/jci.insight.87336)

124 Iyer MR, Cinar R, Katz A, Gao M, Erdelyi K, Jourdan T, Coffey NJ, Pacher P & Kunos G. Design, synthesis, and biological evaluation of novel, non-brain-penetrant, hybrid cannabinoid CB1R inverse agonist/inducible nitric oxide synthase (iNOS) inhibitors for the treatment of liver fibrosis. *Journal of Medicinal Chemistry* 2017 **60** 1126–1141. (doi:10.1021/acs.jmedchem.6b01504)

125 Heimann AS, Gomes I, Dale CS, Pagano RL, Gupta A, de Souza LL, Luchessi AD, Castro LM, Giorgi R, Rioli V *et al*. Hemopressin is an inverse agonist of CB1 cannabinoid receptors. *PNAS* 2007 **104** 20588–20593. (doi:10.1073/pnas.0706980105)

126 Dodd GT, Mancini G, Lutz B & Luckman SM. The peptide hemopressin acts through CB1 cannabinoid receptors to reduce food intake in rats and mice. *Journal of Neuroscience* 2010 **30** 7369–7376. (doi:10.1523/JNEUROSCI.5455-09.2010)

127 Dodd GT, Worth AA, Hodkinson DJ, Srivastava RK, Lutz B, Williams SR & Luckman SM. Central functional response to the novel peptide cannabinoid, hemopressin. *Neuropharmacology* 2013 **71** 27–36. (doi:10.1016/j.neuropharm.2013.03.007)

128 Macedonio G, Stefanucci A, Maccallini C, Mirzaie S, Novellino E & Mollica A. Hemopressin peptides as modulators of the endocannabinoid system and their potential applications as therapeutic tools. *Protein and Peptide Letters* 2016 **23** 1045–1051.

129 Piazza PV, Vallée M, Marsicano G, Felpin FX, Bellocchio L, Cota D, Revest JM, Vitiello S, Spampinato U & Maldonado R. INSERM and University of Bordeaux. Antagonists of CB1 receptor. Patent Publication N° WO/2012/160006. International Application N: PCT/EP2012/059310.

130 Hebert-Chatelain E, Desprez T, Serrat R, Bellocchio L, Soria-Gomez E, Busquets-Garcia A, Pagano Zottola AC, Delamarre A, Cannich A & Vincent P. A cannabinoid link between mitochondria and memory. *Nature* 2016 **539** 555–559. (doi:10.1038/nature20127)

131 Mendizabal-Zubiaga J, Melser S, Benard G, Ramos A, Reguero L, Arrabal S, Elezgarai I, Gerrikagoitia I, Suarez J, Rodriguez De Fonseca F *et al*. Cannabinoid CB1 receptors are localized in striated muscle mitochondria and regulate mitochondrial respiration. *Frontiers in Physiology* 2016 **7** 476. (doi:10.3389/fphys.2016.00476)

132 Shao Z, Yin J, Chapman K, Grzemska M, Clark L, Wang J & Rosenbaum DM. High-resolution crystal structure of the human CB1 cannabinoid receptor. *Nature* 2016 Epub ahead of print. (doi:10.1038/nature20613)

133 Hua T, Vemuri K, Pu M, Qu L, Han GW, Wu Y, Zhao S, Shui W, Li S & Korde A. Crystal structure of the human cannabinoid receptor CB1. *Cell* 2016 **167** 750–762.e714. (doi:10.1016/j.cell.2016.10.004)

134 Kinsella JE. Lipids, membrane receptors, and enzymes: effects of dietary fatty acids. *Journal of Parenteral and Enteral Nutrition* 1990 **14** 200S–217S. (doi:10.1177/014860719001400511)

135 Di Marzo V. Endocannabinoids: synthesis and degradation. *Reviews of Physiology, Biochemistry and Pharmacology* 2008 **160** 1–24. (doi:10.1007/112_0505)

136 Berge K, Piscitelli F, Hoem N, Silvestri C, Meyer I, Banni S & Di Marzo V. Chronic treatment with krill powder reduces plasma triglyceride and anandamide levels in mildly obese men. *Lipids in Health and Disease* 2013 **12** 78. (doi:10.1186/1476-511X-12-78)

137 Pintus S, Murru E, Carta G, Cordeddu L, Batetta B, Accossu S, Pistis D, Uda S, Elena Ghiani M & Mele M. Sheep cheese naturally enriched in alpha-linolenic, conjugated linoleic and vaccenic acids improves the lipid profile and reduces anandamide in the plasma of hypercholesterolaemic subjects. *British Journal of Nutrition* 2013 **109** 1453–1462. (doi:10.1017/S0007114512003224)

138 Mennella I, Ferracane R, Zucco F, Fogliano V & Vitaglione P. Food liking enhances the plasma response of 2-arachidonoylglycerol and of pancreatic polypeptide upon modified sham feeding in humans. *Journal of Nutrition* 2015 **145** 2169–2175. (doi:10.3945/jn.114.207704)

139 Rossi F, Bellini G, Luongo L, Manzo I, Tolone S, Tortora C, Bernardo ME, Grandone A, Conforti A & Docimo L. Cannabinoid receptor 2 as antiobesity target: inflammation, fat storage, and browning modulation. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 3469–3478. (doi:10.1210/jc.2015-4381)

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

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

142 Piomelli D. A fatty gut feeling. *Trends in Endocrinology and Metabolism* 2013 **24** 332–341. (doi:10.1016/j.tem.2013.03.001)

143 Di Marzo V & De Petrocellis L. Endocannabinoids as regulators of transient receptor potential (TRP) channels: a further opportunity to develop new endocannabinoid-based therapeutic drugs. *Current Medicinal Chemistry* 2010 **17** 1430–1449. (doi:10.2174/092986710790980078)

144 Engeli S, Bluher M, Jumpertz R, Wiesner T, Wirtz H, Bosse-Henck A, Stumvoll M, Batkai S, Pacher P & Harvey-White J. Circulating anandamide and blood pressure in patients with obstructive sleep apnea. *Journal of Hypertension* 2012 **30** 2345–2351. (doi:10.1097/HJH.0b013e3283591595)

145 Quercioli A, Pataky Z, Vincenti G, Makoundou V, Di Marzo V, Montecucco F, Carballo S, Thomas A, Staub C & Steffens S. Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity. *European Heart Journal* 2011 **32** 1369–1378. (doi:10.1093/eurheartj/ehr029)

146 Jumpertz R, Wiesner T, Bluher M, Engeli S, Batkai S, Wirtz H, Bosse-Henck A & Stumvoll M. Circulating endocannabinoids and N-acyl-ethanolamides in patients with sleep apnea – specific role of oleoylethanolamide. *Experimental and Clinical Endocrinology and Diabetes* 2010 **118** 591–595. (doi:10.1055/s-0030-1253344)

147 Koethe D, Hoyer C & Leweke FM. The endocannabinoid system as a target for modelling psychosis. *Psychopharmacology* 2009 **206** 551–561. (doi:10.1007/s00213-009-1591-7)