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REVIEW

Examining the role of cannabinoids on osteoporosis: a review

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Abstract

Purpose Prior research studies have shown that the endocannabinoid system, influenced by CBD and THC, plays a role in bone remodeling. As both the research on cannabis and use of cannabis continue to grow, novel medicinal uses of both its constituents as well as the whole plant are being discovered. This review examines the role of cannabinoids on osteoporosis, more specifically, the endocannabinoid system and its role in bone remodeling and the involvement of the cannabinoid receptors 1 and 2 in bone health, as well as the effects of Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and synthetic cannabinoids on bone.

Methods A comprehensive literature search of online databases including PUBMED was utilized.

Results A total of 29 studies investigating the effects of cannabis and/or its constituents as well as the activation or inactivation of cannabinoid receptors 1 and 2 were included and discussed.

Conclusion While many of the mechanisms are still not yet fully understood, both preclinical and clinical studies show that the effects of cannabis mediated through the endocannabinoid system may prove to be an effective treatment option for individuals with osteoporosis.

Keywords Cannabis · Osteoporosis · Cannabinoids · Δ9-Tetrahydrocannabinol (THC) · Cannabidiol (CBD) · Bone

Introduction

The earliest evidence of medicinal use of the cannabis sativa plant dates back to 2700 B.C. [1]. In recent years, research on cannabis has been growing, and the number of documented medicinal properties has expanded. Cannabis contains more than 100 distinct phytocannabinoid compounds that interact with the endocannabinoid system, a network of receptors, signaling molecules, and enzymes [2]. Phytocannabinoids are plant-derived products capable of interacting with mammalian cannabinoid receptors. Of the phytocannabinoids that have been isolated from the cannabis plant to date, the main psychoactive component, Δ9-tetrahydrocannabinol (THC), and the major nonpsychoactive component, cannabidiol (CBD), have been shown to be partial agonists at the cannabinoid receptors, CB1 and CB2 [3]. In addition to these receptors, there are multiple other putative molecular targets for these phytocannabinoids [2, 3]. Clinical studies have explored the therapeutic potential of cannabis use for a range of disorders including; Alzheimer's disease, cancer, epilepsy, seizures, human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS), amyotrophic lateral sclerosis (ALS), Crohn's disease, hepatitis C infection, multiple sclerosis (MS) with muscle spasticity, severe and chronic pain, severe nausea, post-traumatic stress disorder (PTSD), and cachexia, or wasting syndrome [4–19].

Methods

This narrative review utilized multiple search engines including PubMed and the University at Buffalo's Library database to identify manuscripts in the English language matching our

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search criteria. A search on PubMed with the terms cannabis and bone yielded 122 results, cannabis and osteoporosis yielded 18 results, THC and osteoporosis yielded 9 results, THC and bone yielded 115 results, CBD and osteoporosis yielded 24 results, and CBD and bone yielded 166 results. All of the relevant references from these searches are included in this review. These preclinical and clinical studies were all peer-reviewed and published dating from 1990 to present, with valid statistical analyses. Search keywords included "bone, osteoporosis, Cannabis, Tetrahydrocannabinol/ THC, and Cannabidiol/ CBD." A total of 29 research studies were found to be relevant to the scope of this review and were therefore included (Tables 1, 2, 3, 4, and 5). The focus of this review was to report on the role that modulation of the endocannabinoid system (largely by cannabis constituents) may play in the treatment of osteoporosis.

Osteoporosis

Bone is continuously being remodeled through resorption mediated by osteoclasts and formation by osteoblasts. Remodeling is critical for maintaining appropriate bone density, structure, and strength, with disruption of this process leading to bone diseases such as osteoporosis [20]. In 2001, osteoporosis was defined as a "skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture," with bone strength primarily reflecting the integration of bone density and bone quality [21–23]. Currently, the World Health Organization defines osteoporosis as a bone mineral density (BMD) T-score, measured by dual-emission X-ray absorptiometry, as less than − 2.5 [21]. Osteoporosis is characterized by inferior bone quality and microstructure that leads to bone damage and an increased risk for

Table 1 Clinical studies that are representative of the primary outcomes of *cannabis sativa* constituents on bone

Cannabis constituent	Species	Sex	Regimen	Dose	Route of administration	Outcomes	Reference:
Cannabis sativa plant	Human	Both	Chronic	Self-report heavy use	Self-report heavy use	↓BMD, ↓BMI ↑ bone turnover, ↑ fractures	[25]
Cannabis sativa plant	Human	Both	Chronic	Self-report heavy use	Inhalation	↑ bone lysis	[99]
Cannabis sativa plant	Human	Both	Chronic	Heavy users (smoked more than 5000 × in their lifetime)	Inhalation	↓hip and spine BMD ↓BMI ↑CTX and P1NP concentrations ↓ 25(OH)D concentration	[25]
Cannabis sativa plant	Human	56 y/o male	Chronic	1–7 g daily from ages 22–47	Any form of consumption	↓BMD	[99]

Table 2 Preclinical studies that are representative of the primary outcomes of *cannabis sativa* on bone

Cannabis constituent	Species	Sex	Regimen	Dose	Route of Administration	Outcomes	Reference
Equal parts THC and CBD	Rats	Male	2, 4, 6, and 8 weeks	5 mg/kg/day	i.p. injection	↑ maximal force to a degree greater than CBD alone, eliminated effect of CBD alone on work-to-failure	[128]
Marijuana smoke	Rat	Male	Chronic	8 min/day	Marijuana smoke inhalation (MSI)	↓ bone area in threads of titanium implants	[100]
Marijuana leaves	Rats	Male	30 days	3 g for 8 min daily	Inhalation via smoke chamber	↑ bone loss in teeth w/ induced periodontitis No effect on healthy teeth	[139]
AjA	In vitro (cultures from long bones of mice)	N/A	N/A	97% chemically pure AjA	AjA added to cell cultures	↓osteoclast formation	[137]

Table 3 Preclinical studies that are representative of the primary outcomes of *Cannabis sativa* constituents on bone

Cannabis constituent	Species	Sex	Regimen	Dose	Route of administration	Outcomes	Reference
Dronabinol (Synthetic THC)	Rat	Male	3 weeks	10 mg/kg/day	i.p. injection	↓ bone resorption ↑ bone formation ↑ number or osteoclasts and osteoblasts in alveolar bone	[129]
THC	Rat	Female	Chronic	45% fat diet containing 0.4% THC	Oral	↓articular cartilage deterioration ↓MMP3, MMP13, IL1β, and IL6 expression	[127]
THC	Mice (double knockout of Cnr1 and Cnr2)	Both	6 weeks	5 mg/kg/day	i.p. injection	↓ femoral and vertebral length in control and Cnr2 −/− female No significant changes in Cnr1 −/− or Cnr1 −/− Cnr2 −/− females No significant effect in males	[123]
CBD	Rat	Male	Chronic	0.5 or 5 mg/kg/day	I.P. injection	High dose: ↑osteocalcin, ↓CTX, ↑BMD Low dose: no significance	[130]
CBD	Rat	Male	1, 4, and 12 weeks	71.25 ± 3.28% of total 3 mg in 25 days	CBD released from autograft placed in bone	↑ bony-like tissue in defected areas ↑X-ray scores ↑new bone formation/ unions	[131]
CBD	Rats	Male	30 days	5 mg/kg/day	i.p. injection	↓ alveolar bone loss ↓ RANKL expression	[132]
CBD	Mice	Male	8 weeks	10 mg/kg 3×/week	i.p. injection	↑ osteoclast count in GPR55 control, Cnr1 knockout, and Cnr2 knockout. No change in GPR55 knockout ↓ bone resorption ↑ bone volume	[115]
CBD	In vitro (human dental stem cells)	N/A		2 µm		↓ Cell number No significant effect on mineralization ↑osteopontin,↑ osteonectin, ↑ osteocalcin in apical papilla cells ↑ osteocalcin in dental pump cells	[134]
THC or CBD	Rats		2, 4, 6, and 8 weeks	5 mg/kg/day	i.p. injection	↓ callus size in both groups ↑ maximal force in CBD group ↑ work-to-failure in CBD group	[128]

bone fragility and fractures [24]. Defects in microarchitecture, poor intrinsic material properties, defective repair of microdamage, and excessive remodeling are all causes of decreased bone strength and increased susceptibility to fracture [21]. Heavy cannabis use has been associated with low BMD and an increased risk of fractures; however, moderate cannabis use has been shown to increase BMD and bone strength due to its positive effects on bone remodeling [25–27]. Other studies have also reported that cannabis has the potential to help individuals suffering from osteoporosis [21, 28].

According to the National Osteoporosis Foundation, 54 million Americans suffer from low bone mass, increasing their risk for osteoporosis. Up to one in two women and one in four men over the age of 50 will fracture a bone due to osteoporosis, and 44 million Americans have osteoporosis or low bone density [29]. In 2017, it was estimated that over 200 million people worldwide suffer from osteoporosis [29]. Osteoporotic fractures result in 500,000 hospitalizations, 800,000 emergency room visits, 2.6 million physician visits, and 180,000 nursing home placements each year in the USA [30]. It is projected that there will be an overwhelming 6.3 million hip fractures

Table 4 Studies that are representative of the primary outcomes of both the activation and inactivation of CB1R on bone

Cannabinoid receptor	Species	Sex	Activation or inactivation	Outcomes	Reference
CB1R	Mice	Both	Deletion	↓ bone formation ↑peak bone mass	[101]
CB1R	Mice	Both	Inactivation	↑ BMD ↓osteoclast activity With age: ↓osteoblast differentiation ↑bone loss	[25]
CB1R	Mice	Female	Deletion	↑BMD ↓OVX-induced bone loss ↓osteoclast formation ↓bone resorption	[102]
CB1R	Mice	Both	Inactivation	↓bone mass ↑osteoclasts ↓bone formation rate	[79]
CB1R	Mice	Both	Inactivation	↑bone mass in males Slight ↑diaphyseal shaft and medullary cavity diameters in females	[80]
CB1R	Rats	Male	Inactivation	↓bone dysmetabolism	[24]
CB1R	In vitro (MSCs from rats)	N/A	Activation	↑survival of MSCs during osteogenesis	[121]
CB1R	Mice	Both	Inactivation	↑femora length ↑vertebral body length in females	[123]

Table 5 Studies that are representative of the primary outcomes of both the activation and inactivation of CB2R on bone

Cannabinoid receptor	Species	Sex	Activation or inactivation	Outcomes	Reference
CB2R	Mice	Both	Deletion	↓trabecular BVD ↑bone turnover ↓bone mass ↑osteoclast number per bone SA	[105]
CB2R	Mice	Female	Inactivation	↓osteoclast count ↓bone loss	[112]
CB2R	Mice	Female	Activation	↑new bone formation	[117]
CB2R	Mice	Female	Inactivation	↓trabecular BVD	[75]
CB2R	Mice	Both	Inactivation	↑femora length ↑vertebral body length in females Skeletal elongation slowed by THC	[123]
CB2R	In vitro (mouse cultures)	N/A	Activation	↓osteoclast formation ↓RANKL expression	[105]
CB2R	In vitro (mice cultures)	N/A	Inactivation	↓osteoclast differentiation ↓osteoblast differentiation ↓bone resorption	[112]
CB2R	In vitro (human cultures)	N/A	Activation	↓ osteoclasts	[107]
CB2R	In vitro (human cell cultures)	N/A	Activation	↑osteogenic gene transcription ↓RANKL expression	[108]
CB2R	In vitro (human cell cultures)	N/A	Activation	↑osteoclast differentiation	[109]
CB2R	In Vitro (human and mice breast cancer cells)	N/A	Activation	↑osteolytic and osteoblastic factors RANKL and PTH	[110]
CB2R	In vitro (human cultures)	N/A	Activation	↓multinucleated osteoclast count	[140]

in the USA alone by the year 2050 [31], the vast majority of which are due to osteoporosis. Osteoporotic fractures resulted in 12–18 billion dollars in US healthcare costs annually, and it is projected that by the year 2040, osteoporotic fractures will cost the US healthcare system 50 billion dollars [30, 31].

Mechanism of osteoporosis

Estrogen plays a major role in maintaining bone quality as it restrains osteoclast activity and promotes osteoblast activity. Osteoclasts resorb bone during growth and healing and help to regulate skeletal growth and renewal [32]. Since estrogen inhibits the differentiation of macrophages into osteoclasts, decreases in estrogen release the inhibition of osteoclast activity, allowing for increased bone resorption [33, 34]. Estrogen deprivation is also associated with decreased absorption of calcium by the intestines as it also plays a critical role in the regulation of calcium homeostasis [21, 35, 36]. Further, bone metabolism and calcium homeostasis are closely linked. When calcium levels are low, the parathyroid glands release parathyroid hormone (PTH) which stimulates the small intestine to absorb dietary calcium and promotes resorption of bone by osteoclasts thus releasing calcium from the skeleton [37]. The most common cause of decreased estrogen levels is menopause which is characterized by the cessation of menstruation followed by 1 to 2 years of a gradual decline of estrogen produced by the ovaries [21]. This depletion in estrogen is the predominant reason why osteoporosis is the most prevalent in postmenopausal women.

The mammalian skeleton is constantly undergoing a process known as coupled remodeling, where the mineralized matrix is continuously being removed and replaced with newly formed bone in response to damage and changes in load bearing. While healthy adult individuals have a balanced state of bone remodeling, older individuals experience a net increase in bone resorption, leading to bone loss, which can result in osteoporosis. Bone remodeling is also necessary to repair bone microdamage before it enlarges and becomes clinically apparent. Following microdamage, remodeling is initiated by signaling from osteocytes, the most prevalent bone cells [21]. Osteocytes are able to sense mechanical cues via their dendritic projections by changes in fluid flow shear stress (FFSS) and, in turn, secrete paracrine factors such as RANKL and sclerostin, which are both central regulators of bone remodeling [38]. In addition to dendritic processes, in vitro and in vivo studies over the last 2 decades have provided evidence that the osteocyte cytoskeleton, primary cilium, ion channels, and extracellular matrix are also major mechanosensors [39].

RANKL, produced by osteoblasts and osteocytes, binds to the RANK receptor on osteoclast precursors, which then differentiate into osteoclasts. Once formed, osteoclasts attach to the surface of bone and secrete hydrochloric acid and the enzyme cathepsin K to dissolve bone mineral and the bone matrix, respectively. Osteoblasts then lay down layers of bone collagen matrix, called osteoid, to be mineralized and turned to hydroxyapatite [40]. Many of the trapped osteoblasts within the newly formed bone will further differentiate into osteocytes which, as previously mentioned, play an important role in cell signaling, regulating osteoblast and osteoclast function, and sensing mechanical loading [40, 41]. Clinical trials of antiresorptive treatments provide evidence that reducing excess remodeling reduces fracture risk [42].

Pharmacological treatments for osteoporosis

There are a variety of pharmacologic treatments available for osteoporosis, and these drugs predominantly comprise two categories: antiresorptive or anabolic. Antiresorptive drugs function by inhibiting osteoclasts and bone resorption, while anabolic drugs work by stimulating osteoblasts and bone formation [43]. The main type of antiresorptive drugs are bisphosphonates, which are compounds that bind to hydroxyapatite crystals on bone surfaces which are taken up by osteoclasts whose activity is subsequently affected by the drug thus inhibiting bone resorption [23]. Bisphosphonates have been shown to affect bone remodeling and, as a result, have been used for decades as a treatment for osteoporosis and to reduce fracture risk [43, 44]. Intravenous zoledronic acid and intravenous ibandronate have been used as treatment options for severe osteoporosis, as this route ensures that the bisphosphonate is delivered to the bone at sufficiently high concentrations for maximal efficacy [22]. While bisphosphonates can help individuals presenting with osteoporosis, they have been shown to have side effects including osteonecrosis of the jaw, esophageal cancer, and atrial fibrillation [45, 46]. The current FDA-approved antiresorptive agents include the bisphosphonates as well as the monoclonal antibody to RANKL, denosumab [21, 47, 48]. The selective estrogen receptor agonists raloxifene and bazedoxifene as well as estrogen are used for osteoporosis and also decrease bone resorption [49–55]. Teriparatide (PTH1-34) was the first anabolic agent registered for the treatment of osteoporosis [48, 56, 57]. Subsequently abaloparatide (an analogue of PTHrP) was approved as an anabolic treatment for osteoporosis [58, 59]. Romosozumab, a monoclonal antibody to sclerostin, decreases bone resorption and increases bone formation and is the newest drug approved for osteoporosis [60–64].

Endocannabinoid system and cannabis

Endocannabinoid system

The endocannabinoid system (ECS) plays a major role in many cognitive and physiological processes including immunology, psychology, developmental processes,

neuronal plasticity, metabolic regulation, and signal transduction [27, 65, 66]. The ECS is also involved in the regulation of various physiological functions, including bone mass and remodeling [24]. *Cannabis sativa* exerts its effects via the ECS, which consists of cannabinoid receptors, the G-protein-coupled receptors (GCPRs) cannabinoid receptor 1 (CB1R), and cannabinoid receptor 2 (CB2R), as well as endocannabinoids and their related downstream enzymes [24, 67]. CB1R and CB2R are class A GPCRs. These receptors are also involved in cell recognition and communication, which make them prominent drug targets [68]. The CB1R is one of the most highly expressed GCPRs in the central nervous system, especially in the cerebral cortex, basal ganglia, hippocampus, and cerebellum [68]. The two most studied endogenous ligands of the endocannabinoid system (CB1R and CB2R) are N-arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG). There are multiple pathways associated with the biosynthesis and degradation of these ligands, as well as many enzymes responsible for their production and degradation [69]. The activation of the CB1R and CB2R by anandamide and 2-AG aids in the regulation of a multitude of neuronal and glial ion channels, as well as vascular tone and cholesterol. Clinically, the activation of CB1R and CB2R also improves symptoms of schizophrenia and exerts a modulatory effect on the brain's reward system [70–74]. The endocannabinoids anandamide and 2-AG also play a role in bone regulation and are produced by osteoblasts and osteoclasts [75].

Cannabinoid receptor 1

CB1R is the GPCR cannabinoid receptor encoded by the CNR1 gene that is expressed in the peripheral and central nervous system [76]. Activation of the CB1R by its ligands causes coupling to intracellular effector proteins that mediate receptor desensitization, trafficking, and signaling to result in therapeutic outcomes [76]. Activation of the receptor by its ligands can lead to pain regulation, as well as influence neurogenesis, learning and memory, energy balance, and metabolism [68].

Through modulation of both excitatory and inhibitory neurotransmitters, the CB1R is involved in negative feedback and has the capability to influence both bone formation and resorption [77]. Activated by endocannabinoids, anandamide, and 2-AG, CB1R is present mainly in skeletal sympathetic nerve terminals regulating adrenergic tonic restrain of bone formation [78]. In peripheral tissues, CB1R inhibits norepinephrine release, and in bone, it may help regulate norepinephrine release in sympathetic nerve fibers, as immunoreactive CB1R was located close to osteoblasts in sympathetic neurons [79]. Norepinephrine in sympathetic terminals suppresses bone formation due to its activation of osteoblastic beta2-adrenergic receptors; thus, by decreasing norepinephrine through the activation of CB1R, inhibition of bone formation is reduced [80].

Cannabinoid receptor 2

CB2R shares 44% overall identity with CB1R and is expressed in the skeleton, immune system, and inflammatory cells [78, 81]. Given that CB1R and CB2R have cannabinoid agonists and antagonists with their own specific binding sites, they are not functionally identical, but both can mediate the effects of THC [78, 82]. CB2R has been shown to influence pain and inflammation, arthritis, addiction, neuroprotection, cancer, and bone regeneration, along with other possible therapeutic processes [83, 84]. CB2R has received considerable attention in recent years as it shows promising therapeutic properties through selective modulation that avoids the adverse psychotropic effects that have been seen in therapies targeting CB1R [81, 85]. Importantly, Karsak et al. found that a silent single nucleotide polymorphism in humans CB2R is strongly associated with osteoporosis in women [86], suggesting that CBR2 may be a target for new osteoporosis treatment.

Cannabis

Cannabis has been used medically to treat pain, nausea, fever, and gynecological disorders and to stimulate appetite for thousands of years [87]. The cannabis plant and its cannabinoid constituents have been extensively studied on both chemical and biological levels, since the early twentieth century [88]. As a result, the use of cannabis has been clinically evaluated in the treatment of a number of illnesses such as glaucoma, depression, anxiety, Alzheimer's disease, MS, and alleviation of symptoms of HIV/AIDS and cancer [4, 88]. Of the aforementioned illnesses, CBD has been shown to have benefits in anxiety-related disorders and Alzheimer's disease, as well as the alleviation of pain in HIV/AIDS and cancer [5, 16, 89–92].

Δ9-Tetrahydrocannabinol (THC)

Of the roughly 100 cannabinoids found in cannabis, the most prominent psychoactive compound is thought to be THC [93]. It is the predominant phytocannabinoid present in the *cannabis* species and can activate endocannabinoid receptors throughout the body [26]. THC is a partial agonist of CB1R in the central nervous system and CB2R in the immune system. The effects of THC are primarily mediated by activation of cannabinoid receptors which then decrease the concentration of cAMP through inhibition of adenylate cyclase. THC is prescribed in the form of sesame oil for a variety of treatments including the stimulation of appetite in

individuals with AIDS, gastric bypass, chemotherapy, and relief of neuropathic pain [78].

Cannabidiol (CBD)

CBD is another cannabinoid found in the *Cannabis sativa* plant and is the second most abundant compound in the plant [94]. Unlike THC, CBD does not have any psychoactive activity, making it non-intoxicating. But it does have many useful pharmacological properties, such as analgesic and anti-inflammatory effects, as well as efficacy in treating multiple conditions such as anxiety, neuropathic pain, and epilepsy [93, 95]. Further, CBD and its analogs have been shown to reduce immune responses, prevent experimental colitis, reduce beta-amyloid-induced neuroinflammation, reduce inflammation in acute lung injury, and decrease hepatic ischemia–reperfusion [96]. While the exact mechanism by which CBD induces these effects is currently unknown, it is thought that CBD engages different targets through multiple molecular mechanisms such as the targeting GPCRs, ionotropic receptors, enzymes, and nuclear factors, as well as binding intracellularly to transporters, including binding proteins 1, 3, 5, and 7 [94, 95, 97]. Overall, CBD has been shown to be a useful pharmaceutical product with more fewer and less severe side effects compared to THC [95].

Cannabinoid effects on osteoporosis

From 2007 to 2010, the National Health and Nutrition Examination Survey reported that 60% of the population claimed to have used cannabis at some point in their lifetime, making cannabis the most widely used illegal drug in the USA [98]. This survey also revealed that heavy cannabis users were more likely to be male and have a lower BMI and an increased intake of alcohol, tobacco, and other illegal drugs. The study concluded that while individuals with a history of cannabis had other risk factors for low BMD, the use of cannabis itself could not be found to be an independent factor [98]. In contrast to these findings, a cross-sectional study found that heavy cannabis users had a lower hip and spine BMD when compared to non-cannabis using controls. Specifically, multiple regression analysis showed heavy cannabis use to be an independent predictor of low spine and hip BMD (Table 1) [25]. Another clinical study found that heavy cannabis smoking is associated with bone lysis and may cause and accelerate osteoporosis (Table 1) [99]. An additional clinical study showed that heavy cannabis use was associated with reduced BMD and BMI and an increase in bone turnover and fractures [25].

Preclinical research has shown that marijuana smoke inhalation (MSI) significantly lowered bone filling around implants when compared to controls (Table 2) [100]. This had negative impacts on bone healing around titanium implants, which was attributed to reduced bone formation and/or an increase in resorption. Histomorphometric analysis revealed that MSI in rats had lower values for bone area, or bone-to-implant contact, in the threads of the titanium implants when compared to controls [100]. This was attributed to THC's inhibitory effect on CB2R expression on bone cells, in turn, resulting in an overall reduction in bone formation [100]. Based on these clinical and preclinical findings, the use of cannabis as a treatment or prevention of osteoporosis should be carefully monitored.

Expression of the endocannabinoid system in bone

There are multiple components of the endocannabinoid system that are expressed in bone. Both cannabinoid receptors, CB1R and CB2R, as well as the enzymes responsible for endocannabinoid synthesis are expressed in osteoclasts, osteoblasts, and bone marrow cells [101, 102]. In addition, the two main endocannabinoids, 2-AG and anandamide are produced locally in bone by osteoblasts and osteoclasts [78, 80]. In a mouse model, 2-AG was administered both chronically and acutely and, in both instances, led to the activation of CB1R in the sympathetic nerve terminals [103]. Anandamide, a CB2R-selective agonist, stimulates osteoblast proliferation in vitro [78]. Other studies also found that the biosynthetic degrading enzymes of 2-AG and anandamide, NAPE-PLD, and FAAH are also expressed in bone cells [78, 103].

Endocannabinoid system and bone remodeling

Endocannabinoids also have an effect on bone remodeling [24]. For example, inactivation of CB1R leads to an increase in BMD due to reduced osteoclast activity (Table 4) [25]. Other findings report that sympathetic neurons innervating bone express CB1R, suggesting that it may be part of a neural mechanism that helps regulate bone turnover [104, 105]. When CB1R acts on the peripheral sympathetic nerve terminals, it prevents the production of norepinephrine, which in turn leads to an increase in osteoblast activity through inhibition of osteoblast β2-adrenergic receptors [28]. CB1R deficient [CB1(−/−)] mice have increased BMD compared to control mice, as well as defects in osteoclast differentiation which protects them from ovariectomy-induced osteoporosis (Table 4) [102]. These results indicate that the CB1R plays critical roles in both osteoblast and osteoclasts, thereby influencing the balance of bone remodeling [104].

In addition, CB2R also has a role in the regulation of osteoclast activity and bone resorption. CB2R is highly expressed in osteoblasts, osteocytes, and osteoclasts [26]. When CB2R is stimulated on osteoblast precursor cells, it leads to increased numbers of pre-osteoblastic cells. Mature

osteoblasts also respond to CB2R activation by increasing alkaline phosphatase activity which in turn increases matrix mineralization [106]. An in vitro study found a decrease in human multinucleated osteoclast count when CB2R was stimulated (Table 5) [107]. This decrease in osteoclast numbers and activity could in turn reduce the amount of bone resorption. In another in vitro study using human periodontal ligament cells, activation of CB2R resulted in increased osteogenic gene transcription and a decrease in RANKL expression (Table 5) [108]. Further, differentiation of monocytes into mature osteoclasts is also mediated by CB2R signaling (Table 5) [109]. In both human and mouse breast cancer cells, CB2R-selective agonists, HU308 and JWH133, caused an increase in PI3K/AKT activity leading to higher levels of osteolytic and osteogenic factors including RANKL and PTH (Table 5) [110]. Human gene linkage studies have also shown that in some populations there is a correlation between Cnr2 polymorphisms and decreased BMD and osteoporosis [86, 111]. The genes encoding CB2R and Cnr2 have been detected in macrophages and monocytes, or preosteoclasts, as well as mature osteoclasts, osteoblasts, their precursors, and osteocytes within the bone matrix (Table 5) [105, 112]. Cnr1 gene expression is also much lower levels in osteoclasts, osteoblasts, and osteocytes as compared to Cnr2 [79, 105, 113, 114].

Cannabinoid receptors 1 and 2 and bone

Mice lacking either CB1R or CB2R showed abnormal bone phenotypes, such as an increase in BMD as well as protection against ovariectomy-induced bone loss, confirming that the endocannabinoid system has a role in regulating bone mass [102, 115, 116]. An age-matched study involving women with postmenopausal osteoporosis revealed that there were polymorphisms in the genes encoding CB2R and CNR2 [111]. More importantly, they found a missense variant, Gln63Arg, which affects CNR2 expression and activity, to be associated with low BMD [111]. They further showed that CB2R activation may be a stimulator of osteoblast proliferation and osteoclastogenesis. Additionally, activation of CB2R using synthetic agonists stimulates bone formation, while CB2R(−/−) resulted in lower BMD [86, 111].

CB2R also plays a role in the crosstalk between osteoclasts and osteoblasts [110]. CB2R activation in osteoblasts leads to an increase in RANKL and osteoprotegerin (OPG) production (Table 5) [110]. The CB2R(−/−) phenotype resulted in increased bone formation, providing protection from estrogen-induced bone loss in mice (Table 5) [117]. Additionally, ovariectomized mice with a CB2R(−/−) phenotype resulted in reduced trabecular bone loss and an increase in cortical thickness when compared to controls (Table 5) [105]. As such, activation of CB2R may be served as a potential new treatment for osteoporosis.

CB1R is localized on sympathetic nerve endings that innervate bone, in contrast to CB2R expression in bone cells [118]. Recent studies demonstrated that CB1R negatively regulates release of norepinephrine from synaptic nerve terminals which in turn suppresses bone formation through its binding to osteoblastic beta2AR [80]. 2-AG is an activator of CB1R and results in inhibition of norepinephrine release, thus stimulating new bone formation [119, 120]. Also, increased CB1R expression leads to increased survival of mesenchymal stem cells during osteogenesis in vitro (Table 5) [121].

Cannabinoid receptor 1

Several preclinical studies have been performed to gain insight into the endocannabinoid system's role in bone remodeling (Table 5). Using CB1R-deficient (Cnr1(−/−)) mice (CD1 background), it was shown that in females, normal trabecular bone was observed, whereas high bone mass was observed in males [79]. In sexually mature mice, normal bone formation and resorption was observed, indicating that these effects are specific to the developmental phase in which peak bone mass is determined [79, 102, 113]. In contrast, in C57 Cnr1(−/−) mice, both sexes displayed a low bone mass phenotype in addition to an increase in osteoclast number and a decrease in the bone formation rate [79]. Similarly, Cnr1(−/−) mice showed significantly reduced bone mass, bone volume density, trabecular density, and bone formation rates for both sexes. Further, female Cnr1(−/−) mice showed an increase in osteoclast number. In CD1R background Cnr1(−/−) mice, males had a higher bone mass phenotype, whereas females displayed volumetric bone density within normal ranges with a slight increase in diaphyseal shaft and medullary cavity diameters [80].

Female Cnr1(−/−) mice on a C57BL/6 background had low peak bone mass, and the authors speculated that this is a result from genetic differences within this strain, as mice on a CD1 background exhibit high peak bone mass [79]. In addition, osteoclast formation was decreased by the CB1R and CB2R selective antagonists, AM251 and SR144528, respectively, and increased by cannabinoid receptor agonists [101]. Further, it was found that while the CB1R protected against age-related bone loss in ovariectomized mice through regulation of adipocyte and osteoblast differentiation of bone marrow stromal cells, it regulated peak bone mass through the effects on osteoclast activity. Additionally, the selective CB1R antagonist, rimonabant, ameliorates bone dysmetabolism seen in conjunction with obstructive sleep apnea syndrome [24].

In contrast to these findings, a preclinical study showed Cnr1(−/−) mice had increased spine and femur BMD and greater trabecular bone volume at the tibial metaphysis when compared to controls [102]. Further, Cnr1(−/−) mice found

no changes in osteoclast or osteoblast numbers when compared to the control group. Finally, deletion of Cnr1 inhibited osteoclasts by promoting apoptosis through the inhibition of osteoclast survival factor production.

Cannabinoid receptor 2

Several studies have sought to determine effects of the CB2R on bone (Table 5). Interestingly, CB2R deficiency increases both bone loss and formation [25, 105, 117]. Other studies have shown that CB2R agonists can inhibit or reduce bone loss in ovariectomized mice [122]. Anandamide (a selective agonist of CB2R) stimulates osteoblast proliferation in vitro, as well as increases osteoclast numbers [102]. CB2R activation also has an inhibitory effect on osteoclast formation due to reduced monocyte mitosis and repression of RANKL expression in osteoblasts and their progenitors [105].

Cnr2(−/−) mice develop osteoporosis at an accelerated rate due to imbalance of bone resorption and formation [105, 113]. It was suggested that the observed increase of bone loss is a result of higher osteoclast numbers, while at the same time, osteoblast formation and differentiation was not sufficient to compensate for the increased rate of bone resorption. Cell cultures from Cnr2(−/−) mice also displayed decreased osteoclast and osteoblast differentiation, indicating that CB2R has a vital role in differentiation of both cell types [112]. In addition, it was found that when CB2R was inactivated in vitro using an antagonist, AM630, there was a decrease in both osteoclast formation and bone resorption [112].

Another study found that at the end of the linear growth phase, femora were significantly longer in both CB2R(−/−) and CB1R(−/−) females when compared to controls (Tables 4 and 5). In addition, CB2R(−/−) female mice show a significant increase in vertebral body length when compared to control mice, but this effect was not seen in in male mice (Table 5) [123]. Female Cnr2(−/−) mice also display a decrease in trabecular BMD (Table 5), and by the time the mice matured, both sexes displayed decreased BMD, along with trabecular structure transitioning from plate-like to rod-like [75].

Mice lacking CB2R have also shown phenotypes similar to postmenopausal osteoporosis; loss of trabecular BMD and increased bone turnover. The overall loss of BMD and bone mass is due to increased bone resorption in comparison to altered bone formation. It was suggested that the excessive bone resorption in these mice is due to increases in osteoclast number per bone surface area. Female Cnr2(−/−) mice also showed higher peak trabecular bone mass at both the tibial and femoral metaphysis, but not in the lumbar spine. Cnr2(−/−) mice also showed increased cortical bone volume in males but not in females, and in Cnr2(−/−) female mice, cortical bone diameter was significantly smaller in comparison to the controls [105].

These findings are inconsistent with earlier studies that found trabecular bone volume, total volume ratio, and bone turnover to be in normal range but decreased with the addition of high bone turnover rates in another [118]. The CB2R may influence the regulation of osteoblast and osteoclast interactions as studies have shown that the activation of CB2R in osteoblasts lead to changes in the levels of RANKL and OPG [105]. Additionally, the activation of the CB2R by HU308 stimulates new bone formation, in turn protecting against estrogen deficient bone loss [102, 105]. Finally, CB2R agonists also have anti-inflammatory effects and can reduce the expression of bone resorption promoting cytokines, as well as increase the expression of TNF, IL-1 receptor, and its antagonist, which reduced osteoclast formation [105]

Cannabinoid receptors 1 and 2

Combined deficiency of the CB1R and CB2R receptors could possibly protect against age-related bone loss. Mice with combined Cnr1 and Cnr2 deficiency showed an increase in trabecular bone mass and tibial and femoral strength [116]. Ovariectomized Cnr1/2(−/−) mice had a significantly lower decrease in trabecular bone volume when compared to control mice [116]. Additionally, the Cnr1/2(−/−) mice had significantly fewer osteoclasts in comparison to control mice. A deficiency in osteoclasts could be responsible for protecting mice against estrogen deficiency bone loss. While the study did find that double cannabinoid receptor knockout mice had a decrease in new bone formation, as well as an increase in the amount of fat in the bone marrow in comparison to the control mice, the reduction in osteoclasts had the greatest effect on BMD [26, 116].

GPR55

GPR55, a novel cannabinoid receptor, has been shown to be expressed in both human and mouse osteoblasts. While cannabinoids act mainly on the CB1R and CB2R in bone, GPR55 was found to be involved in cannabinoid signaling [28]. GPR55 is sometimes referred to as the "orphan" GPCR and considered the third cannabinoid receptor [124]. GPR55 is activated by some cannabinoids as well as by L-a-lysophosphatidylinositol (LPI) [115]. CBD is an antagonist of GPR55 and when antagonized, osteoblast activity is increased, while osteoclast activity is decreased, resulting in an overall effect of increased bone formation [28]. While GPR55 cannot be classified as a cannabinoid receptor due to its confusing cannabinoid profile, it is a cannabinoid-sensitive target that has been shown to have roles in cancer progression, analgesia, and bone resorption [125]. The GPR55

receptor is found in other tissues, including the central nervous system, intestines, pancreas, liver, and prostate, with its highest levels in brain and gastrointestinal tract [28, 124]. While GPR55 has not been as extensively studied as CB1R and CB2R, it has been established that when activated by ligands, it leads to the release of intracellular calcium [124]. Finally, it was also found that GPR55 plays a role in bone metabolism [115].

Specifically, GPR55 has been found in human osteoblasts and osteoclasts [28]. GPR55 mRNA expression in human osteoclasts is found at similar levels in both males and females [28, 115]. GPR55(−/−) mice showed an increase in both bone volume and tissue volume. Additionally, an impairment in osteoclast function was observed in male GPR55(−/−) mice when compared to male controls [115]. Moreover, CBD-treated mice showed a significant decrease in serum CTX levels, a biochemical marker of bone resorption, along with an increase in both bone and tissue volume, and a decrease in bone resorption. Analysis of long bones from GPR55(−/−) mice revealed significantly higher osteoclast numbers when compared to controls. Finally, osteoclastogenesis from mouse precursors is inhibited by O-1602, an agonist for GPR55, but this compound showed no effect on human osteoclast formation in vitro [115].

Further, GPR55 mRNA expression was found in multinucleated osteoclasts and primary osteoblasts, and treatment with CBD, a GPR55 antagonist, resulted in a significant increases in osteoclast differentiation, while treatment with O-1602, a GPR55 agonist, had an inhibitory effect [115]. Treatment of human osteoclasts with O-1602 also led to an increase in the resorptive activity of osteoclasts, as evidenced by increased area of resorption pits. Treatment with CBD resulted in the inhibition of effects of O-1602 [115]. Another study also found that O-1602 and LPI, agonists of GPR55, had inhibitory effects on osteoclast formation in mice [126].

The effect of CBD, a GPR55 receptor antagonist, on bone was also examined in GPR55(−/−) mice, and males showed an increase in inactive osteoclast numbers but also revealed a significant increase in both trabecular volume and thickness of [115]. Collectively, these studies indicate that CBD and other antagonists of GPR55 may protect against osteoporosis driven by the effects of GPR55 on bone resorption [126]. While these studies have shown promising effects of cannabinoid antagonists on bone remodeling, we still know little about their mechanisms of action [28].

THC effects on bone

In preclinical trials, the products of *Cannabis sativa*, particularly CBD and THC, have been shown to affect bone. Ovariectomized rats treated orally with THC were better protected against the symptoms of osteoarthritis and pain-related behaviors when compared to the placebo-treated control group (Table 3) [127]. THC-treated rats also showed callus size, both mineralized and unmineralized, that was significantly smaller than that of the control animals following fractures (Table 3) [128]. These effects were independent of mass, as THC had no influence on body weight, which is a factor that could potentially affect osteogenesis [128]. Equal parts of THC and CBD significantly increased the maximal force of the femur slightly greater than just CBD alone [128]. In addition, the combination eliminated the increase in work-to-failure seen with CBD treatment alone [128]. Rats treated with a THC analog (dronabinol) showed both a decrease in bone resorption as well as an increase in formation (Table 3) [129]. Additionally, dronabinol-treated rats had a significant increase in osteoclast and osteoblast numbers in alveolar bone when compared to the control group (Table 3) [129]. Finally, cell culture studies showed that THC had a negative effect on osteogenic activity (Table 3) [121]. Treatment with THC, as well as dronabinol, showed positive effects on bone that may be useful when examining treatment options for osteoporosis. While there are not as many studies examining the effects of THC on bone as there are for CBD, they do show promising results. Moreover, treatment with THC in conjunction with CBD may provide greater positive effects on bone health than CBD alone.

CBD effects on bone

Rats treated 4 weeks post-fracture with CBD showed a callus (both mineralized and unmineralized) that was significantly smaller than the controls (Table 3) [128]. In addition, these CBD-treated rats showed a significant increase in maximal force as well as in work-to-failure [128]. In another study, the effects of CBD on osteoporosis in a rat model with spinal cord injury (SCI) (Table 3) were examined, and they found that SCI rats treated with high-dose CBD had significantly higher levels of osteocalcin when compared to untreated controls. Furthermore, SCI rats treated with high-dose CBD showed enhanced BMD in both the femora and tibiae. However, low-dose CBD had no significant effect on osteocalcin, CTX, or femoral or tibial BMD [130]. In another model, CBD treatment following critical-sized bone defects led to complete healing, while defects in the untreated group were either completely empty or only filled with connective tissue (Table 3) [131]. In addition, X-ray scores revealed that there was superior bone formation and union in the group treated with CBD when compared to the untreated group [131]. Another study found that CBD decreased bone resorption in mice, along with an increase in the number of osteoclasts in GPR55 control mice, both Cnr1(−/−) and Cnr2(−/−), but not in Gpr55(−/−) mice (Table 3) [115]. Finally, CBD

was shown to affect RANKL and RANK expression in a periodontitis rat model that had an inhibitory effect on bone resorption as well as on bone volume (Table 3) [132].

In cultured osteoblast-like cell lines, U2OS and MG-63, a dose-dependent increase in Ang1 protein levels was detected in cells treated with CBD compared to the control cells [133]. Additionally, CBD increased the area of calcium deposits, suggesting that CBD promotes both mineralization and calcium deposition in vitro [133]. Taken together, these results indicate that CBD may affect transcription factors involved in osteoblast differentiation [133]. Using human mesenchymal dental stem cells differentiated into osteoblasts, and exposed to CBD and vitamin D (Table 3) [134], a high dose of CBD decreased cell numbers when compared to the controls, indicative of an increase in osteogenic differentiation (Table 3) [134]. However, there was no significant effect on mineralization in this study. Lastly, it was found that in apical papilla cells (APSCs), treatment with CBD resulted in high osteopontin mRNA expression, along with increases in osteonectin and osteocalcin [134]. While the exact mechanism is not completely known, treatment with CBD results in effects that both inhibit or reduce bone loss, as well as contribute towards bone formation. These results indicate good potential for the clinical use of CBD as a treatment option for osteoporosis.

Synthetic cannabinoids: cannabinoid acids

1′1’-dimethylheptyl-THC-1 1-oic acid, also referred to as ajulemic acid (AjA), is a synthetic cannabinoid acid and has analgesic and anti-inflammatory effects [135]. These effects are similar to those of the Δ9 metabolite, but without psychoactive effects [136]. Cannabinoid acids are components of cannabinoids including all the carboxylic acid metabolites and their synthetic analogs [137, 138]. Recent studies show that ajulemic acid, a cannabinoid receptor agonist, has a suppressing effect on osteoclastogenesis [137]. The addition of AjA to precursor RAW264.7 monocytes in vitro resulted in a complete suppression of osteoclastogenesis, in addition to the impairment of monocyte differentiation [137]. Exposure to AjA also leads to apoptosis of RANKL stimulated precursor monocytes and multinucleated osteoclasts [137]. These findings highlight the ability of AjA to suppress both osteoclastogenesis and the number of active osteoclasts in vitro, suggesting that AjA has the possibility of having a protective effect against bone loss in osteoporotic patients [137].

Conclusion

Osteoporosis is a worldwide major health concern. As the health costs associated with this disease continue to rise, gaining knowledge about both the mechanism of osteoporosis and possible treatment options for the disease is critical. Multiple studies have shown that the endocannabinoid system, influenced by CBD and THC, plays a significant role in bone remodeling. As both the use of cannabis and high-quality research on its effects continue to grow, novel medicinal uses of selected components, as well as the plant as a whole, are being elucidated. Lastly, additional clinical research is required in order to answer whether the effects of cannabis-mediated modulation of the endocannabinoid system can serve as a safe and effective treatment option for patients with osteoporosis.

Declarations

Conflicts of interest None.

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