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REVIEWS

Review

Regulation of Adipose Tissue Metabolism by the Endocannabinoid System

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White adipose tissue (WAT) stores excess energy as triglycerides, and brown adipose tissue (BAT) is specialized in dissipating energy as heat. The endocannabinoid system (ECS) is involved in a broad range of physiological processes and is increasingly recognized as a key player in adipose tissue metabolism. High ECS tonus in the fed state is associated with a disadvantageous metabolic phenotype, and this has led to a search for pharmacological strategies to inhibit the ECS. In this review we present recent developments that cast light on the regulation of adipose tissue metabolism by the ECS, and we discuss novel treatment options including the modulation of endocannabinoid synthesis and breakdown enzymes.

Highlights

Activation of BAT thermogenesis and WAT browning protects from adiposity and related cardiometabolic disorders.

The endocannabinoid system is a key player in adipose tissue function.

Endocannabinoids provide an autocrine/paracrine negative feedback loop in adrenergic stimulation of adipose tissue.

The development of modulators of synthesis and breakdown enzymes of endocannabinoids opens up a new window for therapeutic interventions.

Introduction

Since 1975 the prevalence of obesity has nearly tripled. Over 1.9 billion people worldwide are currently overweight, of whom 650 million suffer from obesity (WHO factsheet *Obesity and Overweight* 2017; www.who.int/mediacentre/factsheets/fs311/en/). As a consequence, the incidence of obesity-related disorders such as type 2 diabetes and cardiovascular diseases is rising. Interestingly, over the past three decades a great amount of research has revealed that the endocannabinoid system (ECS) is a central modulator of metabolic physiology, and the ECS is now increasingly recognized as a regulator of adipose tissue function. In fact, **rimonabant** (see Glossary), a cannabinoid 1 receptor (CB1R) inverse agonist, was one of the first drugs that reached the European market to treat obesity. It was successful in reducing fat mass and improving metabolic health, but it was withdrawn 2 years later owing to psychiatric side effects observed in some patients (reviewed in [1]). The discovery of a peripheral mode of action of CB1R led to renewed interest in the ECS being a target for obesity and related disorders [2,3]. We review here recent developments that highlight the role of the ECS in adipose tissue function, and we discuss alternative treatment options that target the ECS to improve cardiometabolic health.

Adipose Tissue Physiology

Role of WAT and BAT in Energy Homeostasis

White adipose tissue (WAT) has long been considered to be an inactive organ, only capable of storing energy in the form of triglycerides (TGs). However, its role as an endocrine organ and its importance for whole-body metabolism has now been well-recognized. WAT is responsible for the synthesis of various hormones, including leptin and adiponectin, which are crucial in regulating satiety and insulin sensitivity, respectively [4]. WAT is also important in regulating energy homeostasis because it is capable of releasing TG-derived fatty acids (FAs) into the bloodstream, which can subsequently be used by other organs as an energy substrate or be packaged in TG-rich **lipoproteins** in the liver.

In contrast to WAT, the main function of **brown adipose tissue** (BAT) is to dissipate energy into heat. BAT is localized in the interscapular, cervical, and paravertebral regions around large

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arteries, where it is able to take up glucose and (TG-derived) FAs from the bloodstream. Via the process of lipogenesis, these nutrients are temporarily stored in intracellular lipid droplets. When the environmental temperature drops, BAT breaks down these stored TGs to produce heat by mitochondrial uncoupling involving **uncoupling protein 1** (UCP-1), a process called adaptive or **non-shivering thermogenesis** (Box 1). To replenish lipid stores, brown adipocytes take up glucose (via GLUT1 and GLUT4), free FAs (via CD36), and TG-derived FAs that are liberated by **lipoprotein lipase** (LPL). Importantly, a decade ago BAT was found to be present and active in adult humans, as visualized by glucose uptake in [^{18}F]fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) scans during cold exposure [5,6].

Browning of WAT

Prolonged cold exposure or β3-adrenergic receptor agonism [7] not only stimulates BAT but also leads to **browning** of WAT either by **transdifferentiation** of existing white adipocytes or by stimulation of precursor cells to differentiate into brite (brown-in-white) adipocytes within WAT [8,9]. The relative contributions of these pathways is a matter of debate, but for the purpose of this review we will call these cells beige cells. These cells are phenotypically different from WAT and BAT by having very low intrinsic expression of BAT-specific genes, such as *UCP-1*, *CIDEA*, and peroxisome proliferator-activated receptor gamma coactivator 1α (*PPARGC1A*), but UCP-1 levels can very rapidly be increased upon sympathetic stimuli via cAMP/PKA signaling, allowing **uncoupled respiration** [10,11]. Like brown adipocytes, beige cells have multilocular intracellular lipid droplets and a high mitochondrial content.

Human BAT Consists of Both Beige and Brown Adipocytes

There has been debate about the cellular origin of human BAT. Some studies suggest that human BAT consists mostly of beige adipocytes, instead of classical brown adipocytes (reviewed in [12,13]). For example, Wu *et al*. [10] demonstrated that expression of the human counterparts of the *Cd137*, *Tbx1*, and *Tmem26* genes, which are almost exclusively expressed in murine beige adipocytes, are highly expressed in human BAT, whereas classical brown markers are not. By contrast, a more recent study showed that human BAT does express classical brown adipocyte-specific markers [14]. This discrepancy may be explained by inter-

Box 1. BAT Physiology

At the cellular level, brown adipocytes are characterized by a high number of mitochondria to achieve a high oxidative capacity, and by multilocular intracellular lipid droplets in which fatty acids are temporarily stored as TGs. Furthermore, a large number of nerve endings of the sympathetic nervous system, spread across the tissue, enables brown adipocytes to quickly respond to a cold environment. Upon cold stimuli, sympathetic outflow towards BAT is increased, thereby stimulating the release of norepinephrine by sympathetic nerve termini in the adipose tissue. This binds to and stimulates β3-adrenergic receptors, which are G protein-coupled receptors, on brown adipocytes, a process that can be mimicked by administration of a β3-adrenergic agonist in mice [7] and humans [89]. β3-Adrenergic activation triggers an intracellular signaling cascade that promotes intracellular lipolysis. Specifically, the activity of intracellular adenylyl cyclase is activated, resulting in a rise in cAMP levels. cAMP subsequently activates protein kinase A (PKA), which then phosphorylates a series of enzymes that are crucial for lipolysis, namely perilipin 1, comparative gene identification-58 (CGI-58), hormone-sensitive lipase (HSL), and adipose triglyceride lipase (ATGL). Phospho-HSL is responsible for the hydrolysis of TGs and diglycerides, the first of which is also mediated by phospho-ATGL and is the rate-limiting step in lipolysis [90]. Liberated FAs undergo β-oxidation within the mitochondrial matrix and allosterically activate UCP-1. UCP-1 is a protein present in the mitochondrial inner membrane that uncouples the mitochondrial electron transport chain from ATP synthesis by facilitating proton leakage into the mitochondrial matrix [91]. An increase in UCP-1 activity therefore results in heat production, also known as uncoupled respiration.

Importantly, thermogenic activation of BAT reduces plasma TG and cholesterol levels, as shown in mice [7,92]. Specifically, TG-derived FA uptake by BAT results in accelerated hepatic clearance of cholesterol-enriched lipoprotein remnants, which thereby reduces the development of diet-induced atherosclerosis, as reviewed by Hoeke *et al*. [93].

Glossary

***N*-Arachidonoylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD):** the main enzyme responsible for anandamide (AEA) synthesis.
Brown adipose tissue (BAT): an endocrine organ specialized in dissipating energy that is stored in the form of triglycerides (TGs) into heat.
Browning: a process in which white adipocytes obtain thermogenic properties.
Diacylglycerol lipase (DAGL): the main enzyme responsible for 2-arachidonoylglycerol (2-AG) synthesis.
Endocannabinoid: an endogenous lipid-based compound that binds to cannabinoid receptors.
Fatty acid amide hydrolase (FAAH): the main enzyme responsible for AEA degradation.
Insulin resistance: a condition in which cells in for example liver, muscle, and adipose tissue fail to respond to insulin.
Lipoprotein: a multimolecular complex built from lipids and protein (s) with the purpose of transporting hydrophobic lipids through the aqueous plasma.
Lipoprotein lipase (LPL): a protein bound to capillaries of metabolically active tissues that hydrolyzes TGs within lipoproteins to liberate fatty acids (FAs) for subsequent uptake by those tissues.
Monoacylglycerol lipase (MAGL): the main enzyme responsible for 2-AG degradation.
Non-shivering thermogenesis: the production of heat without shivering through for example uncoupled respiration in BAT.
Rimonabant: the first cannabinoid receptor type 1 (CB1R) inverse agonist that was approved for use in humans, but which was later withdrawn owing to psychiatric side effects.
Sympathomimetic: a compound that mimics the effect of endogenous agonists of the sympathetic nervous system.
Thermoneutrality: the temperature at which an animal does not need to regulate its body temperature (approximately 25–26°C for humans and 30–32°C for mice).

and intraindividual differences between BAT depots because Cypess *et al*. [15] have shown that the deeper depots in human neck BAT consist mostly of classical BAT, whereas more superficial depots mostly contain beige adipocytes.

The Endocannabinoid System

The ECS is involved in a broad range of physiological processes and is an important player in energy homeostasis because it regulates appetite, nutrient partitioning, and energy expenditure (reviewed in [16–18]). It encompasses G protein-coupled cannabinoid receptors, their ligands (**endocannabinoids**), and the enzymes that are responsible for their biosynthesis and breakdown.

Cannabinoid Receptor Types 1 and 2

The CB1R (encoded by *CNR1*) was first identified in 1990 as a target of Δ^9-tetrahydrocannabinol (THC), the putative psychoactive constituent substance in the *Cannabis* plant [19]. Three years later the cannabinoid receptor type 2 (CB2R) was discovered via sequence homology [20]. CB1R is widely expressed in the central nervous system (CNS) [19] and to a lesser extent in peripheral metabolic tissues, including WAT, BAT, liver, myocardium, and skeletal muscle [21–23]. The presence of the CB1R in adipose tissue was first described in 2003 by two independent groups [24,25], and its expression is higher in differentiated adipocytes than in undifferentiated adipocytes [26], thereby suggesting a direct role of the ECS on adipose tissue function. On the other hand, the CB2R is well known for its immune-regulatory properties [27,28], but its expression and function in other cell types remains mostly unclear.

Synthesis and Breakdown of Endocannabinoids

The two most prominent endogenous ligands of the cannabinoid receptors are 2-arachidonoylglycerol (2-AG) and anandamide (*N*-arachidonoylethanolamine, AEA). These endocannabinoids have the same backbone consisting of the polyunsaturated FA (PUFA) arachidonic acid (AA). Although 2-AG and AEA are both AA derivatives, their pathways of synthesis are distinct and are regulated by different enzymes, allowing differential regulation of their levels. More specifically, AEA is synthesized by hydrolysis of its direct precursor, *N*-arachidonoylphosphatidylethanolamine (NAPE) by the enzyme ***N*-arachidonoylphosphatidylethanolamine-specific phospholipase D** (NAPE-PLD), whereas 2-AG is primarily produced by the hydrolysis of AA-containing diacylglycerols (DAG) by **diacylglycerol lipase** (DAGL). Degradation of AEA and 2-AG is facilitated by hydrolysis by primarily **fatty acid amide hydrolase** (FAAH) and **monoacylglycerol lipase** (MAGL), respectively. These biosynthesis and degradation pathways are more extensively reviewed elsewhere [29,30].

The Involvement of the Endocannabinoid System in the Control of Energy Homeostasis

The ECS has repeatedly been associated with obesity. Circulating AEA and 2-AG levels are higher in obese individuals compared to lean individuals [31], and circulating 2-AG levels positively correlate with measures of obesity such as body mass index (BMI) and body fat percentage, as well as with serum TG levels [21,31–33]. Moreover, circulating endocannabinoid levels in obesity positively correlate with adverse cardiac events [34]. In line with these observations, high-fat diet feeding of mice increases plasma 2-AG and AEA levels in association with weight gain [35,36], and FAAH deficiency in mice promotes energy storage, ectopic lipid storage, and **insulin resistance** [37,38], while mice deficient for CB1R are resistant to high-fat diet-induced obesity [39]. Moreover, systemic blockade of CB1R by the inverse agonist rimonabant reduces adiposity in mice [40] and humans [41].

The ECS exerts its effects on energy metabolism partly via the regulation of appetite and hypothalamic control of energy expenditure (Box 2). However, because CB1R is also

Transdifferentiation: the differentiation of a mature somatic cell into a different mature somatic cell.
Uncoupled respiration: a metabolic state in which catabolic products are used for heat production instead of ATP synthesis.
Uncoupling protein 1 (UCP-1): a regulated proton channel highly expressed in brown adipocytes and to a lesser extent in beige adipocytes that, when activated, uncouples oxidative phosphorylation from ATP synthesis, resulting in heat production.
White adipose tissue (WAT): an endocrine organ specialized in storing excess energy in glucose and in lipids as TGs.

Box 2. Hypothalamic CB1R Signaling Inhibits Brown Fat Thermogenic Activation

Cannabinoid receptors are expressed in hypothalamic regions involved in the regulation of appetite and energy expenditure. Several attempts have been made to distinguish between central and direct peripheral effects on thermogenic BAT activity. Verty *et al*. [73] and Bajzer *et al*. [72] showed that thermogenic BAT activity and body weight loss induced by CB1R antagonism is prevented by sympathetic denervation of the tissue. In addition, Quarta *et al*. [94] demonstrated that mice with conditional CB1R knockout in forebrain neuronal cells exhibit increased sympathetic tone and norepinephrine turnover in BAT, resulting in elevated *Ppargc1a*, *Nrf1*, and *Tfam* mRNA levels compared to wild-type mice, which can promote mitochondrial biogenesis. UCP-1 levels are higher in BAT of these conditional CB1R knockout mice than in wild-type mice, leading to improved cold tolerance associated with increased oxygen consumption [94].

The effect of CB1R in the brain can be assigned to paraventricular nucleus (PVN) neurons in the hypothalamus. Evidence for this was found by ablation of single-minded 1 (Sim1) neurons, that account for the majority of the PVN neurons, which causes obesity by inducing hyperphagia and reducing energy expenditure [95]. Similarly, removing CB1R-dependent inhibition of glutamate release in these neurons by Sim1-specific CB1R deficiency leads to increased overall energy expenditure that is independent of food intake and to higher thermogenesis on a high-fat diet [96]. These findings demonstrate the importance of the ECS in the brain for regulating BAT activity.

The effects of CB1R on appetite may be mediated both centrally and peripherally. On the one hand, stimulation of the ventromedial hypothalamus by anandamide directly increases food intake [97], and the ECS is thought to stimulate food reward and palatability (reviewed in [98]). On the other, adipose-specific CB1R knockout mice have a reduced daily caloric intake, suggesting the presence of a peripheral mechanism that influences appetite, possibly mediated via retrograde signaling from adipose tissue to the brain [59].

expressed in peripheral metabolic tissues including BAT and WAT, the ECS was also expected to play a direct role in these tissues, possibly of even more significance for the regulation of energy homeostasis than for the central effects. This would open paths for developing compounds to target the ECS in peripheral tissues directly, without interfering with the CNS. In the next sections we discuss how circulating endocannabinoids impact on adipose tissue function, and we discuss the production of endocannabinoids by adipose tissue itself and their local effects.

Endocannabinoids Reduce Thermogenic Activity in BAT and WAT

CB1R exhibits its effects mainly through $G\alpha_i$ proteins that are capable of inhibiting adenylyl cyclase and thereby preventing intracellular cAMP production [42]. Because sympathetic stimulation of adipose tissue via norepinephrine induces thermogenic activation of BAT and browning of WAT via stimulation of adenylyl cyclase and subsequent cAMP production, endocannabinoids can thus counteract the effects of norepinephrine on these tissues.

CB1R Signaling in WAT

The overall effect of CB1R stimulation in WAT is to favor fat storage (Figure 1). Several *in vitro* studies have demonstrated that CB1R signaling increases lipogenesis (reviewed in [43–45]) accompanied by elevated LPL activity, which promotes the liberation of FAs from circulating TG-rich lipoproteins and the uptake of these FAs by white adipocytes [24]. Moreover, CB1R signaling in WAT stimulates GLUT4 translocation and activates fatty acid synthase (FAS), resulting in elevated glucose uptake and *de novo* FA synthesis, respectively. In concordance with this, the expression of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor essential for adipocyte differentiation, is also increased upon CB1R signaling [26,46].

CB1R signaling in WAT impairs mitochondrial biogenesis and thereby prevents browning of white adipocytes. Specifically, CB1R stimulation inhibits the phosphorylation of 5'-AMP-activated protein kinase (AMPK) in cultured murine white adipocytes [47]. This decrease in AMPK

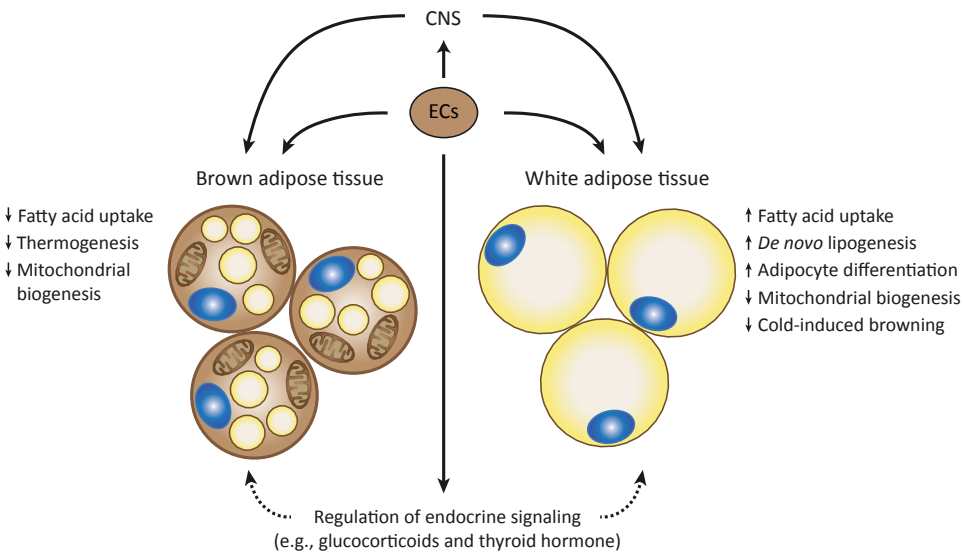

Figure 1. Schematic Overview of the Overall Effects of Endocannabinoids on White and Brown Adipose Tissue. Circulating endocannabinoids (ECs) favor fat storage in white and brown adipose tissue. These effects are mediated in part by direct binding of ECs to the cannabinoid receptor 1 (CB1R) on the white and brown adipocytes. In addition, some of the effects of ECs are mediated via the central nervous system (CNS), and some may be mediated via regulation of endocrine signaling acting on non-adipose tissues.

activity is accompanied by decreased expression of *Ppargc1a*, encoding PGC-1α, and by reduced expression of nuclear respiratory factor-1 (*Nrf1*) and mitochondrial DNA transcription factor A (*Tfam*), genes encoding factors that are considered to be important regulators of mitochondrial biogenesis and thermogenesis [47–50]. Similar effects of CB1R stimulation on mitochondrial biogenesis have been observed in cultured human subcutaneous and visceral adipocytes [47].

Conversely, pharmacological blockade of CB1R in a white adipocyte cell line increases UCP-1 expression, stimulates phosphorylation of AMPK, and promotes expression of PGC-1α [51]. These changes in gene expression are accompanied by induction of mitochondrial biogenesis, together resulting in increased oxygen consumption [51]. In addition, subcutaneous, but not visceral, adipocytes of CB1R knockout mice show higher UCP-1 and PGC-1α expression, higher oxygen consumption, and elevated mitochondrial biogenesis compared to adipocytes derived from wild-type control mice, indicating sensitization towards a brown phenotype [26,52]. This also indicates that CB1R signaling can have differential effects in different adipose tissue depots, and this may be of importance in the evaluation of CB1R modulators [26].

Emerging data point towards an interaction between the ECS and insulin signaling that is independent of weight gain (reviewed in [53]). Peripheral CB1R inhibition by an inverse agonist improves insulin sensitivity [54]. Some studies have suggested that these effects are mediated via an increase in adiponectin levels, an adipokine known for its insulin-sensitizing effects [55]. For example, adiponectin is downregulated upon CB1R stimulation, as shown in 3T3-F442A [26] or 3T3-L1 [56] cells, as well as in cultured mature adipocytes derived from human omental adipose tissue [57], and this is reversed by CB1R blockade [26,54,56–58]. In addition, adipose-specific CB1R knockout mice have higher plasma adiponectin levels than controls [59]. Moreover, CB1R antagonism is ineffective in increasing insulin sensitivity in adiponectin

receptor knockout mice [60]. On the other hand, the insulin-sensitizing effect of rimonabant is not affected in diet-induced obese adiponectin knockout mice [61] and only partially reduced in adiponectin knockout *ob/ob* mice [62]. Although the effects of adiponectin on insulin sensitivity have been well established in mice [63], such a potential role of adiponectin in humans has not been firmly established because no evidence was found for an association between adiponectin levels and insulin sensitivity in a Mendelian randomization study [64]. An alternative mechanism explaining the increased insulin sensitivity upon rimonabant treatment is a shift in macrophage phenotype from a proinflammatory M1 to an anti-inflammatory M2 phenotype [65].

Interestingly, the interaction between the ECS and insulin signaling may be reciprocal because insulin treatment of white adipocytes resulted in a lowering of 2-AG and AEA levels, together with increased mRNA expression of ECS degradation enzymes and a decrease in ECS synthesis enzymes [26]. Similarly, following hyperinsulinemia, *FAAH* gene expression was elevated in subcutaneous adipose tissue of lean but not of obese individuals, whereas *CNR1* gene expression was not altered [66].

CB2R Signaling in WAT

Evidence for a role of CB2R in WAT signaling is scarce, and is mostly linked to the regulation of inflammation. CB2R is expressed in WAT [67] and its expression is increased in obesity, especially within the macrophage-enriched stromal vascular fraction [68]. Importantly, CB2R signaling in immune cells in this tissue may be linked to adipose physiology. For example, CB2R stimulation promotes type 2 T helper cell (Th2) polarization and interleukin-4 secretion, resulting in browning of adipocytes in WAT [69]. In addition, associations could be made between CB2R genetic variants and BMI [69], and treatment of obese mice with a CB2R agonist lowered food intake, reduced body weight, and increased lipolysis, evidenced by higher adipose triglyceride lipase (ATGL) protein expression and reduced adipocyte cell size [70]. Although these studies suggest a beneficial role for CB2R in the regulation of adipose tissue metabolism, more research will be necessary to firmly establish this relationship as well as the relative contribution of CB2R versus CB1R signaling to metabolic homeostasis because most data point in the direction of unfavorable metabolic effects of ECS signaling mediated via CB1R.

CB1R Signaling in BAT

Compared to WAT, evidence for the effects of endocannabinoids on BAT remains scant. CB1R inverse agonism by rimonabant stimulates thermogenesis in brown T37i adipocytes *in vitro*, evidenced by increased oxygen consumption together with elevated UCP-1 expression and glycerol release as a measure of intracellular lipolysis [22]. Stimulation of these brown adipocytes with the CB1R inverse agonist in combination with norepinephrine led to a synergistic increase in intracellular phospho-HSL levels, indicating that CB1R signaling is also coupled to the adrenergic pathway in BAT [22]. Evidence for the effects of CB1R antagonism on thermogenic activation of BAT *in vivo* is also available. CB1R blockade by rimonabant or the peripheral antagonist AM6545 reverses diet-induced obesity [71] and stimulates uptake and combustion of VLDL-TG-derived FA by BAT, an effect preserved at **thermoneutrality** [22]. CB1R blockade also increases BAT UCP-1 expression [22,72]. Consistently, rimonabant treatment increases energy expenditure and BAT temperature [72,73], and Hsiao *et al*. [74] demonstrated that chronic peripheral inhibition of CB1R by BPR0912 induced UCP-1 expression in BAT of mice and elevated core body temperature, indicating increased thermogenic activity of BAT. Importantly, BAT can be visualized in rats using radioactive PET ligands with high affinity for CB1R, thereby demonstrating its dense presence in the tissue *in vivo* [75].

In all, there is a positive association between CB1R blockade and BAT thermogenic activity. Conversely, serum endocannabinoid levels are higher in the South Asian population [76], who are known to have reduced BAT volume and activity, increased visceral adipose tissue depots, and an elevated risk for metabolism-related diseases such as cardiovascular diseases and type 2 diabetes, compared to the white Caucasian population [77,78]. Further studies will be necessary to demonstrate a causal relationship between high ECS tonus and reduced BAT activity.

Endocannabinoids Are Produced and Broken Down by Adipose Tissue Itself

Surprisingly little is known about the origin and regulation of local and circulating endocannabinoid levels [79]. However, although the main organs responsible for plasma endocannabinoid levels have not yet been identified, there are strong indications that adipose tissue contributes to the pool of circulating endocannabinoids [80]. In fact, endocannabinoids produced by adipose tissue may induce local autocrine and paracrine negative feedback loops in response to adrenergic stimulation.

Endocannabinoid Synthesis and Degradation in WAT

Recently, Krott *et al*. [81] reported that cold or adrenergic receptor agonism not only increase expression of *Ucp1* and *Ppargc1a* in WAT, but also of *Cn1r*, which goes together with elevated levels of AEA and 2-AG and of their synthesis enzymes in WAT [81]. Although the intracellular signaling cascade leading to enhanced expression of these genes remains to be identified, the latter finding suggests that the ECS may be activated to dampen the effects of adrenergic signaling in an autocrine/paracrine fashion. Endocannabinoids secreted by WAT will decrease adenylyl cyclase activity, as discussed before, thereby preventing adrenergic-induced browning of WAT [81] (Figure 2, Key Figure).

Further evidence suggesting that WAT produces endocannabinoids is provided by the finding that obese mice have higher plasma 2-AG and AEA levels, have higher adipose tissue 2-AG levels, and also show altered expression of EC synthesis and degradation enzymes in adipose tissue [35]. Similarly, Engeli *et al*. [82] showed in subcutaneous adipose tissue of obese humans increased expression of *DAGLA*, but not of *NAPEPLD*, which are 2-AG and AEA synthesis enzymes, respectively. They also observed a reduction in gene expression of *FAAH* and *MGLL* that encode the AEA- and 2-AG-degrading enzymes, respectively [82]. Interestingly, the inhibitory effects of leptin on lipogenesis have been shown to be mediated via a reduction in AEA synthesis in WAT of mice [83]. Together these data suggest a role for WAT in the production of endocannabinoids.

Endocannabinoid-related compounds derived from adipocytes may also play a role in browning. For example, Geurts *et al*. [84] recently showed that conditional adipocyte-specific *Napepld* knockout mice have higher body fat mass despite equal food intake. In addition, these mice have decreased expression of browning markers in WAT and exhibit impaired adaptation to cold exposure compared to wild-type mice [84]. Surprisingly, AEA levels in WAT of these knockout mice were not different from wild-type mice. The effects of *Napepld* knockout on browning were possibly explained by reduced prostaglandin E2 (PGE2) levels, another product derived from AA. Furthermore, decreased production of other *N*-acylethanolamines (NAEs) in the adipose tissue of these knockout mice altered the gut microbiota composition, thereby suggesting the existence of an adipose tissue to gut microbiota axis. Because the plasma endocannabinoid levels were not reported in these *Napepld* deficient mice, the extent to which WAT is responsible for plasma endocannabinoid levels remains to be determined.

Key Figure

Hypothetical Model Showing How Endocannabinoids (ECs) Inhibit Norepinephrine (NE)-Induced Activation of Brown Adipose Tissue (BAT) Thermogenesis and White Adipose Tissue (WAT) Browning

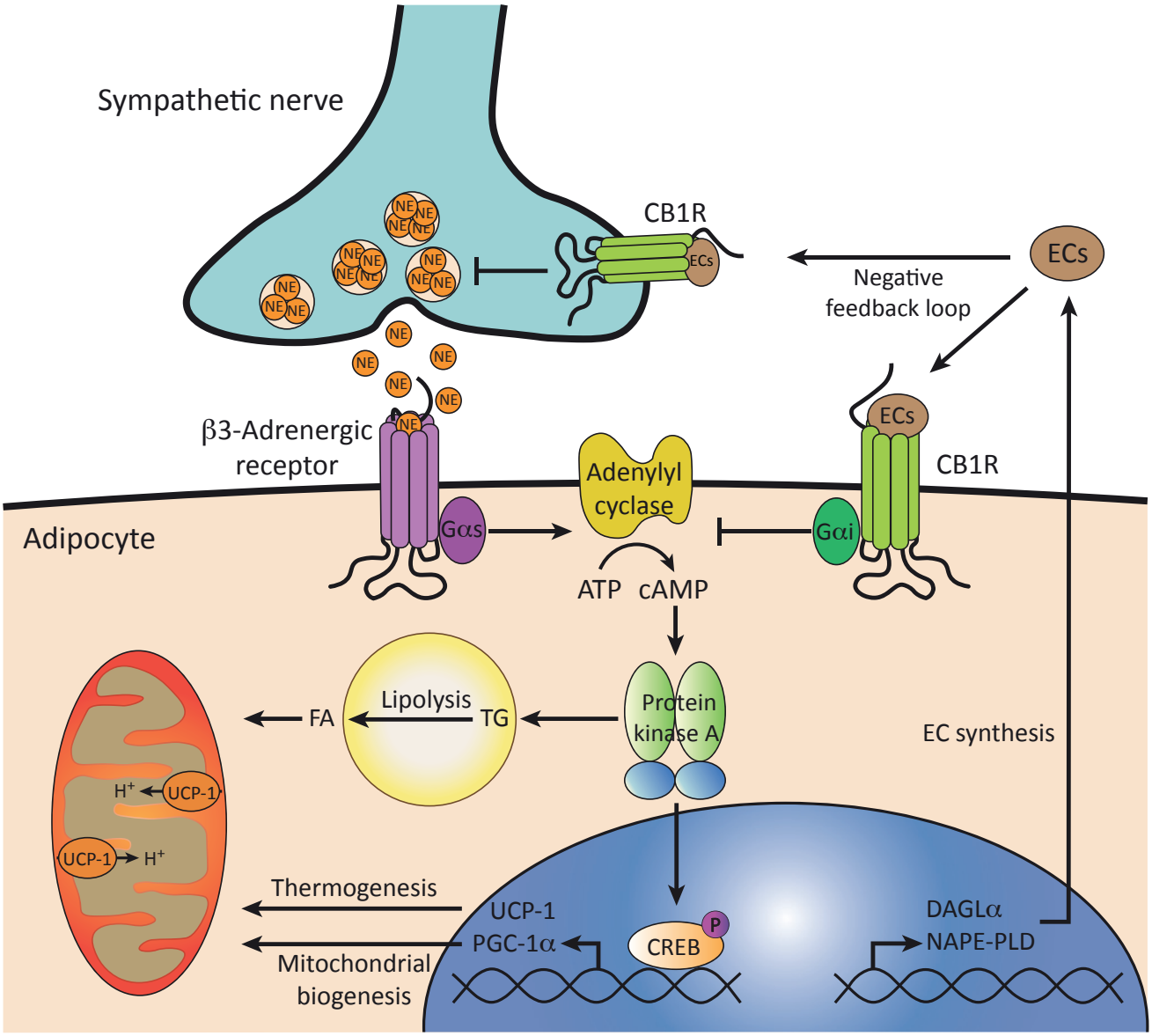

Figure 2. Upon cold exposure, sympathetic nerve endings in the adipose tissue release NE that binds to and stimulates β-adrenergic receptors on adipocytes. Stimulation of these receptors activates adenylyl cyclase, resulting in a rise in cAMP levels and subsequent activation of protein kinase A (PKA). PKA enhances intracellular lipolysis, resulting in the liberation of fatty acids (FAs) from triglycerides (TGs). In addition, PKA phosphorylates CREB which initiates, among others, transcriptional upregulation of uncoupling protein-1 (UCP-1) and PGC-1α, resulting in increased uncoupled respiration and mitochondrial biogenesis, respectively.

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Endocannabinoid Synthesis and Degradation in BAT

Data on the role of BAT in the production of endocannabinoids are limited. Krott *et al*. [81] reported that BAT stimulation via β3-adrenergic receptor agonist CL316,243 increases the levels of AEA and 2-AG in BAT, accompanied by elevated expression of *Dagla* and a suggested increase in the bioavailability of AEA precursors, because they showed that BAT *Napepld* expression decreases upon acute CL316,243 stimulation. They also showed that *Cnr1* gene expression is elevated upon β3-adrenergic receptor stimulation in primary brown adipocytes [81]. This suggests the presence of a similar autocrine and paracrine negative regulatory feedback loop in BAT as in WAT (Figure 2), which may control norepinephrine-stimulated thermogenic BAT activity, thereby preventing excess heat production.

In WAT and BAT this negative feedback loop is of interest for the treatment of cardiometabolic disorders. Targeting the ECS by modulating the biosynthesis and/or degradation of endocannabinoids, for example by the use of DAGL inhibitors [85], may increase its thermogenic activity in BAT, thereby improving metabolic health, without suffering from side effects related to the inverse agonistic nature of most CB1R blockers [86]. In addition, a combination of a **sympathomimetic** or cold exposure with local reduction of ECS tonus could be of interest as a new therapeutic strategy because these interventions act synergistically and therefore may have greater potential than a single intervention.

Endocannabinoids Inhibit Norepinephrine Release in Peripheral Presynaptic Nerves

Finally, there is evidence for an additional effect of endocannabinoids secreted from adipocytes. CB1R is present in sympathetic terminals of nerves innervating WAT and BAT. CB1R signaling in these sympathetic nerve terminals directly inhibits norepinephrine release [87], possibly by activating inwardly rectifying K^+ channel conductance and inhibiting N-type voltage-dependent Ca^{2+} channel conductance [88]. Of note, high-fat diet feeding of mice decreases norepinephrine content in adipose tissue, which can be reversed by pharmacological CB1R blockade [54]. To date, the role of adipocyte-derived endocannabinoids in regulating sympathetic outflow remains a relatively unexplored terrain, but could also partially explain the metabolic benefits of CB1R blockers such as rimonabant.

Concluding Remarks

The ECS is an important player in energy metabolism, and we are only starting to unravel its complex interactions that are not only context-dependent but possibly are also mediated via other tissues and endocrine signaling. The net inhibitory effect of the ECS on thermogenesis, and the net stimulatory effect on adiposity, makes the ECS an attractive target for the treatment of obesity and obesity-related disorders such as type 2 diabetes and cardiovascular diseases, although many questions on how to target the ECS need to be addressed first (see Outstanding Questions). Pharmaceutical targeting of CB1R has proved to be effective in reducing body mass and improving overall metabolic health, as shown by the Rimonabant in Obesity (RIO) trials. However, caution is needed regarding psychiatric side-effects. The withdrawal of rimonabant resulted in discontinuation of numerous trials involving CB1R antagonists. The elucidation of the importance of peripheral CB1R signaling led to renewed interest in CB1R as a therapeutic target and the development of peripherally restricted antagonists. These compounds have been shown to be effective in rodents, and may therefore prove to be useful to

Outstanding Questions

Which organ(s) is (are) the source of circulating endocannabinoids?

Are circulating endocannabinoid levels representative for endocannabinoid signaling in adipose (or other) tissues?

Why are (circulating) endocannabinoids increased in obesity?

Is it possible to therapeutically lower the endocannabinoid tonus without affecting the CNS, for example through peripherally restricted CB1R antagonists?

Is CB2R agonism a potential strategy to reduce inflammation and promote metabolic health?

Is lowering the endocannabinoid tonus in adipose tissues a strategy to stimulate thermogenesis and induce weight loss in humans?

Can we pharmacologically modulate local endocannabinoid production and breakdown in adipose tissues?

Simultaneously, adrenergic stimulation of adipocytes promotes gene expression levels of enzymes involved in the production of ECs. These ECs may subsequently act in a negative feedback loop (i) to inhibit NE release by the sympathetic nerves by binding to the cannabinoid 1 receptor (CB1R) on nerve terminals, and (ii) to inhibit adenylyl cyclase by binding to CB1R on adipocytes.

safely lower ECS tonus in humans in the future. Furthermore, CB2R agonism is a potential strategy to reduce inflammation and promote metabolic health if supported by future research. As an alternative, compounds that inhibit the production or stimulate the degradation of endocannabinoids in adipose tissue may provide novel tools to even more safely modulate the ECS tonus. So far, a couple of inhibitors of endocannabinoid production have been developed and were proven to be effective *in vitro* and to some extent *in vivo* in rodents [85]. Long-term studies evaluating the potential metabolic benefits in metabolically challenged rodents will be necessary to provide proof-of-concept.

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