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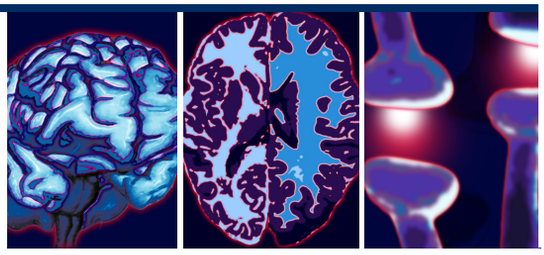

Cannabinoid-1 receptor agonists: a therapeutic option in severe, chronic anorexia nervosa?

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Practice points

- Evidence-based orexigenic therapies are needed for the management of chronic anorexia nervosa (AN).
- The endocannabinoid system (ECS) is a retrograde synapse signaling system with a nearly ubiquitous distribution.
- The first cannabinoid receptors (CB1s) are involved in energy metabolism and appetitive processes.
- Via CB1, the ECS exerts integrative functions in the hypothalamus and hindbrain.
- CB1s are linked to food-related reward processes and hedonic response.
- The ECS is involved in pathways controlling energy metabolism in peripheral tissues.
- The ECS may be involved in the pathogenesis of AN by either impaired receptor ligand synthesis/degradation, dysfunctions of the CB1 or cumulative defects.
- Only one controlled trial of CB1 agonist therapy was performed in subjects with AN, but several controlled studies in anorexia of other causes are showing a positive effect on weight, without major side effects.
- CB1 therapy may alleviate hormonal disturbances in severe, chronic AN.
- Bone mass and fracture risk may be influenced by treatment with CB1 agonists.
- Motor restlessness may be improved by cannabinoid treatment.
- Mood and starvation-induced cognitive deficits may well be improved by CB1 therapy.

SUMMARY Anorexia nervosa is an enigmatic syndrome and has the highest mortality rate among psychiatric disorders. Nutritional rehabilitation remains both the therapeutic cornerstone and the key challenge, but the evidence for effective therapeutic approaches is still very limited. Cannabinoids have been known and used for centuries, with their antidepressant and appetite-stimulating effects being anecdotally quoted in the literature.

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The endogenous cannabinoid system has been characterized in the last two decades, its receptors and endogenous ligands proving to be interconnected with a wide variety of orexigenic and anorectic signaling pathways at both central and peripheral levels. This article aims to justify that endocannabinoid receptor type 1 agonist therapy exerts orexigenic effects and may facilitate nutritional restoration in subjects with severe, chronic anorexia nervosa. In addition, it may favorably influence the neuroendocrine disturbances and cognitive impairments and reduce motor restlessness in severely hyperactive patients.

Anorexia nervosa (AN) is an enigmatic syndrome characterized by disturbed body image, self-starvation, loss of body weight, obsessive thoughts of food, ritualistic patterns of food intake, elevated physical activity, depression, anxiety and emotional rigidity. It has the highest fatality rate among psychiatric disorders, being associated with multiple, profound neuroendocrine disturbances [1].

In chronic AN, nutritional rehabilitation remains the therapeutic cornerstone and at the same time the key challenge, which is the result of feeding-related complications and a high relapse frequency.

Currently, no medications have been approved for the treatment of AN. However, the pharmacological treatment has so far focused on a narrow range of compounds and only a few controlled studies have been performed. Psychoactive drugs such as fluoxetine and olanzapine may give some symptomatic relief, but are far from curing the syndrome. The cannabinoids are among the many new molecular targets that have emerged from the discovery of orexigenic and anorectic signaling pathways and there is increasing evidence suggesting that they play a pivotal role in both central and peripheral processes controlling energy homeostasis and dietary intake.

The aim of this article is to review the potential mechanisms and therapeutic effects of synthetic cannabinoid treatment in chronic, severe AN.

The endocannabinoid system

Despite being used for therapeutic and recreational purposes for millennia, nearly 20 years has separated the elucidation of the structure and stereochemistry of the principal psychoactive ingredient in the cannabis plant – Δ-9-tetrahydrocannabinol (THC) – from the discovery of the first cannabinoid receptor (CB1) in 1988 [2]. In 1993, a second receptor (CB2) was characterized [3]. Both are a class of G-membrane receptors under the G-protein-coupled receptor (GPR) superfamily, sharing 44% of their protein sequences. Mounting evidence suggests that other orphan, non-CB1s and non-CB2s are expressed both in the CNS and the endothelial cells. Among these, GPR55 was described to bind both CB1 and CB2 agonists [4]. CB1 accounts for most of the psychotropic effects of THC [5] and is expressed at particularly high levels in brain regions including the cortex, basal ganglia, cerebellum and hippocampus, but its distribution is not limited to brain circuitry. The receptors, their endogenous ligands and degrading enzymes are what define the endocannabinoid system (ECS).

Because of their lipophilic nature, the endocannabinoids (ECs) cannot be stored in intracellular vesicles and therefore have to be produced 'on demand' in the cell membrane [6]. The postsynaptic release of ECs together with the presynaptic presence of CB1 on both inhibitory and excitatory nerve endings has led to the hypothesis that ECs mediate retrograde synaptic signaling in the adult CNS [7].

Furthermore, based on the observation that CB1 and its ligands are present in brain regions controlling food intake, the ECS has been proposed as a putative modulator of feeding behavior [8]. The ECS appears to have been conserved during evolution and seems to play a critical role in neonatal development. In fact, blocking it with CB1 antagonists prevents suckling of rat pups, leading to fatal starvation [9].

Early studies assessing the acute effects of THC on the food intake in adult humans showed an increased intake, elevated hunger ratings and enhanced food appreciation [10,11]. Conversely, a specific CB1 antagonist known as rimonabant proved to be effective at inducing weight loss in the morbidly obese [12], demonstrating the role of CB1 in mediating the effects of both endo- and exo-genous cannabinoids.

However, the putative cross-talk between ECs and their receptors with the large number of hypothalamic and peripheral peptides known to be involved in appetite regulatory pathways is still under debate.

ECS: modulation of eating behavior & energy metabolism

The ECs appear to play a crucial role in feeding-related neural and hormonal circuitry at several levels, both central and peripheral, being related to integrative functions (hypothalamus and hindbrain), hedonic evaluation of foods (limbic system), gut signaling (intestinal system and pancreas) and adipogenesis (liver and fat tissue) [8].

■ Integrative functions in the hypothalamus & hindbrain

In the paraventricular hypothalamus, CB1 mRNA is coexpressed with mRNA encoding neuropeptides that are known to modulate food intake, such as corticotropin-releasing hormone, cocaine- and amphetamine-regulated transcript, melanin-concentrating hormone and orexin/hypocretin [13].

Among the anorexigenic hormones, leptin seems to be closely related to the ECS. EC levels are high in leptin-deficient mice and are restored by leptin administration, suggesting a negative-feedback relationship [14]. At molecular levels, leptin regulates the excitability of appetite-related neurons by inhibiting voltage-gated calcium entry, thereby decreasing synthesis and release of ECs [15].

Ghrelin acts as a natural antagonist to leptin on neuropeptide Y (NPY) and agouti-related peptide-expressing neurons, resulting in an increase in food intake and bodyweight [16]. Rimonaband impairs the effects of ghrelin [17], suggesting that an intact CB1 is necessary for ghrelin to exert its orexigenic effects [18].

One of the most potent orexigenic hypothalamic neuropeptides is NPY, which exerts its actions on Y receptors parallel to a strong corticotropin-releasing hormone effect [19]. Several lines of evidence suggest a close interaction between the ECS and NPY pathways in the regulation of energy homeostasis. The massive increase in dietary intake induced by hypothalamic NPY administration in wild-type mice is nearly absent in *CB1*-knockout mice [20]. Moreover, CB1 agonists augment NPY release, while CB1 antagonists have the opposite effect [20,21].

■ Food-related reward processes & hedonic response

Food reward is a complex process that involves objective hedonic reactions, an addictive component and associations. The ECS appears to be essential for reward anticipation and initiation of eating, being involved in hedonic reactions and the pleasure associated with food intake [22]. CB1 are expressed peculiarly in brain areas that are directly reward related or involved in the hedonic aspect of eating, such as the shell region of the nucleus accumbens, the hippocampus and the entopeduncular nucleus [9]. Particularly, CB1 or CB2 agonists administered into the shell region of the nucleus accumbens produce a profound hyperphagic response [23].

In rodents, the exogenous stimulation of the hypothalamic CB1 selectively induces feeding of pellets high in fat and sucrose content over standard chow [24]. Conversely, the density of CB1 is downregulated by highly palatable diets in brain areas involved with the hedonic aspects of food [25].

Furthermore, the hyperphagic action of THC is reversed by the nonspecific opioid receptor antagonist, naloxone [26]. The augmentation of the anorexic effect by combined CB1 and opioid receptor blockade strengthens the proposition that the ECS may have important functional relationships with the endogenous opioid system and thus contribute to orosensory reward processes [27].

■ Energy metabolism in peripheral tissues

Several lines of evidence support the peripheral role of the ECS in the regulation of bodyweight. The ECS is suggested to promote fat storage and to decrease energy expenditure [28]. By interacting with leptin, the ECs may induce lipogenesis in liver and white adipose tissue and enhance fatty acid oxidation in muscle together with augmentation of adiponectin [29]. Cota and coworkers observed that wild-type mice exhibit significantly higher amounts of fat mass than *CB1*-knockout mice when fed with the same amount of food [8], so the ECS may determine fat accumulation via CB1, independently from the amount of food ingested [30]. Moreover, *in vitro* blockade of CB1 arrests adipocyte proliferation, suggesting that ECs might tonically stimulate proliferation [30]. Elevation of EC levels promoting preadipocyte differentiation and chronic stimulation of CB1 during adipocyte differentiation accelerates the appearance of an early marker of differentiation, the peroxisome proliferator-activated receptor-γ, while inducing accumulation of lipid droplets [30].

In addition, hepatocytes express CB1, particularly around the centrilobular vein. Via steroid regulatory element-binding protein 1c and its

targets, acetyl-CoA-carboxylase 1 and fatty acid synthase exert a stimulatory effect on fatty acid synthesis and lipogenesis in these cells [30].

β-cells express both CB2 and CB1 [31]. In animal models, stimulation of CB2 reduces insulin release while CB1 only does this to a lesser extent [32]. It seems that the stimulation of systemic and nonpancreatic CB1 induces glucose intolerance either by affecting other factors that regulate insulin release or by inhibiting glucose uptake by tissues in other ways [30]. For instance, glucose uptake is improved by genetic deletion or blockade of the CB1s in the skeletal muscle [33]. Conversely, CB1 antagonism reduces blood glucose and increases β-cell proliferation and mass, as well as enhancing signaling via the insulin receptor in these cells, thus demonstrating a functional interaction between CB1- and insulin receptor-mediated signaling in the regulation of β-cells [34].

CB1 agonists in severe, chronic AN: pathogenetic hypotheses

Anorexia nervosa is considered to be a multidimensional disease determined by a wide interaction between psychological, biological, familial, social, environmental and genetic factors.

As mentioned earlier, the ECS is tightly interconnected with the molecular circuits involved in appetitive processes, but there is still no conclusive evidence regarding their role in the pathogenesis of AN. Inappropriately high plasma levels of ECs were reported in underweight AN individuals [35], suggesting that in these subjects, the ECS may be affected by either impaired receptor–ligand degradation, dysfunctions of the CB1 or cumulative defects. Whether this finding reveals a pathogenetic link to AN or is a consequence of malnutrition is unclear.

The genetic determination in the pathogenesis of AN is supported by family and twin studies that emphasizes a six- to ten-fold increased risk for developing AN in young women whose first-degree relatives had an eating disorder [36], but no genetic mutation has yet been identified. Family-based trials studying the polymorphism of the *CB1* gene revealed that restrictive and binge/purging subtypes of AN may be associated with different alleles of this gene [37].

When analyzing a single specific gene polymorphism for the *CB1* gene and a specific gene polymorphism for one of the major degrading enzymes of cannabinoids, Monteleone and his group found that these two polymorphisms were associated with AN and, moreover, there was a synergistic effect on the biological susceptibility to AN [38].

There are still only a few relatively small studies targeting associations between specific *CB1* gene polymorphisms and AN, making it difficult to determine a clear-cut relationship between these. Moreover, the ECS involves other receptors than CB1 and CB2, and a Japanese study recently described a positive association between AN and a functional polymorphism in the *GPR55* gene, suggesting that also this orphan receptor may play a role in the pathogenesis of AN [39].

Previous clinical experiences with CB1 agonist treatment

Despite the strong evidence regarding the orexigenic effects of CB1 agonist stimulation, the human studies regarding CB1 agonist treatment in severe, chronic AN are surprisingly sparse **(Figure 1)**. Patients suffering from AN have complex psychopathological features that interfere with any therapy intended to overcome voluntary starvation by weight gain and modulation of appetite control systems. We assume that this is the main cause of the striking lack of studies addressing orexigenic therapies in this patient group.

Gross and his group addressed this subject in 1983, five years before CB1 was even described [40]. A total of 11 female patients with primary AN were investigated in a double-blind crossover setting. Relatively high doses of synthetic THC (from 7.5 to a maximum dose of 30 mg/day) were tested against diazepam as an active placebo, despite the fact that both animal [41] and human [42] evidence suggests that benzodiazepines may increase food intake. Moreover, the medication was discontinued during the weekend, possibly to avoid addiction. The daily caloric intake was controlled, and occasionally tube feeding was used. A significant weight gain during both intervention periods was reported, with no significant differences between the two therapies. However, these results might have been biased by using the last recorded weight values for the patients who dropped out of the study as final values. Intriguingly, it seemed that THC induced weight gain to a slightly higher extent than diazepam, with this phenomenon being more pronounced after the second week of the study. Three patients withdrew after experiencing severe dysphoric reactions during active treatment, leading the authors to conclude that THC

was not an efficacious choice in the treatment of primary AN. Despite its obvious design flaws, this study represents the first and only evidence of CB1 agonist treatment in AN [40].

However, there are several studies addressing the orexigenic effects of CB1 agonist treatment in anorexia induced by causes other than AN. By treating ten patients over 28 days, Nelson and coworkers showed a median weight gain of 1.3 kg on relatively small daily doses of THC (2.5 mg) in an interventional Phase II study in patients with cancer-associated anorexia [43]. That same year, a multicenter, double-blind, placebo-controlled parallel-group trial was conducted in 139 patients with AIDS receiving 5 mg THC daily over 6 weeks. It showed a minor mean weight gain in the THC group of 0.5 kg above placebo [44]. In 2006, the Cannabis In Cachexia Study Group conducted a multicenter, Phase III, randomized, double-blind, placebo-controlled study aiming to compare orally administered cannabis extract (2.5 mg THC + 1 mg cannabidiol) and synthetic THC (5 mg daily) with placebo in patients with cancer-related anorexia–cachexia syndrome [45]. They assessed changes on appetite and quality of life, showing that both cannabis extract and THC were well tolerated, but not superior to placebo.

Recently, two double-blind, placebo-controlled interventions conducted in different populations of HIV-positive marijuana smokers demonstrated that both natural and synthetic THC increase the daily caloric intake [46] and body weight [47].

Addressing the possible adverse reactions during CB1 agonist treatment in subjects with AIDS-associated anorexia, Beal and his group specifically noted that THC improved mood in the absence of euphoria [44]. In another study, all cannabinoid preparations produced significant psychotropic effects, except for low-dose (5 mg/day) dronabinol (synthetic THC) [47]. These side effects were positively rated with no impairment of cognitive performance, confirming the observations from a large body of experimental data that suggest a biphasic effect with low doses having anxiolytic and orexigenic effect, and high doses having anxiogenic-like effects [48].

The contrast between AN and other diseases characterized by secondary appetite and weight loss is quite impressive. Patients with AIDS- or cancer-associated cachexia are likely to administer cannabis-derived drugs because they are suffering from depression, nausea and pain.

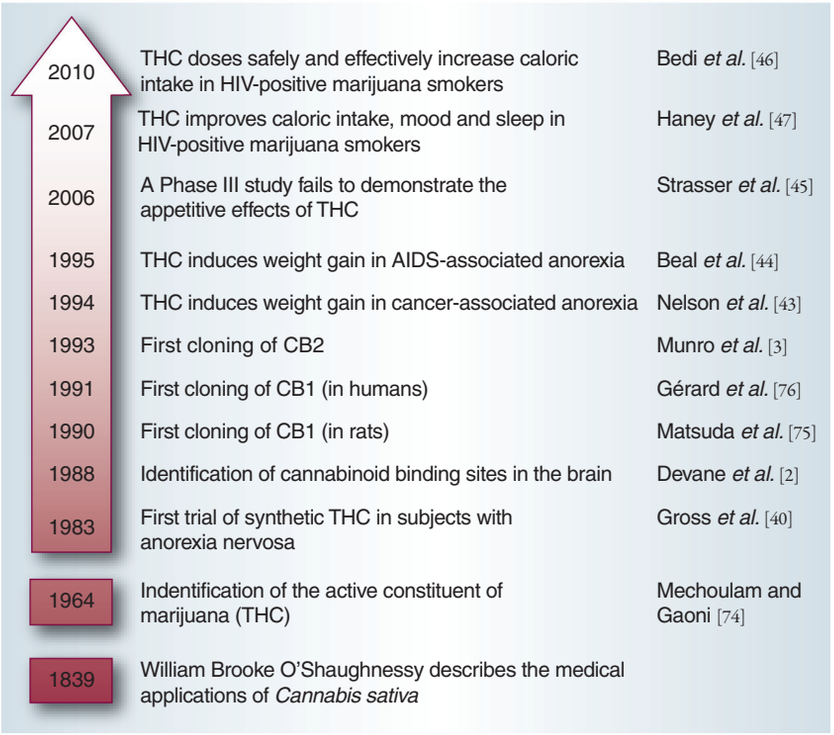

Figure 1. Key points in the research of the orexigenic effects of cannabinoids.
CB: Cannabinoid receptor; THC: Δ-9-tetrahydrocannabinol.

Although not comparable with AN with respect to the etiological and psychological profiles, studies of anorexia in AIDS [44,46,47] and cancer [45] are not only providing evidence of the effects of CB1 agonist treatment in humans, but also proof of the safety of using it in underweight patients.

Possible therapeutic applications of CB1 agonist therapy in AN

By playing a pivotal role in hormonal and neural circuits in both the CNS and peripheral tissues (Figure 2), the ECS may represent a therapeutic target when addressing the profound neuroendocrine disturbances induced by chronic starvation and by being underweight in AN.

■ Hormonal disturbances in severe, chronic AN

All the endocrine axes are affected in AN [1]. The corticotropin-releasing hormone, corticotropin and cortisol levels are high in these patients. Hypothalamic amenorrhea is a diagnostic element [49]. Growth hormone (GH) resistance with high GH levels and low IGF-1 levels have been described [50], and euthyroid sick syndrome is a well-known feature. There is evidence that CB1 ligands are involved in the negative retrograde

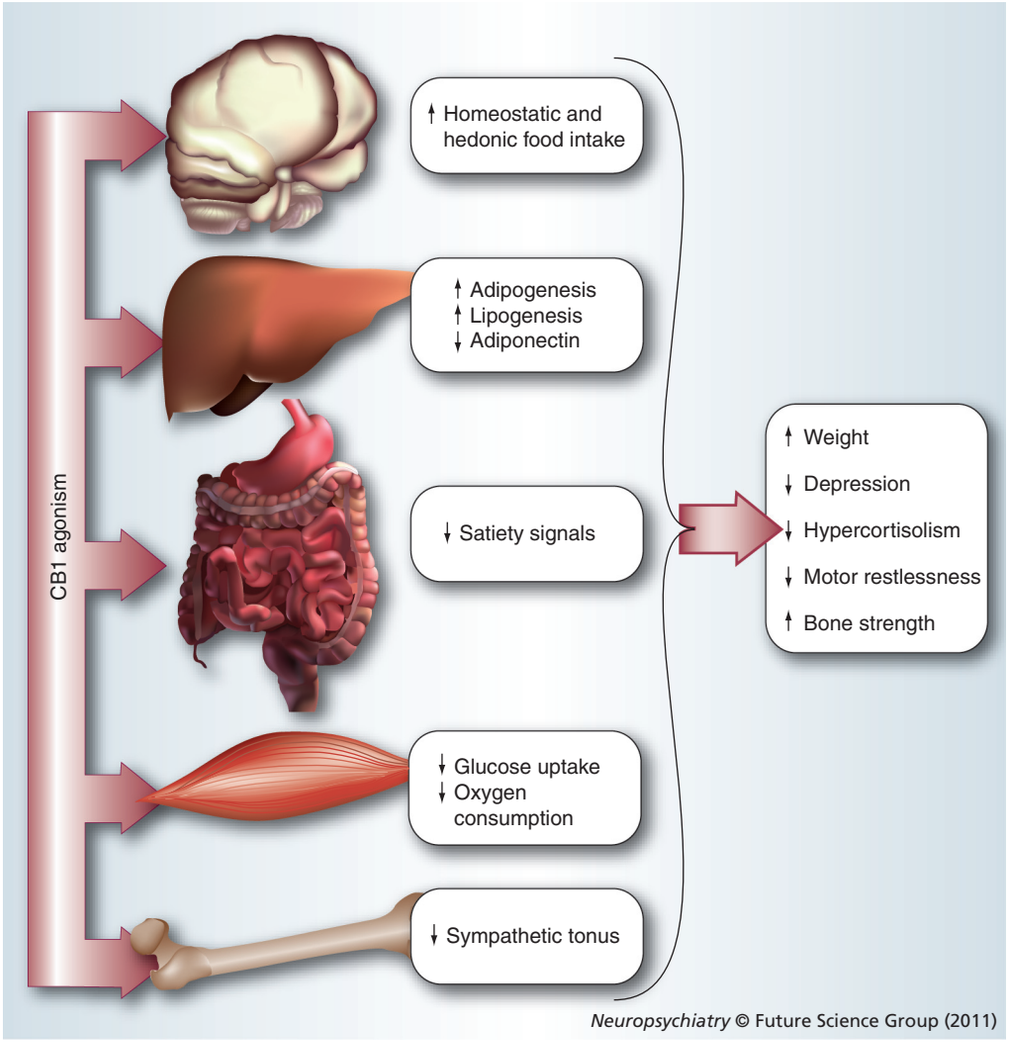

Figure 2. Physiological and potential therapeutic effects of cannabinoid-based drugs in anorexia nervosa.
CB: Cannabinoid receptor.

signaling cascade in the hypothalamic–pituitary–adrenal (HPA) axis [13,51,52]. In the same way, the ECS may play a pivotal role in counteracting the stress-induced HPA activation [53]. Repeated stress results in the loss of this retrograde signaling by corticoid-mediated functional downregulation of the presynaptic CB1s [54], providing a possible molecular explanation for the hypercortisolism in severe, chronic AN. Following these theories, agonistic CB1 stimulation in subjects with chronic AN could counteract both hypercortisolism and other stress-induced hormonal disturbances, but this hypothesis has not yet been addressed in human studies.

In murine studies, the GH secretion seems to be suppressed by ECs. This effect may be exerted through a CB1-mediated stimulation of somatostatin release [55]. Human data regarding GH response to CB1 agonist stimulation are sparse and, to our knowledge, there are no data available regarding the effects of long-term CB1 agonist treatment on the GH axis in patients with AN.

By controlling the hypothalamic–pituitary–thyroid axis, the hypothalamic thyrotropin-releasing hormone-synthesizing neurons are involved in the regulation of energy homeostasis. Recently, the ECS has been shown to exert inhibitory effects on these neurons [56], while specific blockage of CB1 induced the reverse effect, demonstrating that the ECS may act as a negative regulator of thyroid stimulating hormone secretion [57]. Interestingly, using adult rodents with congenitally hypothyroidism, Asúa

and coworkers found that a hyperactive phenotype was associated with a decreased content of hypothalamic CB1 mRNA, suggesting that the ECS may act as a brake in motor activity initiation [58]. Typically presenting with euthyroid sick syndrome, patients with chronic AN are often motorically hyperactive and the animal models mentioned earlier suggest possible interactions between thyroid hormones and CB1 in human physical activity.

■ Bone mass & fracture risk

Severe, chronic AN is associated with a significantly higher fracture risk compared with the background population [59]. Chronic starvation, its consequent neurohormonal and metabolic changes (hypogonadotropic hypogonadism, GH resistance and high cortisol levels, among others) and exaggerated physical activity leads to catabolic bone turnover by increasing bone resorption without an increase in bone formation. The bone marrow is shifted from its active hematopoietic function to inactive, fat-dominated yellow bone marrow. The result is damaged structural integrity and lower skeletal strength, not necessarily matched by low bone mass density as detected by dual-energy x-ray absorptiometry.

The CB1, CB2 and orphan receptors such as GPR55 are all present in the bone marrow and within the metabolically active trabecular compartment. CB1s are commonly expressed on sympathetic nerve terminals and on cells of the immune system within the bone compartment, but they were also detected on osteoblasts, osteoclasts and bone mass-derived adipocytes [60]. By negatively regulating the suppressing effect of norepinephrine on bone formation (induced by binding to osteoblastic β2-adrenergic receptors), CB1 may downregulate sympathetic induced osteoblast inhibition [61].

By this mechanism, CB1 agonist treatment may also affect bone mass and fracture risk in individuals with severe, chronic AN. However, the bone-protective effect induced by downregulating the sympathetic inhibition of bone formation may be shadowed by its stimulating effect on osteoclasts, which has been described in mature rodents with estrogen deficiency [62].

■ Physical activity

In animal models, THC intake decreased spontaneous motor activity and altered walking patterns in a dose-dependent manner [63]. In humans, it seems that the ECS contributes to the motivational aspects of voluntary running, but probably not the long-term changes of emotional behavior induced by voluntary exercise [64]. Conversely, it seems that – at least in rodents – aerobic physical exercise significantly reduces CB1 expression in brain structures such as the striatum and hippocampal formation [65], suggesting that the ECS is directly influenced by the level of physical activity.

In addition, the interactions between the ECS and other neuronal and hormonal circuits controlling feeding and reward processes emphasize its role in the central adaptive reactions to physical activity.

■ Cognition

Since ancient times, *Cannabis sativa* has been known to alleviate symptoms of depression. However, the adverse psychotropic effects and abuse liability of this drug may have prevented its therapeutic application.

In animal models, CB1 agonists appear to impair short-term memory, whereas ECs seem to produce qualitatively different effects on learning and memory [66]. Moreover, anandamide – an endogenous CB1 agonist – reverses impaired learning ability induced by diet restriction in mice [67].

In humans, different cannabis preparations were evaluated across a range of behaviors (eating topography, mood, cognitive performance, physiological measures and sleep) during placebo-controlled, within-subject interventions in HIV-positive marijuana smokers [46,47]. Besides the increase in the daily caloric intake and mood-enhancing effects, no negative effects on cognitive performance were reported [47].

Conclusion

Anorexia nervosa is associated with the highest mortality of psychiatric disorders, often being a psychosomatic kaleidoscope of multiple psychopathological features and complex somatic derangements resulting from chronic starvation. In this article, we emphasize the physiological features of the ECS and the role of CB1 in controlling food intake and eating behavior at both central and peripheral levels. In addition to its roles in energy metabolism, the ECS is also involved in a multitude of processes involved in reward mechanisms, emotional behavior, stress response, gonadal function and bone metabolism.

These putative effects promote CB1 agonist therapy as a valuable therapeutic tool in chronic AN, which, besides its direct orexigenic effect may also ameliorate many of the physiological sequelae of the eating restraint. Previous studies conducted in patients suffering from anorexia of different causes have already shown that CB1 agonist treatment alleviates weight and is safe, well tolerated and not addictive.

It has been suggested that CB1 stimulation may have antidepressant effects and may also alleviate motor hyperactivity induced by chronic starvation. However, its effects on the psychopathological aspects of the chronic undernourished patient are still not understood, partly due to the poor compliance in this patient group and partly due to concerns about the theoretical risk of developing drug addiction.

One particular and common psychological feature of anorexic subjects is their notable ambivalence for interventions that may cause weight gain, making it difficult to assure a sustained compliance [68] and to achieve relevant conclusions.

Regarding the risk of developing drug addiction, the same principal problems have been managed in the widespread therapeutic use of opioids and benzodiazepines – which, after all, have proven to be very useful options.

In addition, several epidemiological studies show that there is an association between regular cannabis use in adolescence and psychosis or psychotic symptoms [69,70], but cannabis use is neither a necessary nor sufficient condition to develop psychotic symptoms [71]. It still remains to be clarified whether the use of cannabis is causally linked to the development of psychosis or whether it is used as an anxiolytic self-medication in predisposed subjects [72].

These concerns were addressed in a authoritative report by the Royal College of Physicians in the UK that concluded that it is important to distinguish between recreational and medical use, since in the latter, the cannabinoid drugs has been shown to be safe [73].

Future perspective

In the light of the discussed breakthroughs in the research into the ECS, we strongly recommend determined trials to elucidate the therapeutic potential of CB1 agonists.

Novel lead candidates targeting other cannabinoid receptors such as GPR55 may also exert agonistic effects on CB1 and may prove to be an innovative class of therapeutics to this effect.

Emerging from the hypothesis of the phytocannabinoid–terpenoid synergy, a new echelon of phytotherapeutic agents might increase the therapeutic index of standalone CB1 agonist therapy, leading to the development of a viable therapeutic tool in chronic AN.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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