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Green Tea and Bone metabolism

Chwan-Li Shen[1], **James K. Yeh**[2], **Jay Cao**[3], and **Jia-Sheng Wang**[4]

[1]Department of Pathology, Texas Tech University Health Sciences Center, Lubbock, TX, USA.

[2]Applied Bench Core Laboratory, Winthrop-University Hospital, Mineola, NY, USA.

[3]USDA ARS Grand Forks Human Nutrition Research Center, Grand Forks, ND, USA.

[4]Department of Environmental Health Science, University of Georgia, Athens, GA, USA.

Abstract

Osteoporosis is a major health problem in both elderly women and men. Epidemiological evidence has shown an association between tea consumption and the prevention of age-related bone loss in elderly women and men. Ingestion of green tea and green tea bioactive compounds may be beneficial in mitigating bone loss of this population and decreasing their risk of osteoporotic fractures. This review describes the effect of green tea or its bioactive components on bone health, with an emphasis on: (i) the prevalence and etiology of osteoporosis, (ii) the role of oxidative stress and antioxidants in osteoporosis, (iii) green tea composition and bioavailability, (iv) the effects of green tea and its active components on osteogenesis, osteoblastogenesis, and osteoclastogenesis from human epidemiological, animal, as well as cell culture studies, (v) possible mechanisms explaining the osteo-protective effects of green tea bioactive compounds, (vi) other bioactive components in tea that benefit bone health, and (vii) a summary and future direction of green tea and bone health research and the translational aspects. In general, tea and its bioactive components might decrease the risk of fracture by improving bone mineral density (BMD) and supporting osteoblastic activities while suppressing osteoclastic activities.

Keywords

tea polyphenols; antioxidant; bone mineral density; osteoporosis; molecular mechanism; human; rat; cell culture

1. Introduction

Osteoporosis is a degenerative bone disease characterized by low bone mass and microarchitectural deterioration of bone tissue that leads to bone fragility and an increased susceptibility to fractures, especially in the hip, spine, and wrist [1]. Osteoporosis research has also reported some gender disparities. Women are four times more likely than men to develop osteoporosis because of a decrease in their estrogen levels after menopause in conjunction with generally lighter and thinner bones [2]. The rapid decrease in bone mineral density (BMD) that

Address reprint requests and correspondence to C-L Shen, BB 198, 3601 4th Street, Department 11of Pathology, Texas Tech University Health Sciences Center, Lubbock, TX 79430−9097, USA. 12E-mail: leslie.shen@ttuhsc.edu.

occurs in the first 3 to 5 years immediately following menopause and the slower decrease that continues throughout the remainder of a woman's life markedly increase the risk of hip or vertebral fracture, which is a major cause of morbidity and mortality in older women [2,3]. Over half of postmenopausal women will experience a bone fracture as the result of osteoporosis [4]. Similarly, one out of four osteoporosis patients is male and 30% of hip fractures occur in men [5]. The pathogenesis of osteoporosis in men is still poorly understood; it has been reported that approximately one third of osteoporotic men have an idiopathic disease [6].

Hip fracture is the most severe consequence of osteoporosis, leading to reduced activities of daily living, lowered quality of life, and increased mortality of patients [7,8]. As the population ages worldwide, osteoporosis has become a serious health threat in many countries [7,8]. It is estimated that almost 44 million American women and men aged 50 and older have osteoporosis and low bone mass. By the year 2010, it is estimated that over 52 million women and men in this same age category will be affected, and, if current trends continue, the number will climb to over 61 million by 2020 [9]. The economic costs due to hip fractures have increased tremendously in the past decade and are predicted to grow [10-12].

To predict the risk of fractures, clinical application of bone densitometry, such as dual-energy x-ray absorptiometry, is generally used to measure BMD at the spine, hip, and femoral neck. This method is also used to monitor the natural progression of diseases that affect BMD, or the therapeutic response to osteoporosis-specific treatments 1 to 2 years after beginning treatment [13-16]. Low areal BMD is the most important risk factor for hip fractures [17].

Recent research has suggested that BMD is positively associated with tea consumption, which may optimize bone health. The bioactive components in tea may benefit bone health in terms of maintaining higher BMD [18-23] and reducing the risk of fracture [24,25]. Specifically, green tea appeared to benefit bone health more than other kinds of tea (e.g., black, oolong), which may be due to decreased oxidative stress [26,27], increased activity of antioxidant enzymes [26], and decreased expression of proinflammatory mediators [26,27]. In this review, we discuss the beneficial osteo-protective effects of green tea and its bioactive components. In addition, the possible mechanisms of osteo-protection of green tea along with its bioactive components are discussed.

2. Role of Oxidative Stress and Antioxidants in Osteoporosis

Both osteoblastic and osteoclastic cells regulate bone metabolism, and both cell types are involved in the development of osteoporosis [28]. Osteoblasts are bone-forming cells located near the surface of the bone that produce cytokines. Cytokines, including macrophage-colony stimulating factor (M-CSF) and receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL), are both essential for osteoclast differentiation, function, and survival [29,30]. Osteoclasts are bone-resorbing multinucleated cells that become tightly attached to mineralized bone surfaces through their integrins and form resorption lacuna by secreting protons, proteases, and superoxide through ruffled borders [31-34]. Bone resorption by activated osteoclasts with subsequent deposition of a new matrix by osteoblasts causes the formation of bone structure and bone remodeling [28]. Imbalance between bone formation and bone resorption is the key pathophysiological event in many metabolic bone disorders in adult humans, including osteoporosis, a result of bone loss [35].

Oxidative stress is a pivotal pathogenic factor for age-related bone loss [36] in mice [37] and rats [26], leading to an increase in osteoblast and osteocyte apoptosis, among other changes, and a decrease in osteoblast numbers and the rate of bone formation via Wnt/β-catenin signaling [37]. Recent studies showed that oxidative stress inhibited osteoblastic differentiation [38, 39] via extracellular signal-regulated kinases (ERK) and ERK-dependent nuclear factor-κB

signaling pathways [40]. Osteoblasts can produce antioxidants, such as glutathione peroxidase, to protect against ROS [41], as well as transforming growth factor-β (TGF-β), which is involved in a reduction of bone resorption [42]. ROS are also involved in bone resorption with a direct contribution of osteoclast-generated superoxide to bone degradation [43,44] and oxidative stress increases differentiation and function of osteoclasts [45].

Several lines of evidence suggest a tight association between oxidative stress and the pathogenesis of osteoporosis in humans. For instance, Polidori et al. [46] reported that osteoporosis due to increased oxidative stress occurred, particularly in severe osteoporotic syndrome in relatively young males (mean age, 33 years old). A marked decrease in plasma antioxidants was also found in aged osteoporotic women [47]. There is also a biochemical link between increased oxidative stress and reduced BMD in men and women 55 years and older [48]. Dietary antioxidant (i.e., ascorbic acid) intake has a beneficial effect on BMD in postmenopausal women [49]. Since oxidative stress can contribute to bone loss, it is important to elucidate the role of antioxidants like green tea in mitigating bone loss during the development of osteoporosis.

3. Green Tea Composition and Bioavailability

Drinking green tea and/or ingesting green tea bioactive compounds may mitigate bone loss in elderly women and men, thereby decreasing their risk of osteoporotic fractures. Tea, the dried leaves of the *Camellia sinensis* species of the Theaceae family, is a popular beverage with an annual production of three billion kilograms. Of the tea produced worldwide, 78% is black tea, which is usually consumed in Western countries; 20% is green tea, which is commonly consumed in Asian countries; and 2% is Oolong tea, which is produced mainly in southern China [50]. Green tea is made by drying fresh leaves (by frying or roasting) at high temperatures to inactivate the oxidizing enzymes. Green tea is a non-oxidized/non-fermented product that contains several polyphenolic components, also called catechins or tea polyphenols, including (−) epigallocatechin gallate (EGCG), (−) epicatechin gallate (ECG), (−) epicatechin (EC), and (−) epigallocatechin (EGC) [50]. EGCG is the most abundant catechin and it has received the most attention from researchers. The tea catechins account for approximately 30% to 40% of the extractable solids of dried green tea leaves [51].

Tea catechins vary across tea tree and preparations, especially for EGCG and ECG contents. Tea with large leaves usually have less EGCG compared tea with small leaves. Tea should be consumed around 50°C. For better quality control, current chemoprevention or intervention trials usually use dry tea extracts in capsules that can be chemically measured for the exact concentration of each tea component. A traditional dose for human studies is 400 to 1500 mg tea per day according to published studies. The composition of green tea is quite stable in it's dry condition. The shelf life for EGCG, EC, ECG, and EGC as well as the mixture is longer than 1 year in a dry condition and can be oxidized in 30 minutes in water.

The bioavailability of EGCG or catechins, however, is relatively low due to its short half-life by nature. EGCG's short half-life *in vivo*, which ranges from 1.87 to 4.58 hours from a 50 to 1600 mg dose (≅ 0.7 to 23 mg/kg body weight, based on 70 kg body weight) [52], might be overcome by repeated administration because of its reported low toxicity and high tolerance by human subjects, even when given in doses as high as 1600 mg (≅ 23 mg/kg body weight) [52]. Drinking one cup of green tea could lead to a level of EGCG of 1 μmol/L in the circulation [24,53,54]. Studies have also demonstrated the maximum achievable *in vivo* EGCG concentrations [52,55]. For example, a 1600 mg oral dose of EGCG under fasting conditions has been reported to achieve a maximum human plasma level of 7.6 μmol/L [52]. This level is eight times higher than the highest reported daily intake from tea [52], making it likely that only pharmaceutically prepared formulations of green tea could reach plasma levels of the

catechin equal to those used in an *in vitro* study. The distribution of catechins concentration is dependent on tissue sites. It is likely that concentrations of catechins at tissue sites are higher than in the blood. For example, 400 to 1000 times greater concentrations of EGCG in the oral cavity, as compared with plasma, have been obtained when a green tea solution (1.2 g of green tea solid per 200 ml of water) is held in the mouth without swallowing [55]. Furthermore, Watkins laboratory reported in a mouse study [56] that tea catechins can accumulate in long bones (e.g., femur and tibia) with continuous tea consumption as short as 16 days, although this short-term period had no impact on BMD.

4. Beneficial Effects of Tea on Bone Health

The health benefits of tea consumption in preventing cancers and cardiovascular diseases have been intensively investigated [57]. However, limited information is available about the protective effect of consumption of green tea or its bioactive components on bone health. In this section, we summarize the impact of tea or green tea and its bioactive components on bone health, including human, animal, and cellular studies.

4.1. Human studies

In terms of BMD, a positive [19,20,22,23,58,63,65,67,132], a weak inversion [62], or no correlation [68,70] between tea drinking and osteoporosis has been reported by a number of human studies (findings summarized in Table 1). However, published results on BMD and tea consumption were based on cross-sectional or retrospective studies and are therefore inconsistent [64], which may compromise the quality of evidence. For instance, Hegarty et al. [20] reported that after adjustment for age and body mass index, the mean BMD of older women (aged 65 to 75 years in the United Kingdom, N = 1,256) at the lumbar spine, greater trochanter, and Ward's triangle was significantly higher in tea drinkers (N = 1,134) than in non tea drinkers (N = 122). Differences at the femoral neck were not significant between tea drinkers and non tea drinkers. These findings were independent of smoking status, use of hormone replacement therapy, coffee drinking, or the addition of milk to the tea. Hegarty's study also found that the magnitude of the effect of drinking tea was notable. Tea drinkers had approximately 5% higher mean BMD at various sites than non tea drinkers (Table 1, reference 20). This effect was equivalent to about half of the difference in BMD observed in women using hormone replacement therapy compared with women who did not use such therapy, or a decrease in age of approximately 5 years, and was associated with a decline in fracture risk of approximately 10% to 20% [20].

A positive relation between tea drinking, regardless of the type of tea, and BMD has also been reported among postmenopausal women in the United States (age 50 to 79 years) [58], Canada (62.9 ± 6 yr) [22], Australia (70 to 85 years) [23], Demark (45 to 58 years) [65], and Japan (71.8 ± 7.5 yr) [19,66], as well as among older Asian men (51.8 ± 13.8 yr) [21] and women (≥ 15 yr) [21,67]. In a cross-sectional study (N = 632 women age ≥ 60 yr), Muraki et al. [19] reported that at an osteoporosis outpatient clinic, patients with the habit of green tea drinking had significantly higher BMD at the lumbar spine than those without the habit, after adjusting for age, body mass index, and other variables related to lifestyle. A prospective analysis over 4 years from an Australian study [23] suggested that tea drinking is associated with the preservation of hip structure in elderly women (see Table 1).

Not all studies have reported positive results for tea drinking. A weak inverse relationship between tea consumption and BMD of the ultradistal radius was found in a study among pre- and perimenopausal women (N = 281, 50 to 60 years of age) in the United States [62]. Kyriazopoulos et al. [68] reported there was no association between tea consumption and BMD or bone mineral content in young Greek men (N = 300, 18 to 30 years of age). Moreover, Hallanger et al. [69] reported that obsessive tea drinking (10 to 40 cups/day containing fluoride

up to 56 mg/day) (N = 4) could lead to toxic concentrations of serum fluoride (> 15 μmol/L), in which increased BMD is not associated with a reduced risk of fractures. However, Saitoglu et al. [70] reported that there was no difference in BMD at the proximal femur and lumbar spine among healthy Turkish tea drinkers (N = 70, 45 to 65 years of age), regardless of bone mineral status (normal, osteopenia, or osteoporosis).

With respect to bone fracture, studies offer conflicting data on the role of tea drinking (Table 1). Results from the Mediterranean Osteoporosis Study showed that drinking up to 3 cups of tea per day was associated with a 30% reduction in the risk of hip fractures in both women (N = 5,618) [24] and men (N = 1,862) [25] over 50 years of age. Keramat et al. [61] demonstrated that tea drinking (≥ 7 cups per day) increased protective factors associated with osteoporosis risk in postmenopausal women (N = 717). Based on a longitudinal follow-up study, Chen et al. [58] concluded that the current level of tea consumption in the United States results in such a weak effect on BMD that it is unlikely to have any significant impact on fracture risk at the hip and forearm/wrist among postmenopausal women (N = 91,465, 50 to 70 years of age). A limitation of Chen's study included a lack of information on decaffeinated tea consumption and nondaily tea drinking in the studied group, which may mask an association between tea consumption and BMD or fractures. Hallstrom et al. [60] further reported that during a mean follow-up of 10.3 years, there was no association between consumption of tea up to 4 cups per day and incidence of osteoporotic fractures in women in Sweden (N = 31,527, 40 to 76 years of age).

These discrepancies among published findings may be due to experimental design differences (hospital-based or longitudinal); inconsistent definitions of tea intake categories; and incomplete adjustments of the confounding lifestyle characteristics that affect BMD or fracture risk, such as exercise, alcohol intake, smoking, and the intake of other nutrients. The differences in measured menopausal status and skeletal sites evaluated may also contribute to the discrepancies. In addition, these studies' lack of quantitative biomarkers for tea ingestion further hinders the validity of their conclusions [71]. Therefore, animal models are adopted to investigate the effect of green tea on bone health, which effectively eliminate possible confounding factors as well as allow for evaluation of bioavailability, efficacy, and related mechanisms through simulating the human consumption of green tea for targeted populations.

4.2. Animal studies

Although there is conflicting information from human studies, animal studies support that green tea may benefit bone health (Table 2) in terms of mitigating bone loss due to aging, aging plus estrogen deficiency, or chronic inflammation, thereby improving clinical symptoms of rheumatoid arthritis, normalizing bone metabolic disorders, and impacting trace element metabolism.

4.2.1. Green Tea Polyphenols (GTP) mitigate aging-induced and aging-plus-estrogen-deficiency-induced bone loss—Estrogen deficiency is another factor that results in oxidative stress to bone compartments during bone remodeling in elderly women. Estrogen is a phenolic compound that shares structural similarities with well-known lipophilic antioxidants, such as α-tocopherol, which enables estrogen to detoxify accumulated ROS [72]. Estrogen decreases oxidative stress in bone cells, and the loss of this sex steroid accelerates the involution of the skeleton by increasing oxidative stress [73]. Studies showed that estrogen deficiency leads to bone loss by lowering thiol antioxidant defenses in osteoclasts [74]. Hydrogen peroxide is the ROS responsible for estrogen-deficiency-induced bone loss [41]. Increasing concentrations of intracellular antioxidants, such as glutathione [74] or catalase [75], prevents bone loss due to estrogen deficiency.

Shen et al. [26] reported that both aging (sham) and aging-plus-estrogen-deficiency (ovariectomized) resulted in bone loss in 15-month-old female rats as shown by a decreased bone formation biomarker serum osteocalcin (OC) and increased bone resorption biomarker serum tartrate-resistant acid phosphatase (TRAP) and urinary calcium. Compared with the non-GTP supplemented groups, GTP supplementation (400 mg/kg body weight) resulted in a higher value of OC and lower values for TRAP and urinary calcium. Furthermore, the same study showed beneficial effects of GTP supplementation in preserving BMD in both cancellous and cortical bone compartments of sham and ovariectomized rats. GTP supplementation resulted in increased trabecular volume, thickness, number, and bone formation of the proximal tibia, periosteal bone formation rate of tibia shaft, and cortical thickness and area of the femur; and decreased trabecular separation and bone erosion of the proximal tibia and endocortical bone eroded surface of tibia shaft, resulting in a larger net bone volume.

In addition, it was found that the positive effects on bone parameters, such as femur BMD, trabecular number of the proximal tibia, bone formation rate, and eroded surface/bone surface at proximal tibia, were observed with both low (80 mg/kg body weight) and high doses (400 mg/kg body weight) of GTP supplementation. These findings demonstrate that GTP supplementation markedly improved femoral BMD, as well as the microarchitecture of trabecular and cortical bone in the tibia and femur, which were negatively impacted by aging in the middle-aged female rats. However, GTP could not completely prevent bone loss due to aging plus estrogen deficiency in ovariectomized rats.

From the same study [26], the impact of GTP in bone mass in ovariectomized 15-month-old rats (estrogen deficiency) may be independent of estrogen and showed no changes in serum estradiol concentration. Interestingly, GTP supplementation on the sham-operated groups (estrogen adequacy) only showed an impact on uterine weight, not serum estradiol concentration, and therefore may be associated with EGCG's [76,77] and ECG's [76,78] weak binding affinity for estrogen receptor(s) (ER), especially ER-α and ER-β.

4.2.2. GTP mitigates chronic-inflammation-induced bone loss—Green tea polyphenols have been found to counteract inflammation-induced bone loss in a recent animal study [27,79]. Shen et al. [27,79] reported that chronic administration of lipopolysaccharide to 3-month-old female rats using time-release pellets for 90 days resulted in a significant increase in the inflammation index in the animals as determined by the total white blood cell count, accompanied by a significant bone loss. Bone loss was demonstrated by decreases in BMD, accompanied by lowered trabecular volume fraction, number, and thickness in the proximal tibia, and increased eroded surface and osteoclast number in the endocortical tibial shafts. In addition, lipopolysaccharide also resulted in a lower value for OC and a higher value for TRAP when compared with those treated with placebo. Supplementation of GTP (400 mg/kg body weight) in the drinking water significantly increased BMD, serum OC, and trabecular volume fraction and number in both the femur and tibia, but decreased serum TRAP, eroded surface, and osteoclast number in endocortical tibial shafts. This study demonstrated that GTP supplementation in drinking water for 12 weeks prevented trabecular bone loss through increased bone turnover by lipopolysaccharide.

4.2.3. EGCG improves clinical symptoms of rheumatoid arthritis—Rheumatoid arthritis (RA), a chronic inflammatory disorder, is characterized by cellular infiltration and proliferation of the synovium, leading to the progressive destruction of the joints through the interaction between infiltrating cells and mediators [80,81]. Local bone erosion is one of the essential pathologic features of RA and is clinically related to functional outcome [82]. Osteoclasts appear to play a major role in bone erosion in RA joints [83,84]. In a model of RA and osteoporosis, Morinobu et al. [85] demonstrated that EGCG decreased bone resorption activity and osteoclast-specific gene expression without affecting cell viability of osteoclasts,

and EGCG treatment ameliorated clinical symptoms and reduced histologic scores in arthritic mice.

4.2.4. Green tea catechin normalizes bone metabolic disorders due to cadmium toxicity—Bone metabolic disorders, such as kidney malfunction, calcium absorption disorders, and osteoporosis, are a major result of chronic cadmium toxicity [86]. During cadmium toxicity, cadmium directly interferes with the function of 1,25-dihydroxycholecalciferol in the intestinal cells, lowers calcium resorption by inhibiting the synthesis of calcium binding protein, and increases calcium excretion due to kidney malfunction, which subsequently causes the loss of bone minerals [86]. Green tea catechin is known for its detoxifying benefits in that catechin binds with metal ions to form an insoluble complex-ionic salt to remove heavy metals. Choi et al. [87] reported that in cadmium-poisoned rats, green tea catechin reduced bone metabolic disorders by suppressing bone turnover rate as well as normalizing BMD, bone mineral content, and bone calcium content at the vertebra, pelvis, tibia, and femur.

4.2.5. Influence of green tea on trace element metabolism—Several compounds found in teas (i.e., polyphenols, methylxanthines, and aluminum) may interact with the utilization of trace elements. Greger et al. [88] reported that ingestion of green tea, which contains lower molecular weight polyphenols than black tea, tended to have less effect on liver copper and plasma ceruloplasmin levels in young rats than ingestion of black tea. From the same study [88], green tea elevated hematocrits in both normal rats and rats that had been repleted with iron for several weeks, yet initially slowed the iron repletion in iron-depleted anemic rats. This observation suggests that the ingestion of tea had a minimal direct effect on iron utilization and absorption as well as iron deposition in the tibia. The small changes in hematocrits induced by the ingestion of tea might relate to changes in copper metabolism. However, ingestion of tea seemed to have no effect on zinc or aluminum absorption. Zeyuan et al. [89] showed that tea and its water extracts had the following effects:

- **i.** Increased absorption of manganese and copper and the content of manganese in the tibia.
- **ii.** Increased content of aluminum in the tibia, but caused no changes in the apparent absorption rates of aluminum.
- **iii.** Inhibited absorption of calcium and iron without affecting calcium and iron in the tibia.
- **iv.** Decreased apparent absorption rates of zinc with little improvement in the content of 11 zinc in the tibia of 12-month-old SD rats.

Similar findings were reported by Hamdaoui [90] on tea's impact on iron and zinc of male rats, and that green tea decoction (i) reduced the concentration of iron in serum, liver, spleen, and femur; (ii) increased the concentration of zinc in serum, kidney, heart, and femur; and (iii) increased serum selenium and whole blood glutathione peroxidase activity of rats. The effect of green tea on iron status may be beneficial in some cases. Evidence suggests that the reduction of iron absorption, especially in patients with low iron requirements, may protect tissue against damage caused by oxygen free radicals and iron-dependent metal lipid peroxidation [91]. Indeed, the cytoprotective effects of GTP against lipid peroxidation arise not only from their antioxidant properties, including the scavenging of oxygen radicals and lipid radicals, but also from their iron-chelating activity.

4.3. Cellular studies

In addition to human and animal studies, the abilities of green tea bioactive components to increase and/or maintain indices of bone formation and to suppress indices of bone resorption were observed in cellular studies (Table 3).

4.3.1. Green tea bioactive components favor bone formation—The phenotype of mature osteoblasts is characterized by their ability to synthesize and secrete molecules of the extracellular matrix. The beneficial effect of EGCG on bone formation has been demonstrated by increasing alkaline phosphatase activity (ALP, a mature osteoblast phenotype) at both the gene expression and protein levels in osteoblastic-like cells, such as MC3T3-E1 cells [87], osteogenic sarcoma, and SaOS-2 cells [93], followed by increased formation of mineralized bone (as shown on both von Kossa and Alizarin red staining) [93]. Similar observations were also reported in mouse bone marrow cells, D1 cells, and 3T3-E1 cells [94], human stem cells [95], and antler progenitor cells [96]. Studies showed that catechin caused a significant elevation of osteoblastic survival as well as a decrease of osteoblastic apoptosis [87], resulting in stimulating cell proliferation and differentiation of osteoblasts [97]. Yamaguchi and Jie [98] reported that the effect of EGCG on bone calcification is biphasic at a high concentration. EGCG (10^{-4} M) appeared to inhibit ALP activity in *ex vivo* femoral-diaphyseal and -metaphyseal tissues, while a low concentration of EGCG (10^{-7} M) had no effect on bone calcium content.

4.3.2. Green tea bioactive components suppress bone resorption—Bone is resorbed mainly by multinucleated osteoclasts. These are formed by fusion of preosteoclasts [99], which are derived from hematopoietic stem cells in the presence of cytokines, such as M-CSF [100,101] and RANKL [29,102]. These cytokines are expressed by osteoblasts/stromal cells and modulate the function and survival of osteoclasts [29,101,103]. Cytokines and signals from integrins also induce the formation of polarized cell structures in osteoclasts that participate in bone resorption [104]. After a period of bone resorption, some osteoclasts die by apoptosis. Thus, regulation of these processes could potentially lead to control of bone resorption. Because enhanced bone resorbing activity and/or recruitment of osteoclasts is responsible for the pathogenesis of bone diseases like osteoporosis, agents that inhibit differentiation or induce apoptosis in osteoclasts could be used as a prophylactic or therapeutic agent for treatment of such diseases [102-104].

Using embryonic mouse calvaria, Delaiss [105] reported that (−)-catechin inhibited the development of bone resorption. Other cellular studies have extended this finding, observing that EGCG: (i) significantly inhibited the survival of differentiated osteoclasts [106] and increased the apoptosis of osteoclasts [107-109]; (ii) induced cell death of osteoclasts in terms of single-strand DNA damage, without affecting osteoblastic cells in a cocultured system of osteoblasts and osteoclasts [107-108]; and (iii) inhibited the differentiation of osteoclasts [110] and the formation of osteoclasts [106,108,110]. In an animal model of RA, EGCG treatment suppressed osteoclast differentiation *in vitro* in a dose-dependent manner, as judged by TRAP-positive multinucleated cell counts [85] (Table 2).

5. Possible mechanisms of green tea on osteo-protection

There are five main possible mechanisms through which green tea protects bone health: (1) by mitigating bone loss through anti-oxidative stress action, (2) by mitigating bone loss through anti-inflammatory action, (3) by enhancing osteoblastogenesis, (4) by suppressing osteoclastogenesis, and (5) probably through osteoimmunological action.

5.1. Mitigating bone loss through anti-oxidative stress action

The most widely recognized properties of GTP are their anti-oxidative activities, owing to their ability to capture and detoxify ROS [111]. Recent studies [26,112] addressed whether GTP supplementation would improve cellular antioxidant enzymes and/or diminish oxidative stress damage, and also whether the impact of GTP supplementation on the anti-oxidative defense system would have a beneficial effect on bone mass and microarchitecture. In one study, after GTP supplementation (400 mg/kg body weight), liver glutathione peroxidase activity, which might prevent oxidative damage during skeletal remodeling, increased in both 15-month-old sham and ovariectomized rats. This is the first study [26,112] to provide strong evidence of GTP's bone-mass-conservation effect due to its antioxidant capacity.

In a model of chronic-inflammation-induced bone loss, GTP mitigated the loss of BMD due to GTP's antioxidative ability (as demonstrated by a decreased urinary 8-hydroxydeoxyguanosine level) [26]. In another study, EGCG in GTP decreased the formation of oxidative-stress-induced calcium stone deposit formation in rats due to EGCG's anti-oxidative effects [113].

5.2. Mitigating bone loss through anti-inflammatory action

Due to its anti-inflammatory activity, GTP has also been proven to be beneficial in the prevention and treatment of a number of inflammatory diseases. A low-grade systemic chronic inflammation occurring in atherosclerosis leading to inflammation can also result in systemic bone loss. In the development of atherosclerosis, normal bone remodeling can be disrupted and bone loss can be caused by chronic elevation of pro-inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-β, γ-interferon (γ-IFN), and prostaglandin (PG) E_2 [114-116]. In general, these mediators act directly on bone or indirectly to increase osteoclastogenesis, prevent osteoclast apoptosis [117], and/or inhibit osteoblastic activity [118].

There is evidence to indicate a possible interaction between bone and the heart vessel, where a common pathophysiological mechanism is shared in both bone loss and atherosclerosis [79,114,119]. Shen et al. [79] reported that lipopolysaccharide (an inducer of chronic inflammation) not only caused low BMD in rats, but also induced a higher degree of fibrosis in heart vessels (a marker of atherosclerosis) of young rats. GTP supplementation in the drinking water (400 mg/kg body weight) was shown to mitigate bone loss and lessen the degree of fibrosis in the heart vessels of young rats. This protective role of GTP may in part be attributed to decreased inflammation [79].

Accumulating evidence indicates that EGCG's impact on osteoporosis is most likely mediated through its ability to inhibit cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS), predominately at the transcriptional level and, to a certain extent, the posttranslational level, although the specific regulation is not fully established [120,121]. At both the cellular and molecular levels, EGCG regulates a number of signaling pathways, including the eicosanoid pathway involving COX-2 and LOX in human colon mucosa and colon tumors [120]. The protective effect of EGCG is due to its ability to decrease lipid peroxidation, oxidative stress, and the production of NO radicals by inhibiting the expression of iNOS [121].

5.3. Increasing osteoblast numbers, osteoblastogenesis, and bone formation

Green tea bioactive components may be beneficial to bone health by promoting an increase in osteoblast numbers and activity of osteoblasts. The evidence suggests that the components in green tea support osteoblastogenesis by increasing osteoblastic survival, proliferation, differentiation, and bone formation (Table 3).

5.3.1. Improving the survival of osteoblasts through inhibiting TNF-α and IL-6 production—Suppressing the production of TNF-α and IL-6 by osteoblasts may increase osteoblast survival. Both cytokines can mediate the effects of many stimulators of bone resorption (i.e., parathyroid hormone, PTH and IL-1) [122,123]. TNF-α inhibits bone formation, collagen synthesis, and ALP activity in osteoblasts [124]. IL-6 promotes the recruitment of osteoclast precursors and their subsequent differentiation into mature osteoclasts. Both TNF-α and IL-6 could modulate the life span of osteoblasts via apoptosis, thus regulating bone metabolism in certain pathologic conditions such as periarticular osteoporosis found in patients with RA [125]. In an *in vitro* study, TNF-α or IL-6 acted on murine osteoblasts and induced apoptosis of these cells [126]. Choi et al. [87] reported that (+)-catechin can promote survival and ALP activity in osteoblastic MC3T3-E1 cells by inhibiting apoptosis of osteoblasts through a reduction in TNF-α and IL-6 production.

5.3.2. Enhancing bone mineralization through Runx2-mediated mechanism—Green tea bioactive components may affect bone strength by enhancing bone mineralization through the Runt-related transcription factor-2 (Runx2)-mediated mechanism. Runx2 (also known as core binding factor a1) regulates differentiation of osteoblasts from multipotent mesenchymal stem cells [127,128]. Runx2 enhances osteoblast differentiation at early stages but inhibits osteoblast maturation at later stages [129] by interacting with osteogenic genes.

In a murine bone marrow mesenchymal stem cell line D1, Chen et al. [94] reported that after 48 hours of EGCG treatment (1 and 10 μmol/L), the mRNA expression of Runx2, osterix, osteocalcin, and ALP was increased. After a long-term 4-week treatment of EGCG, mineralization was confirmed via von Kossa and Alizarin Red S stain [94]. These authors also reported that EGCG increased ALP activities in 3T3-E1 cells after treatment for 14 days [94]. EGCG's stimulatory effect on ALP activity and the mineralization on D1-cell culture further confirmed its post-transcriptional influences on osteogenesis through blocking the synthesis of Runx2 protein. However, depending on the types of cell line, Vali et al. [93] reported that Runx2 expression decreased after 48 hours of EGCG treatment; this reduction was observed with 1 μM and reached a maximal inhibitory effect with 5 μM in a human osteoblast-like cell line (SaOS-2). EGCG may enhance the differentiation of osteoblasts to progress to the maturation level, thereby leading to increase the mineralization of bone matrix and bone formation in general [93].

5.3.3. Increasing osteoblastic activity through Wnt Signaling—Green tea bioactive components may increase osteoblastic activity through activating the Wnt signaling pathway. The Wnt pathway is now known to control bone development and bone mass acquisition at all skeletal sites [130,131], and Wnt signaling drives differentiation of osteo-chondrogenic progenitor cells toward the osteoblast lineage. When the Wnt ligand binds to its receptor (Frizzled) and co-receptor (low-density lipoprotein receptor-related protein [LRP)5/6] [160-162], the signal turns on the Wnt pathway [133], giving the power of Wnt signaling to stimulate osteoblastic differentiation.

In an antler progenitor cell culture, Mount et al. [96] evaluated the effect of EGCG on canonical Wnt signaling on antler progenitor cell differentiation using ALP as a marker. Although ALP is normally used as a marker of the osteoblast phenotype, its activity has also been shown to increase with chondrocyte terminal differentiation [134]. EGCG induced apoptosis of antler progenitor cells; therefore, EGCG increased ALP activity, probably through activating β-catenin of Wnt signaling [96].

5.3.4. Inducing osteoblastogenesis by suppressing the HSP27-mediated mechanism—Green tea bioactive components can favor osteoblastogenesis via heat shock protein (HSP) 27-mediated mechanisms that are involved in bone modulators, such as

transforming growth factor-beta (TGF-β) and PGD_2. During osteoblastogenesis, down-regulation of osteoblastic proliferation is accompanied by a transient increase in the HSP27 mRNA expression [135].

TGF-β is one of the most abundant cytokines in the bone matrix and plays a major role in the development and maintenance of the skeleton, affecting bone cartilage and bone metabolism [136]. Hayashi [137] first demonstrated that EGCG significantly suppressed the TGF-β-stimulated induction of HSP27 through the suppression of the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) pathway in the osteoblast-like MC3T3-E1 cells. However, EGCG had little effect on the TGF-β-stimulated phosphorylation of ERK1/2 and p38 mitogen-activated protein (MAP) kinase in osteoblast-like MC3T3-E1 cells. In addition, EGCG scarcely affected the TGF-β-induced Smad2 phosphorylation, suggesting that EGCG does not act at a point upstream of Smad2-mediated signaling in osteoblasts.

PGD_2 is also a potent regulator of osteoblastic functions [138,139]. Yamauchi [140] reported that EGCG suppressed the PGD_2-stimulated induction of HSP27 via inhibition of p44/p42 MAP kinase, but not p38 MAP kinase or SAPK/JNK in these cells. Although the physiological significance of HSP27 in osteoblasts has not yet been clarified, it is probable that EGCG-induced suppression of the SAPK/JNK or p44/p42 MAP kinase cascades in osteoblasts contributes to the modulation of osteoblastic cell function toward bone formation at least in part by specifically down regulating HSP27 induction.

5.3.5. Enhancing bone formation through activating the VEGF-mediated mechanism—Green tea bioactive component EGCG can enhance osteoblastogenesis via the vascular endothelial growth factor (VEGF)-mediated mechanism that is involved in $PGF_{2\alpha}$ stimulation. VEGF is a heparin-binding angiogenic growth factor displaying high specificity for vascular endothelial cells. VEGF is involved in trabecular bone formation and expansion of the hypertrophic chondrocyte zone in the epiphyseal growth plate of mice [141]. Therefore, the inactivation of VEGF led to complete suppression of blood vessel invasion concomitant with impaired trabecular bone formation and expansion of the hypertrophic chondrocyte zone in the mouse tibial epiphyseal growth plate [141].

In addition to VEGF, $PGF_{2\alpha}$ is known as a potent bone-resorptive agent; it stimulates the proliferation of osteoblasts and inhibits their differentiation [142]. $PGF_{2\alpha}$ has also been shown to stimulate VEGF synthesis through protein kinase C (PKC)-dependent activation of p44/p42 MAP kinase in osteoblast-like MC3T3-E1 cells [143]. Tokuda et al. [143] reported that EGCG upregulates $PGF_{2\alpha}$-stimulated VEGF synthesis via amplifying activation of SAPK/JNK, but neither p44/p42 MAP kinase nor p38 MAP kinase, in osteoblasts.

5.4. Suppressing osteoclastogenesis and osteoclastic activity

The bioactive components in green tea appear to decrease the actions of osteoclasts in vivo (Table 2) and reduce osteoclastogenesis in cell culture (Table 3). The effects of green tea include suppressing bone resorption, increasing apoptosis of osteoclasts, and inhibiting the formation of osteoclasts.

5.4.1. Inhibiting bone resorption by stabilizing collagen—Collagen is the main organic constituent of the extracellular matrix of bone [144]. The basic building block of the bone matrix fiber network is type I collagen, a triple-helical molecule containing α1(I) and α2 (I) chains. The removal of collagen by tissue collagenase [145] and cystine-proteinases [146] is a necessary step for bone resorption. Therefore, an increase in the resistance of collagen to the action of collagenase can prevent collagen degradation, which reduces bone resorption.

Delaisse et al. [105] reported that the (+)-catechin inhibited bone resorption and prevented osteoclast activation by acting on bone collagen. In this study, the inhibitory effect of (+)-catechin may have been due to its oxidation products that bind to the thin layer of unmineralized collagen to separate resting osteoblasts from the mineralized matrix and prevent their removal by osteoblast-secreted collagenase. This could be sufficient to inhibit the whole process of bone resorption by preventing osteoclast activation.

5.4.2. Increasing apoptosis of osteoclasts through caspase activation-dependent mechanisms—The number of osteoclasts in an organism depends on the relative rates of osteoclastogenesis and apoptosis. Apoptosis of osteoclasts is regulated by the activation of caspases [147,148]. There are two main pathways leading to the activation of caspase-3 (a key executioner), including the mitochondria (receptor-independent) pathway and the death receptor with its ligand pathway (i.e., TNF and Fas ligand) [149,150]. Yun [151] reported that EGCG significantly inhibited the survival of osteoclasts differentiated from RAW 264.7 cells and induced the apoptosis of osteoclasts as shown by DNA fragmentation. EGCG-induced apoptosis in RAW 264.7 cell-derived osteoclasts was mediated in part through the activation of caspase-3. ECGC stimulates the activation of caspase-3, the elevation of caspase-3 activity, and the cleavage of pro-caspase-3.

The activation of NF-κB in cells provides cell survival signals and protects cells from apoptosis [152,153], whereas the inhibition of NF-κB activity in cells dramatically reduces cell growth [153,154]. Hafeez [109] demonstrated that high-dose GTP effectively reduced cell proliferation and induction of apoptosis via decreasing nuclear DNA binding of NF-κB/p65 and lowering of NF-κB/p65 and p50 levels in the cytoplasm and nucleus of human osteosarcoma SaOS-2 cells. GTP inhibits NF-κB activation through suppressing inhibitor of kappaB kinase activation and increasing phosphorylation of IκB-α in SaOS-2 cells. Inhibition of NF-κB decreases Bcl-2 protein expression and increases the levels of Bax, thus shifting the Bax/Bcl-2 ratio in favor of apoptosis. From the same study, GTP activated both caspase-3 and -8. These findings suggest that GTP may induce osteoclasts' apoptosis by involving a caspase-dependent mechanism with downregulation of NF-κB [109].

5.4.3. Increasing apoptosis of osteoclasts through the Fenton reaction—Hydroxyl radicals generated via the Fenton reaction from hydrogen peroxide (H_2O_2) and ferrous ions are produced by a variety of cells, including osteoclastic cells. The resulting hydroxyl radicals are reactive and may initiate and propagate the degenerative reaction in cell membranes known as lipid peroxidation. Such lipid peroxidation would contribute to oxidative stress in cells. Nakagawa et al. [107] first demonstrated that EGCG triggers the Fenton reaction to form highly reactive hydroxyl radicals from H_2O_2 and Fe^{2+} [$H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^{-} + F^{e3+}$], and the resulting hydroxyl radical activates caspase-3 activity and induces single-strand DNA breakage (a hallmark of cell death) in osteoclasts. These results indicate that the Fenton reaction is primarily involved in EGCG-induced osteoclastic cell death.

5.4.4 Inhibiting the formation of osteoclasts via the MMP pathway—Green tea bioactive components can suppress the formation of osteoclasts by inhibiting the release of matrix metalloproteinases (MMPs) by osteoblasts. Both collagenase (i.e., MMP-1 and MMP-13) and gelatinase A (MMP-2) and B (MMP-9) have been considered the principal MMPs in the digestion of bone collagen by osteoblasts [155,156]. In a co-culture system of mouse bone marrow cells and calvarial primary osteoblastic cells, Yun et al. [106] demonstrated that EGCG inhibited the expression of *Porphyromonas gingivalis*-induced MMP-9 mRNA of osteoblasts, not MMP-2 and MMP-13, and EGCG inhibited osteoclastic formation, but had no inhibitory effect on the cell viability of either the co-culture system or primary osteoblastic cells. These findings suggest that EGCG may inhibit the alveolar bone

resorption that occurs in periodontal diseases by inhibiting the expression of MMP-9 in osteoblasts and the formation of osteoclasts.

5.4.5 Suppressing bone resorption by inhibiting IL-6 production—Green tea bioactive component EGCG can suppress bone resorption by inhibiting IL-6 production by osteoblasts. In bone metabolism, IL-6, the most potent osteoclastogenic factor, stimulates bone resorption and induces osteoclast formation [157]. Tokuda et al. [158] reported that EGCG suppresses endothelin-1-induced IL-6 synthesis in osteoblasts via an inhibition of p44/p42 MAP kinase activation in osteoblastic-like MC3T3-E1 cells, and the inhibitory effect is exerted at a point between PKC and Raf-1 in the endothelin-1 signaling cascade. These same authors [159] also reported that EGCG inhibits basic-fibroblast-growth-factor-2-induced IL-6 synthesis at least partly via attenuation of the p44/p42 MAP kinase pathway and the p38 MAP kinase pathway in osteoblasts. Takai et al. [160] found that EGCG significantly reduced the IL-6 synthesis and IL-6 mRNA expression induced by platelet-derived growth factor-BB through suppression of the SAPK/JNK pathway in osteoblast-like MC3T3-E1 cells. Moreover, Ishida [161] reported that EGCG controls inflammatory bone resorption in chronic osteomyelitis by inhibiting the production of IL-6 and RANKL in osteoblasts infected with *S. aureus*.

5.5. Modulating osteoimmunological activity

Observation of accelerated bone loss caused by inflammatory diseases, such as RA, contributed enormously to the emergence of the field of osteoimmunology. Excessive bone loss is not only present in inflammatory diseases but also in autoimmune diseases and cancer. The prevalent skeletal disorder, osteoporosis, is associated with alterations in the immune system [162]. Although GTP has not been evaluated from the aspect of osteoimmunology, we speculate that there are two ways that GTPs may modulate osteoimmunological activity: first, by inhibiting differentiation of osteoclasts through RANKL signaling, and second, by modulating the production of cytokines by immune cells. Our speculation can be a possible new application to explore how GTP may influence bone health through osteoimmunological activity.

5.5.1. Inhibiting differentiation of osteoclasts through RANKL signaling—Green tea bioactive components can suppress osteoclastic differentiation via RANKL signaling. The differentiation of the macrophage polykaryon into osteoclasts is principally regulated by three cytokines: RANKL, macrophage-colony stimulating factor (M-CSF), and osteoprotegerin (OPG) [163,164]. During osteoclast development under RANKL stimulation, the nuclear factor of activated T cells c1 (NF-ATc1) is up-regulated, and this up-regulation is considered to be a master regulator for inducing osteoclast-specific genes such as TRAP, calcitonin receptor (CTR), carbonic anhydrase II, cathepsin K, and αv and β3 integrins [165].

One example of how EGCG is involved in suppressing osteoclasts via RANKL is what happens in the joints of RA patients. Under arthritic conditions, factors such as RANKL and inflammatory cytokines produced by T cells, synovial fibroblasts, and activated macrophages, facilitate osteoclast formation [166]. Thus, suppressing osteoclast development or inhibiting RANKL and M-CSF expression in inflamed joints is an option for reducing bone erosion in RA joints. In an experimental arthritis model using mice, Morinobu et al. [85] reported that EGCG reduced the generation of TRAP-positive multinucleated cells, bone resorption activity, and osteoclast-specific gene expression without affecting cell viability. EGCG down-regulated RANKL-induced expression of NF-ATc1, but not of NF-κB, c-FOS, and c-Jun, resulting in blocking differentiation of monocytes into osteoclasts, as judged by decreased bone resorption. In addition, EGCG inhibited the mRNA expression of TRAP, CTR, carbonic anhydrase II, cathepsin K, and αv and β3 integrins that are induced by RANKL. Lin et al. [110] also recently reported that EGCG significantly suppressed the RANKL-induced differentiation of

osteoclasts and pit formation in murine RAW 264.7 cells (a murine preosteoclast cell line) and bone marrow macrophages (precursor of osteoclasts) at the early stage of osteoclastogenesis. EGCG blocked RANKL signaling by significantly reducing RANKL-induced NF-κB transcriptional activity and the nuclear transport of NF-κB, a transcription factor known to be essential for the development of osteoclasts.

5.5.2. Modulating the production of cytokines by immune cells—EGCG has modulating effects on cytokine production by immune cells. An increased production of mononuclear cell immune cytokine products (such as IL-1, IL-6, IL-12, and TNF-1α) contributes to the postmenopausal enhancement of bone resorption. In addition to changes of cytokine production by monocytes, T-cell abnormalities have been reported in patients with osteoporosis [167]. Eghbali-Fatourechi [168] reported that the surface expression of RANKL on marrow stromal cells, B cells, and T cells was significantly higher in early postmenopausal women when compared with premenopausal or estrogen-treated women. These findings suggest that upregulation of RANKL on stromal cells and lymphocytes in the bone marrow could mediate increased bone resorption due to estrogen deficiency. Wu et al. [169] reported that EGCG upregulated the production of T-helper cell cytokines, IL-12, and TNF-α, which are important for antimicrobial cell-mediated immunity in murine alveolar macrophage cell lines (MH-S cells). Matsunaga et al. [170] found that EGCG downregulated the production of IL-10, an IL associated with Th2 helper cells that is important in humoral antibody-based immunity. Matsunaga et al. [170] also showed that EGCG stimulated the production of IFN-γ by macrophage cells.

Besides T-cells, EGCG also has an impact in dendritic cells (DC) and antigen-presenting cells, which play key roles as the immune sentinels by initiating T-cell responses against microbial pathogens and tumors [171]. In a model of primary murine bone marrow-derived DCs, EGCG has an inhibitory effect on production of the TNF-α and IL-12 in stimulated DCs. Ahn et al. [172] reported that EGCG suppressed the LPS-induced phenotypic and functional maturation of murine DCs through inhibition of expression of MAPK (ERK, p38, and JNK) and of NF-κB activation. The cytokine modulatory effects of EGCG on pro-inflammatory cytokines appears to be host-cell specific. For example, EGCG decreased LPS-induced TNF-α production in a dose-dependent manner in the murine macrophage cell line, RAW 264.7, and similarly inhibited LPS-induced TNF-α production in elicited BALB/c mouse peritoneal macrophages, effects attributed in part by blocking NF-κB activation [173].

6. Other bioactive components in tea that benefit bone health

Besides the catechins, tea is also an important source of flavonoids, caffeine, and dietary fluoride [174]. Fluoride intake can alleviate osteoporotic progression [175]. Fluoride concentration in tea brewed in fluoride-free water ranges from negligible to four parts per million, depending on the type and amount of tea used. Three or more cups of tea daily would be expected to increase fluoride intake by up to 4 mg daily. Therefore, the relatively high fluoride content of the tea leaves may enhance the protective effect of tea on BMD. However, overdose of fluoride from excessive tea drinking (10 to 40 cups/day containing fluoride up to 56 mg/day) had a detrimental effect on BMD [69].

Tea is a potential source of flavonoids, including phytoestrogen, isoflavone [176], and lignans [177], which are reported to have several biological actions, including a weak estrogenic effect [178]. Tea-derived flavonoids and lignans may improve BMD [105,179,180], particularly in older women with low concentrations of endogenous estrogen. Compared with American and European women, Japanese women have a diet that is higher in isoflavonoids [181]; high dietary isoflavonoid intake and its subsequent estrogenic effect may be a reason for the infrequent occurrence of hot flashes and other menopausal symptoms in Japanese women

[181]. Relatively weak estrogenic effects of isoflavonoids in tea may not have a noticeable effect on BMD in premenopausal women, who have high amounts of endogenous estrogen, such as predominated in the group studied by Hernandez et al. [182], or in men, in whom androgens predominate, but may be important in maintaining BMD in older women who have low levels of endogenous estrogen [22].

Another bioactive component of tea is caffeine. Some studies suggest that caffeine intake is inversely related to BMD [62,182], but these findings were not supported by other studies [183,184]. This discrepancy may be due partially to the addition of milk to coffee, which could ameliorate the adverse effects of coffee drinking [20,182]. Although the caffeine content of the tea leaf is higher (2% to 3% versus 1%) compared with that of roasted coffee, tea is diluted more for drinking. The average caffeine content of a tea beverage in the United States is about 30 to 45 mg/cup, while that of a coffee beverage is about 60 to 129 mg/cup [185]. Hence, an adverse effect of caffeine from tea on BMD may be less significant. However, it is possible that at higher tea intakes, caffeine may attenuate the benefit of other bioactive components of tea.

7. Summary and future research

Osteoporosis is the result of an imbalance in the ratio with more resorption than formation. Enhancing the activity of osteoblasts, plus reducing that of the osteoclasts, may help restore the balance in bone metabolism and limit bone loss in the development of osteoporosis. There is mounting evidence that green tea contains many bioactive ingredients that support some protection against osteoporosis. This is supported by data from *in vitro*, *ex vivo*, and *in vivo* animal studies and human epidemiological findings. The beneficial effects of tea bioactive products appear to be mediated through antioxidant or anti-inflammatory pathways and their related signaling pathways in the various cells comprised of bone compartments (Figure 1).

These significant beneficial effects on bone suggest that GTP may serve as an effective dietary supplement to prevent BMD loss in patients with low bone mass. It is worthy to point out that even though green tea and its metabolites are found to be useful in treating bone loss, there is still a gap in our knowledge that needs to be filled in regard to the translation of findings in animal observations and how this is applied to human populations. Evidence from all animal studies only shows an increase in BMD without testing bone strength and anti-fracture capacity; these animal data mainly focus on long bones, while the published human data are for spine and hip. In addition, there is still limited data supporting the BMD increment and anti-fracture effect of green tea from longitudinal studies. In future human studies, green tea and its active ingredients should be given for long-term periods, the bioavailability should be monitored via validated biomarkers, and efficacy in terms of bone mass and micro-architecture should be evaluated through advanced imaging technology in order to ensure their possible benefits in treating osteoporosis.

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Abbreviation

ALP, alkaline phosphatase
BMD, bone mineral density
COX, cyclooxygenase
EC, (−) epicatechin

CTR, calcitonin receptor
DC, dendritic cells
ECG, (−) epicatechin gallate
EGC, (−) epigallocatechin
EGCG, (−) epigallocatechin gallate
ER, estrogen receptor
ERK, extracellular signal-regulated kinases
GTP, green tea polyphenols
H_2O_2, hydrogen peroxide
HSP, heat shock protein
γ-IFN, γ-interferon
IL, interleukin
iNOS, inducible nitric oxide synthase
JNK, c-Jun N-terminal kinase
LOX, lipoxygenase
MAP, mitogen-activated protein
M-CSF, macrophage-colony stimulating factor
MMP, matrix metalloproteinases
NF-ATc1, nuclear factor of activated T cells c1
NF-kB, receptor activator of nuclear factor-kB
OC, osteocalcin
PG, prostaglandin
PKC, protein kinase C
PTH, parathyroid hormone
RA, rheumatoid arthritis
RANKL, receptor activator of nuclear factor-kB ligand
ROS, reactive oxygen species
Runx2, Runt-related transcription factor-2
SAPK, stress-activated protein kinase
TGF-β, transforming growth factor-beta
TNF-α, tumor necrosis factor-α
TRAP, tartrate-resistant acid phosphatase
VEGF, vascular endothelial growth factor

Abbreviation

(−), inhibitory effect
(+), stimulatory effect
"×", indicates blocking the pathway
ALP, alkaline phosphatase
COX-2, cyclooxygenase-2
DC, dendritic cells
EC, endothelial cells
HSP27, heat shock protein 27
γ-IFN, γ-interferon
IL, interleukin
iNOS, inducible nitric oxide synthase
LOX, lipoxygenase
M-CSF, macrophage-colony stimulating factor
MMP, matrix metalloproteinases
NF-κB, receptor activator of nuclear factor-κB
OB-P, osteoblast precursor
OB, osteoblast

OC-P, osteoclast precursor
OC, osteoclast
PGE_2, prostaglandin E_2
RANKL, receptor activator of nuclear factor-κB ligand
ROS, reactive oxygen species
Runx2, Run-related transcription factor-2
TGF-β, transforming growth factor-β
TNF-α, tumor necrosis factor-α
VEGF, vascular endothelial growth factor

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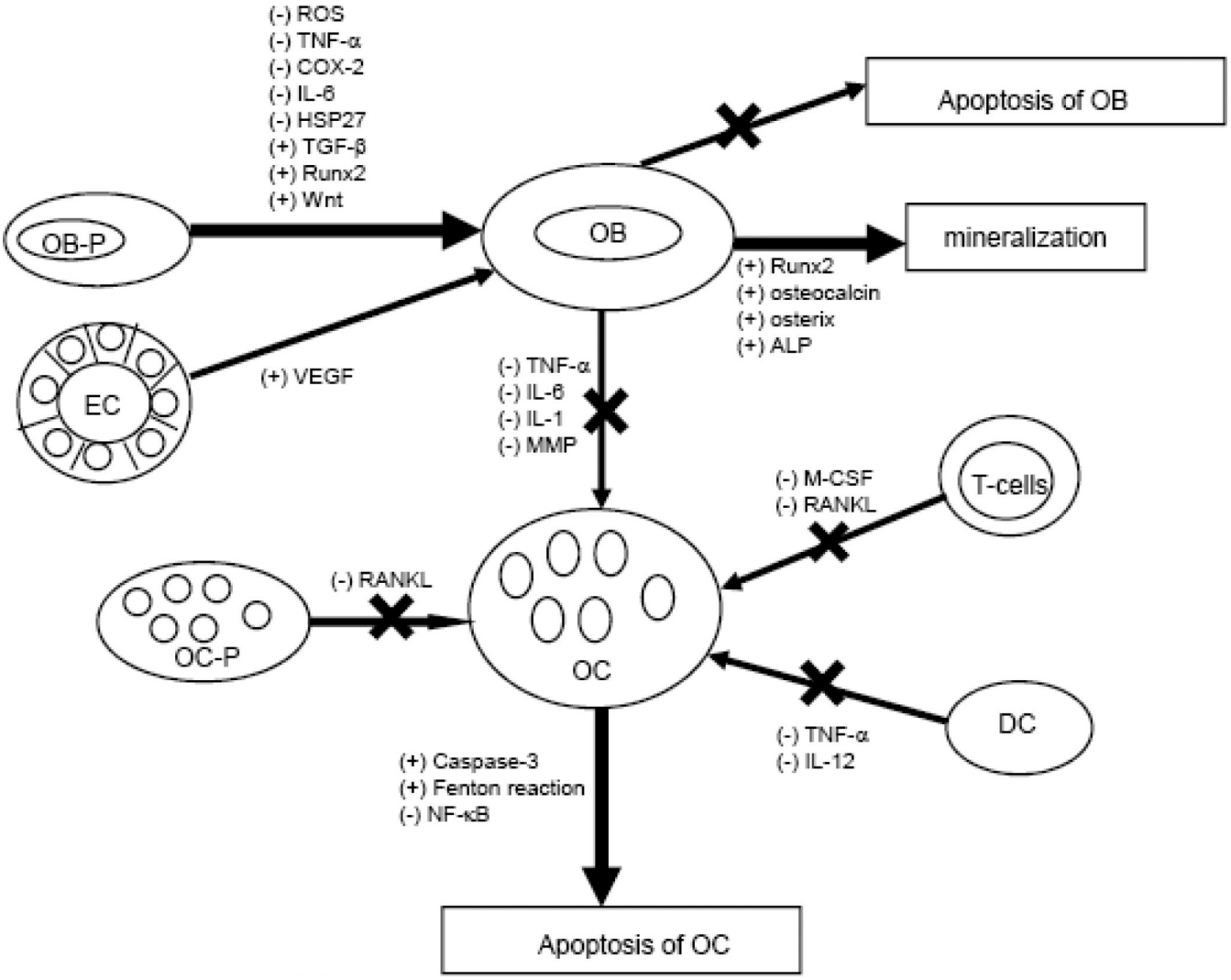

Figure 1.

Possible actions (stimulatory or inhibitory) of green tea bioactive component (EGCG) in osteoblasts (OB) and osteoclasts (OC). Green tea bioactive components appear to promote bone formation by decreasing oxidative stress (ROS) and pro-inflammatory mediators (TNF-α, COX-2), and by increasing OB activity and survival (HSP27, TGFβ, Runx2, Wnt), resulting in enhanced mineralization (Runx2, osteocalcin, osterix, ALP). Green tea bioactive components suppress bone resorption by inhibiting OC formation (MMP) via increasing OC apoptosis (caspases, Fenton) that results in suppressing osteoclastogenesis. In addition, EC is involved in stimulating osteoblastogenesis, while T-cells and DC are involved in suppressing osteoclastogenesis.

Table 1

Epidemiological studies of tea drinking and bone health

Mode	Study design	Number of subjects	cups/day	Endpoint(s)	Reference
Bone mineral density (BMD)					
Tea drinking	Older women in United Kingdom	N = 1,256 (65−77 yr)	FFQ	(i) An increase in BMD at lumbar, spine, trochanter, and Ward's triangle in tea drinkers	20
		122 non-tea drinkers	0 (n=122)		
	Cross-sectional study	1134 tea drinkers	1−3 (n=438)	(ii) No changes in BMD at femoral neck in tea drinkers	
			4−6 (n=567)	(iii) ≅ 5% higher BMD in tea drinker than non-tea drinkers	
			>6 (n=129)		
Tea drinking	Postmenopausal women in USA	N = 4,979	FFQ	(i) A significant trend of increased total BMD with a higher level of tea consumption	58
		(50−79 yr)	<1 (n=3,683)		
	Prospective study (4.1 yr follow-up)		1 (n=566)	(ii) No difference in BMD between hip and lumbar spine	
			2−3 (n=588)		
			≥4 (n=142)		
Tea drinking	Postmenopausal women in Canada	N = 62 (62.9±6 yr)	7-d FR	(i) 10% increase in BMD at lumbar spine in tea drinkers than non-tea drinkers	22
			0−12		
	Cross-sectional study			(ii) 14% increase in BMD at femoral neck in tea drinkers than non-tea drinkers	
Tea drinking	Elderly women in Australia	N = 1027	FFQ	(i) 2.8% increase in BMD at total hip and trochanter in tea drinkers than non-tea drinkers	23
		172 non-tea drinkers	0−5		
	Cross-sectional study	855 tea drinkers		(ii) No changes in BMD at neck/ intertrochanter	
Tea drinking	Elderly women in Australia	N = 164 (70−85 yr)	24-hr dietary recall	(i) Tea drinkers lost 1.6% BMD at hip	23
		42 non-tea drinkers		(ii) Non-tea drinkers lost 4.0% BMD at hip	
	Prospective study (5 yr follow-up)	122 tea drinkers			

Mode	Study design	Number of subjects	cups/day	Endpoint(s)	Reference
Tea drinking	Perimenopausal Women in Demark	N = 2,016 (45−58 yr) (50.1±2.8 yr)	FR	(i) Protective effect on femoral neck and lumbar spine (L2-l4) when T-scores > −0.75	65
			0−30		
	Cross-sectional study			(ii) No association when T-scores ≤ −0.75	
Green tea	Elderly women in Japan	N = 632 (≥ 60 yr)	FFQ	T-scores at lumbar spine for non-green tea drinkers (−2.17±2.08) vs. green tea drinkers (−1.59±2.70)	19
		52 non-green tea drinkers	< 5 days/wk		
	Cross-sectional study	580 green tea drinkers	≥ 5 days/wk		
Tea drinking	Postmenopausal Women in Turkey	N = 724 (57.6±9.6 yr)	FFQ	(i) Habitual tea drinking may have a positive effect on BMD	63
		non-tea drinkers	0−2		
	Cross-sectional study (IPPOT study)	tea drinkers	≥ 2	(ii) T-scores for non-tea drinkers: −1.09±1.66 vs. tea drinkers: −1.51 ±1.68	
		42.5% normal			
		27.2% osteopenia			
		30.2% osteoporosis			
Tea drinking	Asian elderly	N = 1,037 (≥ 30 yr) (51.8±13.8 yr)	Years of tea	(i) 6−10 yr: an increase BMD in lumbar spine	132
	Cross-sectional study		0 yr (n=532)	(ii) >10 yr: an increase in BMD in total body, lumbar spine, hip	
		497 men, 540 women	1−5 yr (n=226)		
		non-habitual tea drinkers	6−10 yr (n=152)		
		vs. habitual tea drinkers	>10 yr (n=124)		
Tea drinking	Chinese women	N = 1,432 (≥ 15 yr)	NP	Tea drinking may protect low BMD	67
	Cross-sectional study				
Tea drinking	Pre- and peri-Menopausal women in USA	N = 281 (50−60 yr)	FFQ	(i) Inverse association between ultradistal BMD and tea intake	62
		(52.5±1.7 yr)	NP		
				(ii) No associations between midshaft BMD and tea intake.	
	Cross-sectional study				
Tea drinking	Healthy men in Greek	N = 300 (18−30 yr)	FFQ	No association between tea consumption and BMD or BMC at radius	68

Mode	Study design	Number of subjects	cups/day	Endpoint(s)	Reference
	Cross-sectional study		0−2		
Tea drinking	Healthy men in Turkey	N = 70 (45−65 yr)	FFQ	No difference in BMD at proximal femur and lumbar	70
	Cross-sectional study	17 normal	up to 20		
		30 osteopenia			
		23 osteoporosis			
Risk of Fractures					
Tea drinking	Women in European	N = 5,618 (≥ 50 yr)	FFQ	Tea consumption was associated with a decrease in risk of hip fractures	24
	Case-control study (MEDOS study)	3,532 controls (77.7±8.8 yr)	Never		
		2,086 cases (78.1±9.4 yr)	Sometimes		
			1−2		
			≥ 3		
Tea drinking	Men in European	N = 1,862 (≥ 50 yr)	FFQ	Tea consumption was associated with a decrease in risk of hip fractures for any tea consumption at any age	25
	Case-control study (MEDOS study)	1,132 controls (74.1±10.1 yr)	Never		
		730 cases (73.9±10.6 yr)	Sometimes		
			1−2		
			≥ 3		
Tea drinking	Postmenopausal Women	N = 363	FFQ	Tea consumption (≥ 7 cups/day) to be significant protective factors of osteoporosis risk	61
		185 normal (55.7±6.0)	NP		
	Case-control study	178 osteopenia (58.2±7.1)			
Tea drinking	Postmenopausal women in USA	N = 91,465 (50−79 yr)	FFQ	(i) Effect of habitual tea drinking on BMD was small	58
			<1 (n=68,188)		
	Cohort study		1 (n=11,363)	(ii) No association between tea drinking and risk of fractures at hip/ forearm/wrist	
	Cross-sectional study		2−3 (n=9,480)		
	(Women's Health Initiative Observational Study)		≥ 4 (n=2,434)		

Mode	Study design	Number of subjects	cups/day	Endpoint(s)	Reference
Tea drinking	Women in Swedish	N = 31,527 (40−76 yr)	FFQ	No association between consumption of tea and incidence of osteoporotic fractures	60
	Prospective study (10.3 yr follow-up)		<1 (n=2,520)		
			1 (n=4,128)		
			2−3 (n=18,703)		
			≥ 4 (n=5,887)		
Tea drinking	Postmenopausal women in USA	N = 4 (50−67 yr)	FR	(i) No association between increased BMD and a reduced risk of fractures	69
			10−40		
	Case report			(ii) Toxic serum fluoride levels (> 15 μmol/L)	

FFQ, food frequency questionnaires; FR, food record; BMD, bone mineral density; NP, not provided.

Table 2

Animal studies of supplementation of green tea or green tea polyphenols and bone health

Bioactive	Animal model	Dose	Duration	Endpoint(s)/Mechanism(s)	Reference Compound
GTP	sham and OVX 14-mo-old female rats	80, 400 mg/kg BW	16 weeks	↑ femur BMD[a] ↑ serum OC, ↓ serum TRAP, ↓ urinary Ca ↑ BV/TV, BFR, Tb.N and Tb.Th of proximal tibia[b] ↓ Tb.Sp and erosion of proximal tibia[b] Via ↓8-OHdG, ↑GPX activity	26,112
GTP	chronic LPS-infected 3-mo-old female rats	400 mg/kg BW	12 weeks	↑ femur BMD[a] ↑ serum OC, ↓serum TRAP ↑ BV/TV, Tb.N and Tb.Th of femur and tibia[b] ↓ eroded surface and osteoclastic number of tibia shifts[b] Via ↓ 8-OHdG, COX-2, and TNF-α production	27,79
EGCG	antibody-induced arthritic model 6 wk-old male mice	20 mg/kg BW intraperitoneally	15 days	↓ bone resorption activity[c] ↓ osteoclast differentiation by TRAP stain[c] ↔ osteoclast cell viability[c] Via ↓ expression of NF-ATC1	85
Green tea catechin	chronic Cd-poisoned young male rats *	250 mg, 500 mg/kg BW**	20 weeks	normalized bone metabolic disorders in BMD[a], BMC[a], bone Ca, urinary DYD, serum OC caused by Cd intoxication	87
Green tea extract	hindlimb suspension 15-wk-old male mice	1500 mg/kg BW**	16 days	Tea catechins accumulated in femur, tibia ↔ femur BMD[a]	56
Green tea	Young rats*	0, 350, 1170, 3500 mg/kg BW**	2 weeks	↑ Zn in tibia ↔ Fe, Cu, and Al in tibia	88
Green tea extract	12-mo-old SD rats	400, 800, 1600 mg/kg BW	7 weeks	↑ Mn and Al in tibia ↔ Ca and Fe in tibia Via modulating apparent absorption	89
Green tea decoction	male rats*	10 g/Kg BW**	6 weeks	↓ Fe in serum, liver, femur, kidney, heart ↑ Zn in serum, liver, femur, kidney, heart	90

Bioactive	Animal model	Dose	Duration	Endpoint(s)/Mechanism(s)	Reference Compound
				↑ Se in serum	

Abbreviation: BFR, bone formation rate; BMC, bone mineral content; BMD, bone mineral density; BV/TV, bone total volume; BW, body weight; Cd, cadmium; COX-2, cyclooxygenase-2; DYD, deoxypyridinoline; GTP, green tea polyphenols; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; OC, osteocalcin; TRAP, tartrate-resistant acid phosphatase; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; TNF-α, tumor necrosis factor-α.

[a] measured by DEXA

[b] measured by histomorphometry

[c] measured by histology.

[*] information on the age of rats or mice not available.

[**] Since there are no information available, we estimated that each rat consumed 30 g feed, 25 mL water every day, and 300 g average body weight. We estimated that each mice consumed 30 g feed / 100 g body weight every day and 30 g average body weight.

Table 3

Effect of green tea bioactive compounds on bone health and related molecular mechanisms

Bioactive	Model	Dose used in medium	Dose duration	Endpoint(s)/Mechanism(s)	Reference compounds
Bone formation (osteoblastogenesis)					
(+)-catechin	MC3T3-E1 cells	10^{-4}–10^{-5} mol/L	48 hours	↑ ALP activity, ↑ osteoblast survial	87
				↓ apoptosis of osteoblasts	
				via ↓ IL-6 and TNF-α production	
EGCG	SaOS-2 cells	1−5 μmol/L	8 days	↑ ALP activity	93
			17 days	↑ formation of mineralized bone nodules including area and number	
				via ↓ Runx2 expression	
EGCG	D1 cells	10 μmol/L	up to 4 weeks	↑ALP activity, ↑mineralization	94
				via ↑mRNA expression of Runx2, osterix, osteocalcin, ALP	
EGCG	3T3-E1 cells	10 μmol/L	14 days	↑ALP activity	94
EGCG	antler progenitor cells	25 μmol/L	24 hours	↑ ALP activity	96
				via Wnt pathway	
EGCG	rat femoral tissue	10^{-4}–10^{-7} mol/L	24 hours	↔calcium content	98
		10^{-4} mol/L		↓ ALP activity	
EGCG	MC3T3-E1 cells	30 μmol/L	60 min	↓ TGF-β-stimulated HSP27 induction	137
				via ↓ SAPK/JNK pathway	
EGCG	MC3T3-E1 cells	100 μmol/L	60 min	↓ PGD_2-induced HSP27 induction	140
				via ↓ p44/p42 MAPK pathway	
EGCG	MC3T3-E1 cells	10−100 μmol/L	60 min	↑ $PGF_{2\alpha}$-induced VEGF synthesis	143
				via ↑ SAPK/JNK activation	
EGCG	NRG cells infected with Staphylococcus aureus	272 μmol/L	4 hours	↓ IL-6 production	161
				via ↓ RNAKL expression	
			48 hours	↓ osteomyelitis (osteoblast infection)	
				via ↓ inflammation	
Bone resorption (osteoclastogenesis)					
(+) catechin	embryonic mouse calvaria	0.1−1 mmol/L	18 hours	↓ development of bone resoprtion induced by PTH or retinoic acid	105
				via collagen-stabilizing properties of catechin	

Bioactive	Model	Dose used in medium	Dose duration	Endpoint(s)/Mechanism(s)	Reference compounds
EGCG	RAW264.7 cells	50 μmol/L	24 hours	↓ survival of osteoclasts ↑ apoptosis of osteoclasts via activation of caspase-3	151
GTP	SaOS-2 cells	43−130 μmol/L	48 hours	↑ apoptosis of SAOS-2 via caspase-3 activation with ↓NF-κB	109
EGCG	RAW 264.7 cells	10−100 μmol/L	2 hours	↓ osteoclastic differentiation via ↓ RANKL-induced NF-κB transcriptional and nuclear translocation	110
EGCG	DC cells	10−100 μmol/L	24 hours	↓ maturation of DC via ↓ MAPK and NF-κB activation	172
EGCG	osteoclast-like multinucleated cells	25−100 μmol/L	24 hours	↑ apoptosis of osteoclasts ↔ oastoblasts via Fenton reaction	107,108
EGCG	bone marrow with primary osteoclasts	20 μmol/L	up to 3 days	↓ formation of osteoclasts via ↓ expression of MMP-9	106
EGCG	MC3T3-E1 cells	10−100 μmol/L	60 min	↓ ET-1-induced IL-6 synthesis via ↓ p44/p42 MAPK pathway	158
EGCG	MC3T3-E1 cells	100 μmol/L	60 min	↓ FGF-2-stimulated IL-6 synthesis via ↓ p44/p42 MAPK pathway	159
EGCG	MC3T3-E1 cells	30 μmol/L	60 min	↓ PGDF-BB-stimulated IL-6 synthesis via ↓ SAPK/JNK expression	160

Abbreviation: ALP, alkaline phosphatase activity; DC, dendritic cells; EGCG, (−) epigallocatechin gallate; ET-1, endothelin-1; FGF, fibroblast growth factor; HSP27, heat shock protein 27; IL-6, interleukin-6; NF-κB, receptor activator of nuclear factor-κB; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinases; PGD_2, prostaglandin D_2; ; PGDF-BB, platelet-derived growth factor-BB; PTH, parathyroid hormone; Runx2, Runt-related transcription factor-2; SAPK/JNK, stress-activated protein kinase/c-Jun N-terminal kinase; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; RANKL, receptor activator of nuclear factor-κB.