

## REVIEW

# The promise and dilemma of cannabinoid therapy: lessons from animal studies of bone disease

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The endocannabinoid system plays an important role in numerous physiological processes and represents a potential drug target for diseases ranging from brain disorders to cancer. Recent preclinical studies implicated endocannabinoids and their receptors in the regulation of bone cell activity and in the pathogenesis of bone loss. Cells and intervening nerves in the skeleton express cannabinoid receptors and the machinery for the synthesis and breakdown of endocannabinoids. In healthy adult mice, pharmacological and genetic inactivation of the cannabinoid type 1 receptor (CB1) and putative cannabinoid receptor GPR55 (G protein-coupled receptor 55) inhibit osteoclastic bone resorption and increase bone mass, suggesting that both receptors have a negative role in early bone development. Although no distinct abnormalities in bone development were observed in healthy adult mice deficient in cannabinoid type 2 receptors (CB2), pharmacological blockage of this receptor was effective in suppressing bone loss associated with increased bone turnover, particularly in mouse models of osteoporosis, arthritis and osteolytic bone disease. In the aging skeleton, CB1 deficiency causes accelerated osteoporosis characterized mainly by a significant reduction in bone formation coupled to enhanced adipocyte accumulation in the bone marrow. A similar acceleration of bone loss was also reported in aging CB2-deficient mice but found to be associated with enhanced bone turnover. This perspective describes the role of cannabinoid ligands and their receptors in bone metabolism and highlights the promise and dilemma of therapeutic exploitation of the endocannabinoid system for treatment of bone disorders.

*BoneKEy Reports* 1, Article number: 224 (2012) | doi:10.1038/bonekey.2012.224

## Background

The endocannabinoid system is a complex system of endogenous mediators, membrane receptors and metabolizing enzymes (reviewed in Klein *et al.*,<sup>1</sup> Grant and Cahn,<sup>2</sup> Di Marzo<sup>3</sup> and Pertwee<sup>4</sup>). The endogenous cannabinoid (endocannabinoid) ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are present in the central and peripheral nervous system, and both are implicated in the regulation of a variety of physiological processes, including neurotransmission, pain perception, learning, memory, cardiovascular homeostasis, appetite, motor function and the immune response.<sup>1–4</sup> Both AEA and 2-AG are synthesized and released from membrane phospholipid precursors,<sup>5,6</sup> undergo cellular uptake by cells and are broken down intracellularly by membrane-bound enzymes, namely fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MGL) and diacylglycerol lipase.<sup>7,8</sup> A number of recent studies reported that AEA and 2-AG together with their metabolizing enzymes are present in the skeleton within the trabecular compartment, and both osteoblasts and osteoclasts

were reported to produce both endocannabinoids *in vitro*.<sup>9–12</sup> A growing list of synthetic non-classical cannabinoid receptor agonists such as CP55,940, JWH133 and HU308 and inverse agonists/antagonists, including SR141716A (also known as Rimonabant or Acomplia), AM251 and AM630 were reported to influence bone cell differentiation and activity *in vitro* and bone turnover in mouse models of bone disease (**Table 1**).<sup>11–19</sup>

Endocannabinoids and their related ligands activate two classic cannabinoid receptors, cannabinoid type 1 receptor (CB1) and type 2 (CB2), both of which are members of the G protein-coupled receptor (GPR) family coupled to adenylyl cyclase and cyclic adenosine monophosphate.<sup>20–22</sup> CB1 receptors are expressed primarily on neurons in the brain, spinal cord and peripheral nervous system,<sup>23</sup> but they are also found in the spleen, tonsils, immune cells, reproductive tissues, gastrointestinal tissues, heart, lung and adrenal gland.<sup>23,24</sup> CB2 receptors are found in the localized areas of the brain,<sup>25</sup> but they are expressed predominately in peripheral tissues such as the spleen and a number of immune cells.<sup>26,27</sup> The 'orphan' GPR55

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Received 30 August 2012; revised 12 October 2012; accepted 16 October 2012; published online 21 November 2012

**Table 1** The role of cannabinoid receptor ligands in the regulation of osteoclast, osteoblast and adipocyte differentiation and activity *in vitro* and *in vivo*

Ligands	Receptor	Osteoclast number	Osteoclast activity	Osteoblast number	Adipocyte number
<b>Agonists<sup>a</sup></b>					
Anandamide	CB1/CB2/GPR55/TRPV1	↑	↑	↑	NT
2-AG	CB1/CB2/GPR55	↑	↑	↑	NT
Δ9-Tetrahydrocannabinol	CB1/CB2	NT	NT	NT	NT
CP55,490	CB1/CB2	↑↓	↑	↑	↓
WIN55,212	CB1	NT	NT	↑	NT
HU308	CB2	↑↓	NT	↑	↓
JWH133	CB2	↑	NT	↑	↓
JWH015	CB2	NT	↑	↑	↓
Lysophosphatidyl inositol	GPR55	↓	↑	NT	NT
O-1602	GPR55	↓	↑	↑	NT
<b>Antagonists<sup>a</sup></b>					
Cannabidiol	GPR55	↑↓	↓↓	NT	NT
AM630	CB2 > CB1/GPR55	↓↓↑	↓↓	↓	NT
SR144528	CB2 > CB1	↓↓	↓↓	↓	NT
AM251	CB1 > CB2/GPR55	↓↓↑	↓↓	↓	↑
SR141716A <sup>b</sup>	CB1 > CB2	↓↓	↓↓	↓	NT

Abbreviations: CB1, cannabinoid type 1 receptor; CB2, cannabinoid type 2 receptor; GPR55, G protein-coupled receptor 55; NT, non-tested; TRPV1, transient receptor potential vanilloid type 1. The data presented in this table are assembled from references.<sup>10–19,36</sup>

<sup>a</sup>The cannabinoid receptor antagonists listed in this table are also known to act as agonists on other receptors. <sup>b</sup>SR141716A is also known as Rimonabant or Acomplia. Black and red arrows denote *in vitro* and *in vivo* data, respectively.

has been implicated recently as a third cannabinoid receptor.<sup>28–30</sup> The GPR55 receptor is expressed in the brain and a number of peripheral tissues, including adrenals, small intestine, liver, spleen, pancreas and prostate.<sup>28–30</sup> GPR55 is activated by a number of cannabinoid ligands, including AEA and 2-AG (Table 1).<sup>28–30</sup> The classic cannabinoid receptors CB1 and CB2 and the putative cannabinoid receptor GPR55 are known to activate a variety of other second messengers such as phospholipase C, mitogen-activated phosphatase kinase, nuclear factor kappa B, Rho and Rac1, PI3 kinase/Akt, calcium and potassium channels, *N*-methyl-D-aspartate receptors and ceramide synthesis (reviewed in Demuth and Molleman<sup>31</sup>). Endocannabinoids—in particular AEA—are also known to bind to and activate a number of channels, including potassium, calcium and vanilloid type 1 channel (transient receptor potential vanilloid type 1 (TRPV1)).<sup>32,33</sup> Moreover, recent studies showed that CB2 selective agonists induce mitogenic effects in osteoblasts via activation of a Gi protein-cyclin D1 and extracellular signal-regulated kinase 1 (ERK1)/2 axis.<sup>34,35</sup> Over recent years, a number of preclinical studies implicated CB1, CB2, GPR55 and other cannabinoid-related receptors in the regulation of bone metabolism. This perspective describes the role of the skeletal endocannabinoid system as a regulator of bone remodeling and discusses novel therapeutic strategies for the prevention and treatment of bone disorders based on targeting the endocannabinoid system.

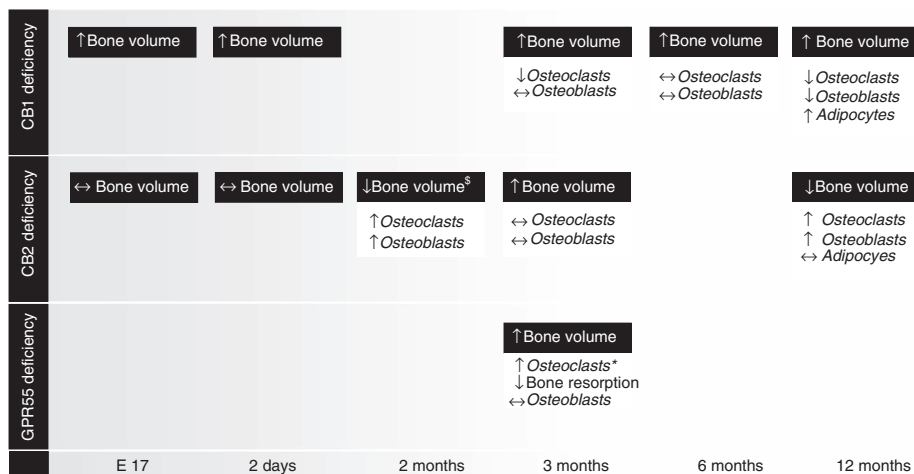
### Targeting Cannabinoid Receptor Type 1 for the Treatment of Bone Disease

A number of preclinical studies reported that the CB1 has a role in bone development and turnover in health and disease. CB1 receptors are present on nerve fibers intervening bone, cells of the immune system and bone cells, including osteoblasts, osteoclasts and bone marrow-derived adipocytes.<sup>1,10,11,15,27,36</sup> Studies in our laboratories showed that adult male and female AB/H mice deficient in CB1 exhibit high

peak bone mass characterized by a significant increase in trabecular bone mass, but no changes were observed in the cortical compartment.<sup>13,15</sup> A detailed histomorphometric analysis of the tibial metaphysis revealed that mice deficient in CB1 receptors have fewer osteoclasts and reduced bone resorption.<sup>13</sup> Following these reports, other workers suggested that the role of the CB1 receptor on bone metabolism may depend upon gender and genetic background. Indeed, both males and females CB1 knockout mice on C57BL/6 background showed low bone mass.<sup>36</sup> Puzzled by this report, we conducted a detailed analysis of bone mass and architecture in CB1-deficient mice using the out-bred mouse strain CD1. These studies confirmed the role of CB1 on early bone development and showed that CB1 deficiency during embryonic development (embryonic day 17) and early skeletal development (days 1–7 and 1–3 months) are associated with a significant increase in bone mass (Hof *et al.*,<sup>13</sup> Idris *et al.*<sup>13,15</sup> and Idris *et al.* unpublished data) (Figure 1).

Detailed micro-computed tomography scanning of trabecular bone in the 3-month-old mouse vertebral and long bone showed a significant increase in trabecular number and volume coupled to a profound reduction in trabecular separation,<sup>15</sup> suggesting a role of this receptor on skeletal growth during early adulthood. The increase in bone mass in the trabecular compartment due to CB1 deficiency was observed in both genders but was significantly evident in females.<sup>13,15</sup> Based on these findings, we are reasonably convinced that CB1 receptors have a negative role in bone turnover during early skeletal growth and in adult mice, but genetic variants, particularly in the inbred C57BL/6 strain of mice, may affect the severity of the abnormal bone phenotype associated with these receptors.

Encouraged by the positive phenotypic abnormalities associated with CB1 deficiency, we and others extensively investigated the effects of cannabinoid receptor blockage on bone loss in mouse models of bone disease. Treatment with the CB1-selective inverse agonist/antagonist AM251 protected



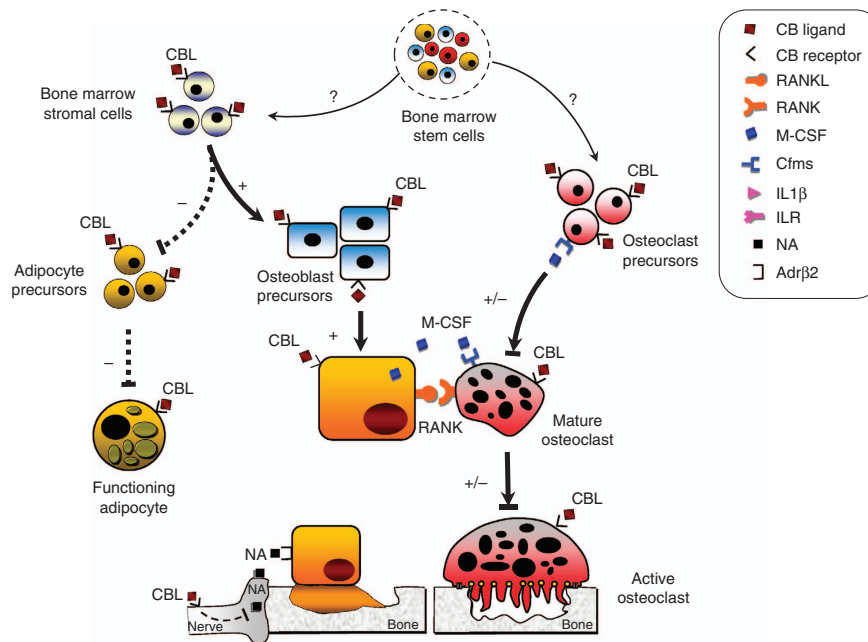
**Figure 1** The regulation of bone metabolism by cannabinoid receptors in mouse models of bone disease. The classic cannabinoid receptors CB1 and CB2 and the putative cannabinoid receptor GPR55 have a role in regulating bone cell differentiation and bone turnover throughout life. During skeletal growth and early adulthood (embryonic day 17 (E17) to 3 months), CB1 and GPR55 deficiency in mice are associated with increased trabecular bone mass mainly due to a significant reduction in bone resorption. During the early stage of bone development and adulthood, osteoblast number and bone formation markers remain unaffected following CB1 and GPR55 deficiency in mice. Conflicting data were reported in young adult CB2-deficient mice. Although our studies showed no changes in trabecular and cortical bone mass in adult mice up to the age of 3 months, Ofek *et al.* reported that CB2-deficient mice showed a trend toward a reduction in trabecular bone volume at the age of 2 months mainly due to increased bone turnover. During late adulthood and after menopause in female mice, CB1 deficiency is associated with excessive bone loss as bone formation is reduced relative to bone resorption. Although osteoclast number and bone resorption remain lower in CB1-deficient mice throughout life, osteoporosis develops as the result of increased adipocyte differentiation and reduced osteoblast differentiation, indicating that with increasing age, CB1 promotes osteoblast differentiation and inhibits adipocyte differentiation. Age-dependent bone loss was also reported following CB2 deficiency in mice and was attributed to increased bone turnover. No data are available for the effects of aging in GPR55-deficient mice. \*Denotes inactive and non-resorbing osteoclasts. <sup>§</sup>Denotes a trend toward a reduction in bone volume. Data shown in this figure are assembled from references.<sup>13–15,17–19,36</sup>

against ovariectomy-induced bone loss in adult mice by suppressing bone resorption.<sup>13,15</sup> The skeletal effects associated with this agent were likely to be mediated by CB1 receptors as genetic inactivation of the same receptor also protected against ovariectomy-induced bone loss in the same model.<sup>13,15</sup> Recent *in vitro* studies showed CB1-selective inverse agonists/antagonists such as AM251 and SR141716A (Rimonabant or Acomplia) suppress bone resorption by inhibiting osteoclast formation, fusion, polarization and survival (Figure 2).<sup>13,14</sup>

Furthermore, osteoclasts generated from CB1-deficient mice were found to be resistant to the effects of these agents, indicating that the mechanism of osteoclast inhibition was mediated, at least in part, by the CB1 receptor.<sup>13</sup> Interestingly, none of the CB1 blockers tested in osteoclast cultures showed any inhibitory effects towards the survival of macrophage colony-stimulating factor (M-CSF)-dependent osteoclast precursors at concentrations inhibitory to osteoclast formation, indicating an osteoclast-specific effect. The CB1 receptor was also reported to be involved in the regulation of osteoblast support for osteoclastogenesis as osteoclast formation was significantly reduced in osteoblast/bone marrow co-cultures in which osteoblasts were prepared from CB1-deficient mice.<sup>15</sup> Altogether, these studies indicate that CB1-selective inverse agonists/antagonists show promise for the treatment of excessive bone loss due to their abilities to suppress osteoclast formation and bone resorption (Figure 2).

One intriguing issue regarding the role of CB1 receptors in bone was the lack of change in the number of osteoblast and bone formation markers in CB1-deficient adult mice in comparison to wild-type littermates.<sup>15</sup> In stark contrast to this, *in vitro* studies showed bone marrow stromal cells from

CB1-deficient mice had a significantly reduced capacity to form mineralized bone nodules when cultured in osteogenic medium and had lower expression of the osteoblast-specific alkaline phosphatase and core binding factor alpha1 (Cbfa1/Runx2).<sup>15</sup> In keeping with these observations, the endocannabinoids AEA and 2-AG and a number of synthetic cannabinoid ligands were reported to stimulate early differentiation of bone marrow-derived osteoblast precursors and enhance bone nodule formation in osteoblast cultures *in vitro* (Figure 2),<sup>16,17,37</sup> whereas CB1-selective inverse agonist/antagonists suppressed osteoblast number and function acting on CB1 receptors.<sup>14–16</sup> A study by Tam *et al.*<sup>10</sup> reported that deficiency in CB1—not CB2—receptors was associated with a defect in bone formation in a mouse model of traumatic brain injury. The authors of this report went on to demonstrate that suppression of bone formation in CB1 knockout mice was accompanied by low levels of noradrenaline, a known inhibitor of osteoblast activity.<sup>10,38,39</sup> Moreover, studies in aging mice showed that CB1 deficiency is associated with a profound reduction in osteoblast number and bone formation leading to a significant loss in bone (Figure 2).<sup>13,15</sup> In this study, CB1 deficiency was also found to be associated with a striking accumulation of adipocytes in the bone marrow compartment of aging mice mainly due to decreased capacity of early mesenchymal cell precursor cells to differentiate into osteoblasts.<sup>15</sup> There is also evidence to suggest that CB1 receptors may protect—at least in part—against the inhibitory effect of leptin on osteoblast activity and bone formation as genetic inactivation of CB1 receptors was found to be associated with reduced levels of leptin in mice.<sup>40,41</sup> Taken together, these studies suggest that CB1 receptors have a role in bone resorption during early bone development but, with increasing age, promote osteoblast



**Figure 2** Current models of regulation of bone cell differentiation and activity by cannabinoid ligands (CBL). Cannabinoid receptor agonists act on cannabinoid receptors expressed on pre-osteoblasts present in the bone marrow, thereby stimulating osteoblast proliferation, differentiation and function. Cannabinoid agonists are also capable of regulating osteoblast function indirectly by inhibiting the production of the catecholamine noradrenaline, an inhibitor of osteoblast differentiation and function. Mature osteoblasts produce endocannabinoids (AEA and 2-AG) and RANKL (receptor activator of nuclear factor kappa-B ligand), which stimulate osteoclast formation. Cannabinoid receptor agonists were reported to both stimulate and inhibit osteoclast formation and bone resorption acting directly on mature osteoclasts and their precursors. See text for detailed description and references. IL, interleukin.

differentiation and inhibit adipocyte differentiation. There is also emerging evidence linking CB1 receptor to glucocorticoid-induced osteoblast dysfunction. Studies in the osteoblast-line cells MC3T3-E1 *in vitro* have shown that CB1 blockage attenuated the deleterious actions of glucocorticoid treatment on survival and activity.<sup>42</sup> Further mechanistic studies revealed that CB1 regulates glucocorticoid-induced dysfunction via ERK/GSK-3 $\beta$  (glycogen synthase kinase-3 $\beta$ )/Runx2 pathways.<sup>42</sup> In light of these findings, it is tempting to speculate that CB1 receptors could potentially serve as targets for both anabolic and anti-resorptive therapies, depending on the age of the patient and type of osteoporosis. However, future studies are still needed to establish which systemic factors—other than noradrenaline, leptin and estrogen—influence the effects of cannabinoid ligands on bone remodeling.

### Targeting Cannabinoid Type 2 Receptors for the Treatment of Bone Disease

The cannabinoid type 2 receptor (CB2) is found predominately on peripheral blood mononucleated cells, immune cells and bone cells, including osteoclasts, M-CSF-dependent osteoclast precursors, osteoblasts and osteocytes.<sup>11,13,16,17,27,43–45</sup> However, despite their abundance in the bone micro-environment, we and others failed to observe overwhelming evidence for bone abnormalities in neonate and adult mice deficient in CB2 receptors (**Figure 1**) (Idris *et al.*,<sup>14</sup> Ofek *et al.*<sup>17</sup> and Sophocleous *et al.*, unpublished data). Unlike CB1-deficient mice that were characterized by a significant reduction in body weight due to the role of CB1 on appetite, CB2-deficient mice are healthy and their weight was indistinguishable from

their wild-type littermates (Ofek *et al.*,<sup>17</sup> Tam *et al.*<sup>36</sup> and Sophocleous *et al.*, unpublished data). In models of bone disease, however, a number of workers implicated CB2 in the regulation of bone cell differentiation and bone mass. For example, we reported that bone mass was lost to a greater extent in wild-type mice compared with CB2 knockout littermates following ovariectomy in adult mice.<sup>14</sup> Furthermore, treatment with the CB2-selective inverse agonist/antagonist AM630 completely protected against bone loss in wild-type mice at a dose of 0.1 mg kg<sup>-1</sup> day<sup>-1</sup> and 1 mg kg<sup>-1</sup> day<sup>-1</sup>, but the effect of this agent was significantly blunted in CB2-deficient mice at 0.1 mg kg<sup>-1</sup> but not at 1.0 mg kg<sup>-1</sup>.<sup>14</sup> This shows that genetic inactivation or pharmacological blockage of CB2 receptors in adult mice is sufficient to partially—yet significantly—protect from ovariectomy-induced bone loss.<sup>14,37</sup> In keeping with this, osteoclasts generated from CB2-deficient mice were resistant to the inhibitory effects of the CB2-selective inverse agonist/antagonist AM630 *in vitro*, thereby confirming that CB2 blockage inhibits osteoclast formation.<sup>14</sup> On the other hand, the CB2-selective agonists JWH133 and HU308 stimulated osteoclast formation *in vitro* and partially reversed the inhibitory effects of AM630 on osteoclast formation (Idris *et al.*,<sup>14</sup> Ofek *et al.*<sup>17</sup> and Sophocleous *et al.*, unpublished data). In a similar vein, pharmacological studies in mouse models of inflammation showed that treatment with the CB2-selective inverse agonists/antagonists Sch.036 and AM630 was sufficient to suppress osteoclast number and prevent bone damage.<sup>46,47</sup> Moreover, other workers reported that the CB2 blocker AM630, which prevented ovariectomy-induced bone loss in our studies,<sup>14</sup> reduced osteoclast number and bone damage following

injection of titanium particles in a mouse model of osteolytic bone disease.<sup>48</sup> Altogether, these studies indicate that CB2 receptors have a role in regulating osteoclast formation and bone resorption under conditions of increased bone turnover and, therefore, peripherally active CB2 blockers could be of value for treatment of bone disease associated with excessive osteoclastic bone resorption.

In stark contradiction to the above findings, there are a number of conflicting reports that the CB2-selective agonists HU308 and AM1241 protect against and/or rescue bone loss in ovariectomized mice mainly by increasing bone formation markers.<sup>17,37</sup> In agreement with this, the selective agonist HU308 was found to stimulate bone nodule formation and exert a mitogenic effect in cultures of newborn mouse calvarial osteoblasts and osteoblast-like cells.<sup>17,49</sup> However, we and others showed that this agent is capable of both stimulating and inhibiting osteoclast formation *in vitro* and *in vivo* depending on the concentration/dose of the compound tested (Idris *et al.*,<sup>14</sup> Ofek *et al.*<sup>17</sup> and Sophocleous *et al.*, unpublished data). Studies also showed that the CB2-selective agent AM1241 acts as an agonist at human CB2 receptors and as an inverse agonist/antagonist at mouse and rat CB2 receptors.<sup>50,51</sup> Although further work is still required to explore the mechanism(s) by which CB2-selective agonists may affect bone loss in adult mice, there is sufficient evidence to show that activation of CB2 receptors may protect from bone loss in the aging skeleton. CB2-deficient mice develop age-related osteoporosis characterized mainly by increased bone turnover (**Figure 1**) (Idris *et al.*,<sup>14</sup> Ofek *et al.*<sup>17</sup> and Sophocleous *et al.*, unpublished data).

Further support for the role of CB2 receptors in age-related osteoporosis come from genetic association studies that revealed a strong genetic association between polymorphisms in CNR2 (CB2)—not CNR1 (CB1)—receptors and postmenopausal osteoporosis in humans.<sup>52,53</sup> Based on these studies, it is clear that peripheral CB2 receptors have a protective role in the aging skeleton and therefore targeting these receptors may represent an attractive and novel approach for the treatment of bone disease, such as postmenopausal osteoporosis.

### Targeting Cannabinoid Type 1 and 2 Receptors for the Treatment of Bone Disease

In a recent study, Sophocleous *et al.*<sup>54</sup> have reported in an abstract form that combined deficiency of the CB1 and CB2 receptors enhances peak bone mass but increases age-related bone loss. In this study, female CD1 mice deficient in both CB1 and CB2 receptors had significantly higher peak bone mass than wild-type controls due to a significant decrease in osteoclast number and activity. Interestingly, these differences in peak bone mass and bone resorption observed were quantitatively similar to those previously observed in single knockouts of CB1<sup>13,15</sup> and CB2<sup>54</sup> in the same background. By 12 months of age, female deficient in both CB1 and CB2 receptors had significantly lower trabecular bone mass and histomorphometric analysis showed that this was associated with a dramatic increase in bone marrow fat accumulation and a reduction in osteoblast numbers and bone formation rate. These differences in bone mass and bone cell activity observed in female deficient in both CB1 and CB2 receptors were quantitatively similar to those previously observed in single

knockouts of CB1 but not CB2.<sup>15</sup> Altogether, these data indicate that combined CB1 and CB2 deficiency enhances peak bone mass by an effect on bone resorption but predisposes to age-related osteoporosis by promoting adipocyte differentiation at the expense of osteoblast differentiation in the bone marrow compartment. This study demonstrates that CB1 and CB2 have overlapping but distinctive roles in skeletal homeostasis and show that CB1 in particular has a key role in regulating osteoblast and adipocyte differentiation in the bone marrow compartment.

### Other Ways to Target the Endocannabinoid System for the Treatment of Bone Disease

**The putative cannabinoid receptor GPR55.** Endocannabinoids and their synthetic analogs bind to and activate the orphan GPR55<sup>28,55</sup> (**Table 1**). A recent study by Whyte *et al.*<sup>18</sup> showed that GPR55 is expressed in human and mouse osteoclasts, and adult male mice deficient in this receptor exhibit a significant increase in peak bone mass due to a defect on osteoclast activity. In the same study, the authors went on to show that GPR55 deficiency causes no observable phenotypic abnormalities in osteoblast number and bone formation.<sup>18</sup> In fact, skeletal abnormalities observed in GPR55-deficient mice appear remarkably similar to those observed in CB1-deficient mice of a similar age.<sup>13,15</sup> Functional studies in osteoclast cultures showed that GPR55 receptor activation using the GPR55 selective agonist lysophosphatidyl inositol both stimulated and inhibited osteoclast formation.<sup>18</sup> On the other hand, GPR55 receptor inactivation using the antagonist cannabidiol inhibited osteoclast activity in rodent models of postmenopausal osteoporosis and periodontitis.<sup>18,56</sup> Based on findings from this study, it seems that non-psychoactive GPR55 antagonists such as cannabidiol could be of value for the prevention and treatment of excessive bone loss, but more studies are needed.

**Endocannabinoid metabolism.** Endocannabinoid-metabolizing enzymes are present in the skeleton, and both osteoblasts and osteoclasts were reported to produce AEA and 2-AG in culture.<sup>9–11,57</sup> Complementary to these findings, a number of cell types within the bone micro-environment, including osteoblasts, osteoclasts, osteocytes, stromal cells and adipocytes were found to express the endocannabinoid-metabolizing enzymes *N*-acylphosphatidylethanolamine-phospholipase D, fatty acid amide hydrolase, diacylglycerol lipase and monoacylglycerol lipase (Tam *et al.*,<sup>10</sup> Rossi *et al.*<sup>11</sup> and Sophocleous *et al.*, unpublished data). Interestingly, in a recent study by Rossi *et al.*<sup>11</sup>, treatment with the FAAH inhibitor URB597 significantly increased levels of AEA and 2-AG and enhanced human osteoclast formation *in vitro*. Although this finding broadly suggests that inhibition of endocannabinoid metabolism is associated with an increase in osteoclast formation, future studies examining the effects of FAAH and MGL deficiency on bone development in mice would conclusively demonstrate whether the machinery for the synthesis and breakdown of endocannabinoids could be targeted for the treatment of bone diseases.

**The TRPV1 channel.** The TRPV1 ion channel is a member of a family of cation channels that are predominately expressed by

sensory nerve fibers and implicated in the regulation of pain perception, inflammation and cardiovascular homeostasis (reviewed in Wong *et al.*<sup>58</sup>). The TRPV1 channel is activated in response to physical abrasion, heat, protons, capsaicin and the endocannabinoid AEA.<sup>32</sup> Studies showed that TRPV1-expressing fibers innervate bone, and pharmacological and genetic inactivation of TRPV1 reduce bone pain in animal models of osteolysis.<sup>59,60</sup> We and others have demonstrated recently that osteoclasts and osteoblasts express TRPV1, and in our studies, the TRPV1 blocker capsazepine inhibited ovariectomy-induced bone loss in mice by reducing indices of bone resorption and bone formation.<sup>11,19</sup> Bearing in mind that AEA activates TRPV1 and that cannabinoid receptors and TRPV1 are co-expressed in bone cells,<sup>11,19</sup> it is possible that some of the skeletal effects associated with cannabinoid receptors may actually be mediated via TRPV1. Although most current knowledge of the role of TRPV1 originates from a small number of mouse studies, there is evidence that this channel could potentially serve as a target for treatment of osteolytic bone disease and the pain associated with bone metastases.

### Conclusion and Future Directions

Although only studied in preclinical models of bone disease, it is becoming clear that the endocannabinoid system has a role in bone metabolism, and therefore CB1, CB2 and GPR55 receptors and their related channels could potentially serve as targets for cannabinoid-based bone therapy. Most studies in adult mouse models of bone disease showed that blockage of CB1, CB2 and GPR55 receptors and their related channel TRPV1 increase bone mass and/or protect against bone loss, suggesting that pharmacological blockers of these receptors may serve as anti-resorptive agents. Naturally, an important part of anti-resorptive therapy is to achieve inhibition of bone resorption without directly suppressing osteoblast and bone formation. Studies on the long-term effects of CB1 and CB2 receptor inactivation suggest that pharmacological blockers of CB1 and CB2 receptors are likely to cause bone loss by inhibiting osteoblast differentiation and reducing bone formation. Accordingly, further studies are needed to assess the long-term effects of pharmacological blockage of cannabinoid receptors on osteoblast differentiation and bone formation in aging osteoporotic mice.

An encouraging and positive finding from studies carried out in aging mice is that activation of CB1 and CB2 receptors seems to exert a protective effect against age-dependent bone loss. This indicates that cannabinoid receptor agonists may serve as bone anabolic agents in the aging skeleton. Taking into account that clinical targeting of CB1 is limited due to reports of physiological and behavioral side effects associated with CB1-selective blockers such as Rimobant and Acomplia,<sup>61</sup> and that findings from genetic studies showing that polymorphisms in CNR2 (CB2)—but not CNR1 (CB1)—receptors are associated with osteoporosis in humans,<sup>52,53</sup> it would be desirable to develop and assess anabolic actions of peripherally active and non-psychoactive CB2-selective ligands as well as CB1-selective ligands that do not cross the blood–brain barrier. There are also a number of other challenges to be addressed. For example, only a skillful combination of positive effects (that is, an increase in bone formation) with CB2 receptor selectivity will provide strong drug candidates for cannabinoid-based bone

anabolic therapy. Future studies should also focus on comparing and contrasting the efficacy of existing and novel cannabinoid ligands for bone anabolic and bone anti-resorptive potential and on developing peripherally active non-psychoactive agents that could potentially be tested in the discovery phase or in clinical trials. Studies on cannabinoid-related receptors—such as GPR55 and TRPV1—and their role in endocannabinoid signaling in bone cells might also offer further possibilities of therapeutic manipulation. The outcomes of these studies would ultimately help to develop novel strategies for harnessing the endocannabinoid system's full potential as a therapeutic target for the treatment of bone disorders.

### Conflict of Interest

The author is a co-inventor on a patent claiming the use of cannabinoid receptor ligands as treatments for bone disease.

### Acknowledgements

This research is supported by grants from the Arthritis Research Campaign and a fellowship from the European Calcified Tissue International Society (ECTS) and Amgen awarded to AI.

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