

## Bone mineral density, polyphenols and caffeine: a reassessment

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Several studies have shown beneficial associations between tea consumption and bone mineral density (BMD) and fracture risk. Current investigations into potential mechanisms of benefit are focused upon the F and polyphenol components of tea. However, previous studies have pointed towards caffeine consumption as a potential risk factor for low BMD and high fracture risk. Tea, therefore, represents an interesting paradox as a mildly caffeinated beverage that may enhance bone health. Fruit and vegetable intake has also been associated with BMD, and it is now apparent that several fruit and vegetable components, including polyphenols, may contribute positively to bone health. Evidence surrounding the function(s) of polyphenol-rich foods in bone health is examined, along with more recent studies challenging the relevance of caffeine consumption to *in vivo* Ca balance. Plant foods rich in polyphenols such as tea, fruit and vegetables, as significant factors in a healthy diet and lifestyle, may have positive roles in bone health, and the negative role of caffeine may have been overestimated. The present review covers evidence of dietary mediation in positive and negative aspects of bone health, in particular the roles of tea, fruit and vegetables, and of caffeine, flavonoids and polyphenols as components of these foods. Since the deleterious effects of caffeine appear to have been overstated, especially in respect of the positive effects of flavonoids, it is concluded that a reassessment of the role of caffeinated beverages may be necessary.

### Bone health: Flavonoids: Polyphenols: Caffeine

#### Introduction

In an ageing Western society, the treatment of age-progression diseases (cancer and heart disease, for example) has become a major issue. Similarly, the maintenance of good bone health with age becomes increasingly important. In osteoporosis, bone becomes increasingly porous, resulting in both greater chance and severity of bone fracture at the hip, spine, forearm and shoulder<sup>1</sup>. Bone fractures can mean reduced mobility, discomfort and a higher risk of early mortality. Osteoporosis can also pressure society as a whole, with the estimated annual cost to the UK of hip fracture alone exceeding £1.7 billion<sup>2</sup>. The sum is likely to increase as life expectancy increases and the medical options extend. Elderly women are the prime demographic group at risk from osteoporosis, because they can lose between 10 and 15% of their bone every 10 years after the menopause<sup>3</sup>. The reasons for this are that women tend to have lower peak bone mass than men, and that levels of oestrogen (a hormone with a positive effect on bone health) are decreased during and after menopause. However, the consequences of the hormonal changes occurring during

pregnancy and because women tend to live longer than men are factors that complicate the issue.

Bone tissue is in a constant state of flux. As well as the various mechanical roles performed by the skeleton (Table 1), it must also act as a Ca depository for the rest of the body, with Ca being removed and replaced as required. The state of bone flux within an individual can be described in terms of bone mineral density (BMD). Bone metabolism is controlled by a variety of growth hormones, sex steroid hormones (such as oestrogens), thyroxine, corticosteroids and insulin. However, three hormones play vital roles – 1,25-dihydroxycholecalciferol, parathyroid hormone and calcitonin<sup>4</sup>. As well as affecting dietary Ca adsorption efficiencies, these hormones also influence the three cell types relevant to bone formation and metabolism (Fig. 1): osteoblasts (bone formation), osteocytes (bone maintenance) and osteoclasts (bone resorption). Imbalances in bone metabolism can cause either improper bone formation or accelerated bone loss<sup>5</sup>. The balance between the formation and resorption of bone tissue is affected by genetic and environmental (for example, diet and lifestyle) factors.

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**Abbreviations:** BMC, bone mineral concentration; BMD, bone mineral density; EGCG, epigallocatechin gallate; FN, femoral neck; HRT, hormone replacement therapy; LS, lumbar spine; ppm, parts per million.

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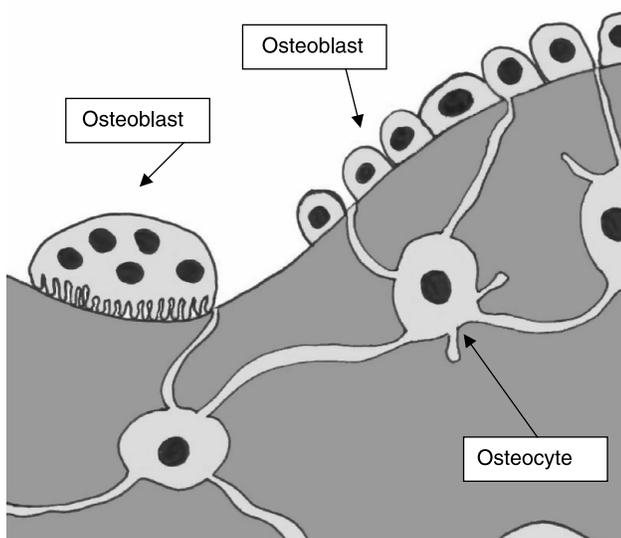
**Table 1.** Roles of calcium and calcium balance in bone health

	Approximate distribution of total body Ca (%)	Typical roles of Ca <i>in vivo</i>
Bone tissue (skeleton, etc)	99	Supports and protects other organs Site for blood cell production Combines with muscle and connective tissue to enable mobility
Rest of body	1	A wide range of cellular reactions, including: Blood clotting Neurotransmitter secretion Normal nerve conduction Neuromuscular system and muscle contraction Some enzyme-mediated reactions Structural role in organelles and membranes

The importance of diet to bone health is well established. However, recent evidence from both epidemiological and experimental studies would suggest that links between diet and bone health go beyond the scope of adequate nutrition (i.e. sufficient Ca and vitamin D), with non-nutrient food components (for example, polyphenols) also playing a role. It is not within the scope of the present review to provide an all-inclusive detailing of the field of diet and bone health. Indeed throughout the text, where appropriate, the reader is directed to comprehensive works of other authors. It is the purpose of the present review, rather, to highlight the evidence indicating a need for a reassessment of traditional perceptions surrounding tea, caffeine, fruit and vegetables with regard to bone health.

### Tea and bone health

A growing body of evidence suggests that tea consumption might be beneficial with regard to BMD.



**Fig. 1.** Principal cell types relevant to bone formation and metabolism: osteoblasts (bone formation), osteocytes (bone maintenance) and osteoclasts (bone resorption).

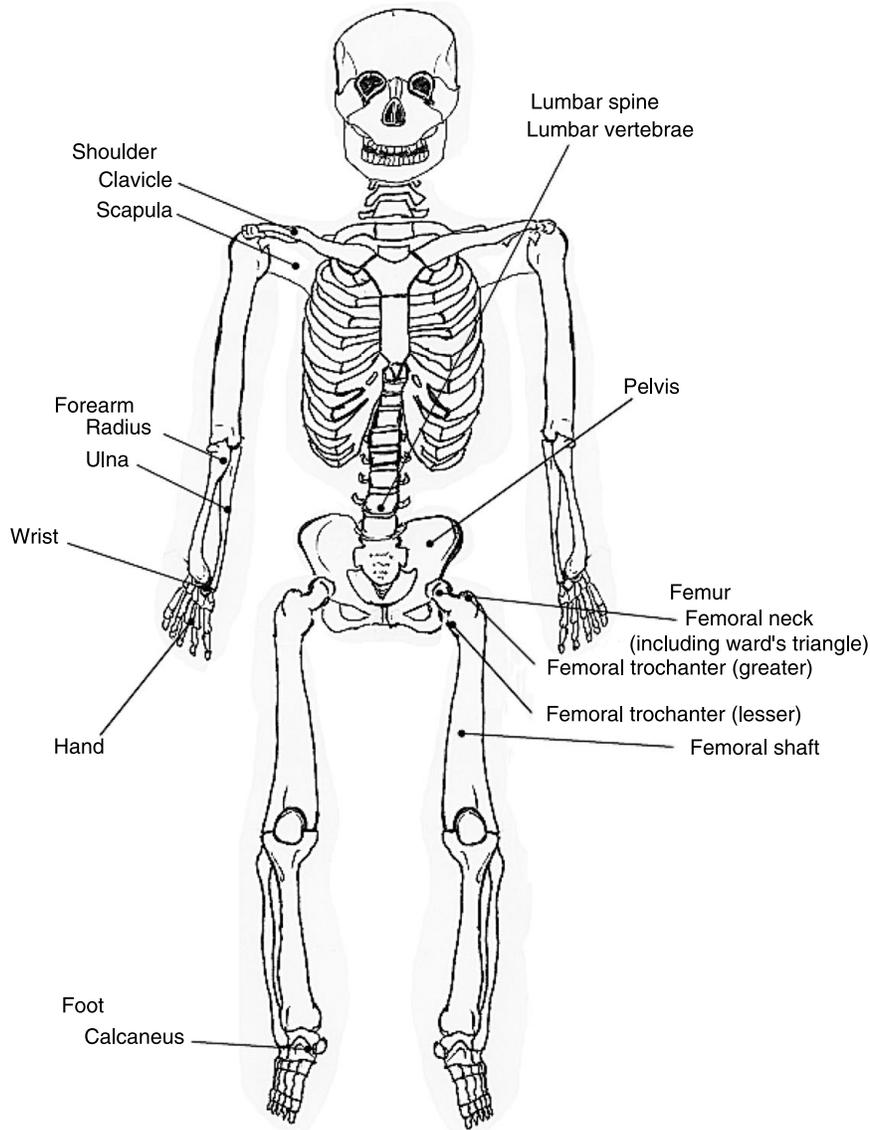
### Epidemiological studies

Several studies have been performed showing the association between tea drinking and BMD. For example in the Mediterranean Osteoporosis Study (MEDOS), Johnell *et al.*<sup>6</sup> found that the consumption of tea was associated with a significantly decreased risk of fracture in a cohort of women aged over 50 years from southern Europe. Whilst in the UK and Ireland black tea is frequently consumed with milk, meaning that tea drinkers have increased Ca intake, in most other countries (including those of the MEDOS cohort), black tea is consumed without milk<sup>7</sup>. It is therefore possible that tea may benefit bone health beyond the provision of extra Ca from added milk.

In studying a cohort of sixty-two postmenopausal women who had not received hormone replacement therapy (HRT), Hoover *et al.*<sup>8</sup> found that of all dietary factors measured tea consumption was most strongly (and positively) related to lumbar and femoral (Fig. 2) bone density, whilst Ca intake and absorption failed to show a statistically significant relationship to overall bone density (once adjusted for relationships between bone density and number of years since menopause, body weight and lean body mass), although a significant relationship did persist when femoral BMD was taken alone.

Similarly, a study of 1256 British women aged between 65 and 76 years<sup>9</sup> found that BMD at all sites tested was considerably higher in tea drinkers compared with non-tea drinkers. The relationship remained significant in three out of four sites after adjustment for factors such as BMI and age, and was independent of smoking status, use of HRT, coffee consumption and the addition of milk to tea (except at the greater trochanter, where BMD values were higher in those that added milk to tea compared with black tea drinkers and non-tea drinkers). However, the relationship between tea consumption and BMD was independent of the volume of tea consumed on a daily basis. Less than 10% of the cohort were non-tea drinkers, and so it is possible that this relatively small sub-population was not representative of non-tea drinkers in the wider UK population in terms of BMD. Coffee was consumed by 81% of tea drinkers and 88% of non-tea drinkers. No significant relationship between tea consumption and coffee consumption was found.

In addition to the evidence above, it should also be remembered that the amount of milk added to tea is often very small. Typically, 25 ml (whole) milk is present in a 190 ml cup of tea<sup>10</sup>. Given whole milk has an average



**Fig. 2.** Common skeletal regions pertinent to bone-health research.

Ca content of 120 mg per 100 ml<sup>11</sup>, one cup of tea provides just 30 mg Ca – just over 4% of the 700 mg reference nutrient intake for men and women. Tea is therefore unlikely to be a significant dietary source of Ca except in those with particularly poor diets, or unless copious volumes are consumed daily.

It is possible that the BMD-related advantages of tea drinking are cumulative, with benefits being proportional to habit length. For example, in a study of 1037 men and women aged over 30 years<sup>12</sup>, those that had habitually drunk tea for 6–10 years had significantly higher lumbar spine (LS) BMD compared with non-habitual tea drinkers, whilst those with a habit exceeding a decade had higher BMD at four separate sites compared with other groups. The relationship held after adjustment for other confounding factors, with tea consumption predicting 0.5–5.1% variation in BMD. No significant difference in BMD was shown between non-habitual drinkers and those with 1–5

years of habit. No significant difference could be found between those drinking black tea (9%) and those drinking green or oolong tea (91%). However, it was suggested that the number of black tea drinkers in the study may have been too small to significantly represent BMD. Importantly, the study used a group in which only ninety-six individuals out of 502 habitual tea drinkers (19%) added milk to tea.

It is also possible that various types of tea may confer beneficial effects on BMD. Muraki *et al.*<sup>13</sup> showed in a study of 655 Japanese women over the age of 60 years that consumption of green tea on >5 d/week was associated with a significantly higher BMD compared with those consuming green tea less frequently. The relationship was independent of biometric confounding factors, diet, exercise, and use of tobacco, alcohol and treatments for osteoporosis. However, as 91.6% of women studied fell into the high consumption classification, it is possible that infrequent tea drinkers were under-represented.

Table 2. Polyphenol and caffeine contents of caffeinated beverages

Beverage	Predominant polyphenol group(s)	Caffeine content (mg/l)	Caffeine per typical serving (mg)	Reference
Tea				
Black	Flavonoids: flavan-3-ols (including catechins, theaflavins, thearubigins); flavanols (including quercetin); flavones	177–303	29.2–50 (190 ml cup, including milk)	Peterson <i>et al.</i> (2005) <sup>*94</sup> ; Astill <i>et al.</i> (2001) <sup>†95</sup>
Green	Flavonoids: flavan-3-ols (catechins); flavanols (including quercetin); flavones	40–211	8–40.1 (190 ml cup, water only)	Peterson <i>et al.</i> (2005) <sup>*94</sup> ; Astill <i>et al.</i> (2001) <sup>†95</sup>
Coffee				
Foast and ground	Phenolic acids: chlorogenic acids; ferulic acids	600 (average)	99 (190 ml cup including milk)	Clifford (1999) <sup>*96</sup> ; Nebesny & Budryn (2003) <sup>*97</sup> ; Debry (1994) <sup>†98</sup>
Instant		420 (average)	69 (190 ml cup including milk)	
Carbonated soft drinks		81.5–171	26.9–56.4 (330 ml can)	Pena <i>et al.</i> (2005) <sup>†99</sup>
Cola (diet and regular)		21–2175	5.3–543.8 (250 ml can)	Pena <i>et al.</i> (2005) <sup>†99</sup>
'Energy' drinks	Variable – functional beverages may include polyphenols			

\* Polyphenol references.

† Caffeine references.

In a study of 2016 postmenopausal women<sup>14</sup> tea consumption (unspecified type) was shown to have a protective effect in the femoral neck (FN) region, but not the spinal region. However, an inverse relationship between tea and coffee intake ( $r = -0.38$ ;  $P < 0.001$ ) was also found. Chen *et al.*<sup>15</sup> found a similarly inverse trend between tea and coffee drinking in a cohort of postmenopausal women from the USA. Therefore as Vestergaard *et al.*<sup>14</sup> suggest, the apparent benefits of tea consumption may simply be an index of low coffee (and thus lower caffeine) intake (Table 2).

Not all epidemiological studies show direct associations between tea consumption and bone health. For example, Chen *et al.*<sup>16</sup> found that tea drinking was not significantly associated with BMD in postmenopausal women from the USA. However, tea consumption was positively related to total body and spinal BMD in women who either used HRT or had used HRT in the past, leading to the suggestion that HRT and tea consumption may have a synergistic effect upon BMD. It is possible, however, that women receiving HRT are associated with different lifestyles to those not undergoing treatment and tea consumption and HRT may not be interacting directly. In a later study, Chen *et al.*<sup>15</sup> found that whilst tea consumption was significantly and positively related to total body BMD ( $P < 0.05$ ) in a subgroup of 4979 women from the USA (aged 50–79 years), Cox proportional hazard models (accounting for various confounding factors) showed no significant relationship between tea consumption and risk of hip or wrist or forearm fracture in the overall cohort of 91465 women. Chen *et al.*<sup>15</sup> concluded that the effect of habitual tea drinking upon BMD was small and did not affect the risk of fracture in the population studied.

#### Putative mechanisms relating tea to bone mineral density

It is likely that the benefits to bone health of tea consumption go beyond the provision of extra Ca from added milk. Therefore, the effects of tea upon BMD are the result of one or more components of tea infusion. Whilst the mechanism(s) for such effects remain to be established, several have been proposed.

**Fluorine and positive bone health.** F occurs as salts (fluorides) *in vivo*, becoming incorporated into bone mineral by substituting for the hydroxyl group in hydroxyapatite to form fluorapatite, thus increasing overall stability<sup>17</sup>. Fluoride has also been shown to stimulate the formation of new bone and can inhibit or reverse dental carries, and fluoride has been used either alone or combined with Ca, vitamin D or oestrogen to treat osteoporosis<sup>18</sup>. Most fresh foods contain just 0.01–1 parts per million (ppm) fluoride<sup>18</sup>, though fish and tea are exceptions. Whilst no RDA value for fluoride intake has been suggested, 10% of UK drinking water is fortified with fluoride to a level of 1 ppm, primarily to benefit dental health.

In a study of several teas, Fung *et al.*<sup>19</sup> reported a variety of fluoride contents for different tea types infused in double-distilled water at 1% (w/v) for 5 min (Table 3). From the results of Fung *et al.*<sup>19</sup> the average fluoride content of black teas from Sri Lanka (Twinings) and the UK (Lipton and Rickshaw) was 0.93 mg/l tea liquor (reaching 1.5 mg/l upon

**Table 3.** Fluorine content of 1% tea infusions (adapted from Fung *et al.*<sup>19</sup>)

Tea type	Region or country of origin	Dissolvable F in tea liquor (mg/l)
Chinese green	Zhejiang	1.22
	Guangdong	1.20
	Guangdong	1.18
Chinese oolong	Guangdong	0.82
	Yunnan	0.59
	Yunnan	0.58
Chinese pureh	Yunnan	0.90
	Yunnan	1.13
	Yunnan	0.94
Chinese black	Guangdong	1.02
	Yunnan	1.25
	Yunnan	1.35
Twinnings (black)	Sri Lanka	0.91
Lipton (black)	England	0.94
Rickshaw (black)	England	0.93
Chinese brick	Hunan	2.28
	Hunan	2.18

infusion for 360 min), which equates to 0.15 mg fluoride per cup. Similarly, Duckworth & Duckworth<sup>20</sup> investigated the F contents of tea samples taken from fifty selected UK households. On each of 3 consecutive days, consumption data (via questionnaire) and an infused tea sample were obtained. Fluoride concentrations of tea samples (made with drinking water containing <0.15 ppm fluoride) varied widely from 0.44 to 2.78 mg per litre tea liquor (0.07–0.46 mg/cup). The authors calculated that daily intake of fluoride from tea ranged from 0.04 to 2.71 mg/d, and that daily intake increased with age (mean daily intakes ranged from 0.3 mg for those below 7 years, to 0.85 mg for those above 60 years). However, the authors stated that the small size of each age group (the study used 213 subjects in total) denies any extrapolation of consumption trends to the greater UK population.

**Fluorosis.** However, the dose gap between activity and toxicity for fluoride is narrow<sup>17</sup> and excessive consumption of fluoride can lead to a condition termed 'fluorosis'. Doses in excess of 8 mg/d<sup>17</sup> can lead to bone diseases such as osteosclerosis (an abnormal hardening or increased density of bone) and osteoporosis, and calcification of ligaments and tendons resulting in joint pain, stiffness, muscle impairment and eventual abnormalities of the spine, legs and arms<sup>21</sup>.

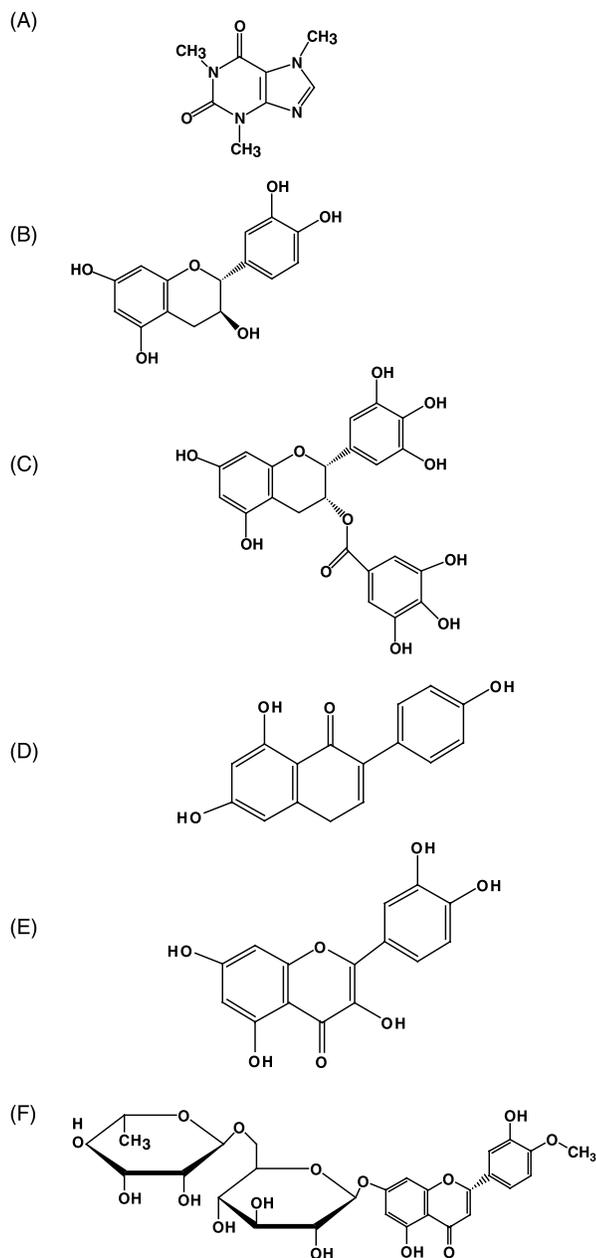
Tea drinking has been associated with fluorosis in certain geographical areas of the world. For example, Cao *et al.*<sup>22</sup> reported that consumption of 'brick tea' (low-quality tea leaves, pressed into blocks) was the major cause of fluorosis in the Tibetan province of Naqu County. Foods containing brick tea accounted for 99% of the daily intake of fluoride (reaching 12 mg in adults), with 89% of the 111 randomly sampled adults (male and female, aged 30–78 years) studied showing clinical symptoms of fluorosis. Similarly, Zhang *et al.*<sup>23</sup> found that bone mass was significantly lower at the calcaneus (measured using quantitative ultrasound analysis) and in both the dominant and non-dominant hands (measured using metacarpal cortical index) in women from a grassland area (*n* 38) compared with those in an urban area (*n* 46) within Inner Mongolia. High fluoride concentrations

of brick tea (2.61–10.87 ppm) were significantly correlated to dominant and non-dominant hand metacarpal cortical index in the grassland group. In an earlier paper Cao *et al.*<sup>24</sup> stated that educating populations with a long history of brick tea consumption may do little to change their consumption habits, suggesting instead that the provision of a low-fluoride substitute tea may be a better approach to reducing the occurrence of fluorosis. Rats given a low-fluoride brick tea rather than a normal brick tea (210 mg soluble fluoride per kg dried tea *v.* 503.5 mg/kg respectively), were observed to have no signs of dental fluorosis after 1 year, compared with a 75% incidence in the group consuming normal brick tea. The authors suggested that a low-fluoride brick tea is likely only to prevent development of fluorosis rather than reverse the condition.

Associations between tea and fluorosis are mainly restricted to the consumption of brick tea in areas where the diets of the populations are much more restricted than those of Western populations. For example, the Tibetans living at high altitude in the study of Cao *et al.*<sup>22</sup> did not have access to vegetables, gaining much of their energy from buttered tea and zamba (tea mixed with fried highland barley flour). However, an incident of skeletal fluorosis has recently been linked to high consumption of instant teas in the USA<sup>25</sup>, in which four out of ten preparations tested had fluoride levels exceeding the 2.4 ppm upper limit for bottled beverages (as set by the US Food and Drug Administration), with one preparation exceeding the 4 ppm safety limit for drinking water as set by the US Environmental Protection Agency. Nevertheless, whilst excessive consumption of tea will always bring the risk of fluorosis, it is suggested that the risk to Western populations is relatively small.

**Flavonoids.** Tea is a rich source of flavonoids which may benefit bone density both through the inhibition of bone resorption and the stimulation of bone growth. The predominant flavonoids within tea are catechins. Green tea typically contains 32–40% catechins by dry weight, whilst black tea contains 10–12% catechins<sup>26</sup>. Epigallocatechin gallate (EGCG) is the major catechin, accounting for between 9 and 13% of green tea on a dry weight basis. Tea (both green and black) also contains significant quantities of flavonols, methylxanthines (for example, caffeine), organic acids and volatiles. It contains a number of complex polyphenols such as thearubigins and theaflavins, and is also the only known source of the non-essential amino acid theanine. Several studies concerning the use of tea flavonoids as potential therapeutic agents for osteoporosis have been performed. Fig. 3 shows the structures of certain tea flavonoids and other relevant polyphenols.

**Flavonoids: effects on bone health.** Catechins have been shown to affect bone metabolism both *in vitro* and *in vivo*. For example, pre-treating calvaria (from embryonic mice) with (+)-catechin (0.1–1 mM) resulted in a dose-dependent inhibition of bone resorption after addition of parathyroid hormone *in vitro*<sup>27</sup>. In the same study, a similar pre-treatment with (+)-catechin (0.8 mM) was shown to inhibit bone resorption caused by the addition of PG E<sub>2</sub> or retinoic acid. Addition of (+)-catechin (0.8 mM) to calvaria



**Fig. 3.** Structures of particular compounds pertinent to bone health. (A) Caffeine (an alkaloid found in coffee, tea and mate); (B) catechin (a flavan-3-ol found in tea); (C) epigallocatechin gallate (a flavan-3-ol found in tea); (D) genistein (an isoflavone found glycosylated in soya and soya products along with related phyto-oestrogens daidzein, glycitein, formononetin and biochanin A); (E) quercetin (a flavonol found glycosylated in onions, tomatoes and tea); (F) hesperidin (hesperetin 7-rhamnoglucoside, a flavanone glycoside found in citrus fruits).

pre-treated with either parathyroid hormone or retinoic acid was also shown to inhibit resorption of bone.

Polyphenols (including catechin, epicatechin and EGCG) were shown to influence the proliferation of osteoblasts in cell culture *in vitro* over a wide range of concentrations (0.001 nM to 10  $\mu$ M). At low concentration, osteoblast proliferation was increased. The polyphenols tested also stimulated the generation of osteoclasts at low concentration.

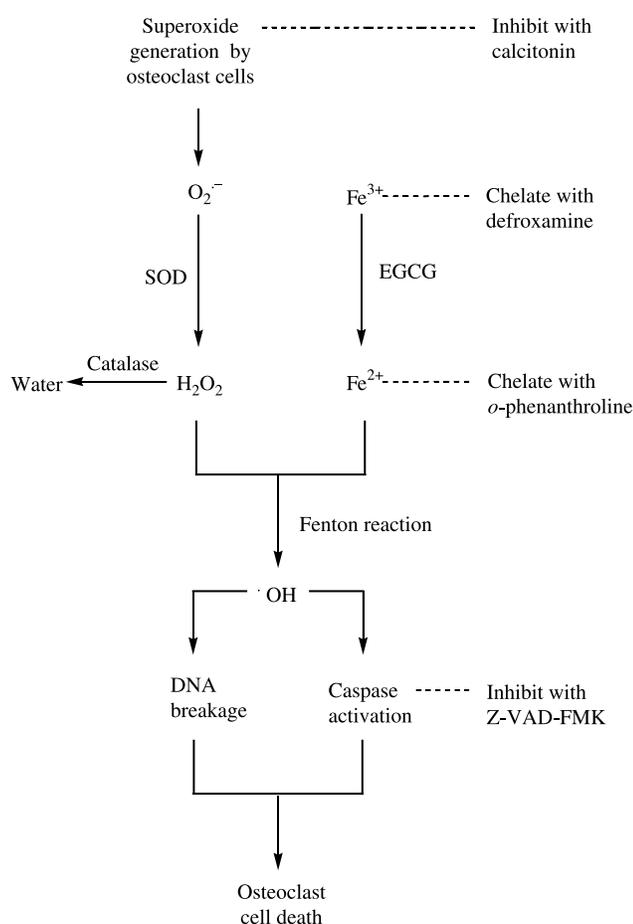
However, upon increasing polyphenol concentration, catechin and EGCG were shown to decrease osteoclast generation<sup>28</sup>. In the same study, green tea extracts (0.0016–0.2  $\mu$ l/ml) were also shown to inhibit the generation of osteoclasts in cell culture in a dose-dependent manner and increased the proliferation and activity of osteoblasts at low concentrations (although proliferation and activity were decreased at higher concentrations).

Similarly, various catechins were shown to reduce levels of osteoclast cells without affecting levels of osteoblasts *in vitro*<sup>29</sup>. The most potent of the catechins was EGCG, which induced apoptosis in over 90 % of osteoclasts when present at 100  $\mu$ M. Apoptosis was shown to be induced by increasing protease (specifically caspase) activity. However, as the addition of a synthetic pan-caspase inhibitor (z-Val-Ala-Asp-xxfluoromethyl ketone) only partially suppressed EGCG-mediated apoptosis but completely inhibited the activation of caspase, it was suggested that other pathways to EGCG-mediated cell death may exist. Nakagawa *et al.*<sup>29</sup> went on to show that the EGCG-mediated activation of caspase was through the reduction of Fe (III) to Fe (II), which could in turn take part in the Fenton reaction, thus increasing oxidative stress (Fig. 4).

Catechin has also been shown to increase the viability of osteoblastic MC3T3-E1 cells in a dose-dependent manner over a range of 1 nM–1 mM<sup>30</sup>. Treatment with catechin (0.1 mM) increased alkaline phosphatase activity (an indicator of osteoblastic activity) and reduced osteoblastic apoptosis induced by the addition of 0.001  $\mu$ M-TNF- $\alpha$ . Interestingly, catechin was also shown to reduce the secretion of cytokines involved in osteoclast formation and bone resorption (TNF- $\alpha$  and IL-6) by MC3T3-E1 cells.

In rats exposed to Cd over 20 weeks (in order to increase bone resorption and thus decrease BMD<sup>31</sup>), urinary deoxypyridinoline (an index of bone resorption):creatinine ratios were increased in those consuming a catechin-free diet, or a diet containing just 0.25 % catechin. However, in rats fed a diet containing larger doses of catechin (0.5 %), urinary deoxypyridinoline:creatinine ratios were similar to that of the control group. Supplementation with catechin resulted in rises of total BMD values similar to that of the control group, with non-supplemented rats showing a 10 % lower rise in BMD. Total bone mineral concentration (BMC) increases were 60 % lower in the non-supplemented group, with catechin-supplemented rats experiencing similar rises in BMC values to the control group in all bone types tested except for vertebra (which were still lower than control). Total bone Ca showed similar trends to BMC. It was thus concluded that catechin normalised BMD, BMC and bone Ca content in rats poisoned with Cd. In contrast, Takita *et al.*<sup>32</sup> found that EGCG implanted into rats caused increased cartilage formation and decreased bone formation. EGCG was shown to decrease angiogenesis and osteogenesis but increase chondrogenesis.

The tea flavonoid rutin and its aglycone quercetin (Fig. 3) have also been shown to modulate the activity of bone cells *in vitro* and *in vivo*. During an 11 d incubation, both rutin and quercetin (10 nM) significantly inhibited the formation of osteoclasts in porcine bone marrow cells treated with 1,25-dihydroxyvitamin D<sub>3</sub> (10 nM)<sup>33</sup>. In the same study, a 48 h treatment of 11 d old osteoclast cells with rutin or



**Fig. 4.** Proposed mechanism of epigallocatechin gallate (EGCG)-mediated cell death in osteoclasts (adapted<sup>29</sup>). EGCG promotes the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ .  $\text{Fe}^{2+}$  combines with  $\text{H}_2\text{O}_2$  (converted from superoxide by superoxide dismutase (SOD)) in the Fenton reaction. Resultant hydroxyl radicals then cause DNA breakage and activation of caspase, both causing the death of osteoclast cells. Z-VAD-FMK, benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone.

quercetin (10 nM) was also shown to significantly reduce bone resorption. Wattel *et al.*<sup>34</sup> also showed quercetin to be a potent inhibitor of osteoclastic activity in cells prepared from rabbit bone (50% inhibitory concentration 1.6  $\mu\text{M}$ ). Upon further analysis using purified cells, it was found that the flavonols induced osteoclastic apoptosis in a dose-dependent manner. However, Notoya *et al.*<sup>35</sup> reported that quercetin also inhibited the proliferation, differentiation and mineralisation of rat calvarial osteoblast-like cells *in vitro*. It would appear that further *in vivo* research is therefore required to better determine the role(s) of quercetin and its metabolites in human bone health.

The addition of rutin (at 0.25%) to the diet of rats for 90 d was shown to prevent femoral trabecular bone loss following ovariectomy<sup>36</sup>. Furthermore, urinary excretion of deoxyypyridinoline (an index of bone resorption) and Ca were increased in ovariectomised rats compared with rutin-fed and control rats, whilst plasma levels of osteocalcin were increased in rutin-fed rats compared with control rats, suggesting that osteoblastic activity was stimulated by rutin.

**Flavonoids: tea polyphenols and direct antioxidant activity.** Tea polyphenols may also promote bone health through direct antioxidant action. For example, a mixture of polyphenols (mainly comprising a variety of catechins) extracted from green tea was shown to minimise significantly reductions in the viability of osteoblasts (isolated from neonatal Sprague–Dawley rat calvariae) *in vitro* as a result of oxidative stress<sup>37</sup>. Addition of  $\text{H}_2\text{O}_2$  (100 mmol/l) to cells for 24 h resulted in a reduction in viability of approximately 85% assessed using flow cytometry analysis and an approximate 90% reduction assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. However, pre-treatment of cells with a green tea polyphenol mixture (200  $\mu\text{g}/\text{ml}$ ) for 1 h at 37°C resulted in reductions in viability of approximately 25 and 40% as assessed by flow cytometry analysis and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide respectively. Park *et al.*<sup>37</sup> also repeated the experiment using a xanthine oxidase–xanthine system as a free radical generator. Using enzyme (40 U/l) plus 250  $\mu\text{M}$  of substrate gave reductions in osteoblast viability of approximately 50 and 55% as assessed by flow cytometry analysis and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide respectively, compared with equivalent reductions of approximately 20 and 5% observed in green tea polyphenol-treated cells.

In reviewing the potential of tea to benefit BMD, Wu *et al.*<sup>12</sup> also noted that various studies show that flavonoids within tea may have the potential to alter mineral metabolism. Such behaviour may be direct or indirect in nature. Which of the mechanisms have significant effects *in vivo* remains to be established. It is also important to note that, as Wu *et al.*<sup>12</sup> have suggested, some or all of these mechanisms might work independently or in synergy.

#### Caffeine and bone health

Besides being flavonoid-rich, tea is also mildly caffeinated (Table 2). Several epidemiological studies have shown a correlation between consumption of high-caffeine beverages and low BMD (see below). Therefore tea may represent an interesting paradox, in that it is a caffeinated beverage that appears to benefit bone health. Research into the mechanisms by which caffeine might affect BMD has yielded inconclusive results, so any potential negative effects of caffeine upon bone health remain highly controversial.

**Epidemiological studies.** Several epidemiological studies show caffeine consumption as a potential risk factor for low BMD and fracture. Yano *et al.*<sup>38</sup> found that Ca, milk, vitamin C and vitamin D intake were positively associated with bone mineral content after adjustment for biometric confounding factors, and the use of thiazide (a diuretic used to treat hypertension), history of non-violent fracture and strenuous exercise (males) and use of oestrogen (females) in a cohort of 2120 elderly residents of the USA of Japanese origin. The strength of the association was sex- and site-dependent. However, caffeine consumption was associated with a low bone mineral content at the distal radius and ulna regions in women. In the Framingham study<sup>39</sup>, a cohort of

3170 men and women from the USA were examined every 2 years over a 12-year period, with relationships being adjusted for confounding factors (such as smoking, alcohol consumption, use of oestrogen and biometric data) but not others (such as Ca intake, exercise and use of medication promoting bone loss). A significant increased relative risk of hip fracture (1.53%) over the 2-year period following examination was found for those with caffeine consumption in excess of the equivalent of two cups of coffee per d. Furthermore, it was found that those who changed from a high to low caffeine consumption reduced their relative risk of hip fracture.

Along with mechanistic data (see section on 'Putative mechanisms for the effects of caffeine upon bone mineral density'), such studies have combined to form the traditional assertion that to ensure good bone health, caffeine consumption should be limited. More recent studies, however, have brought the relevance of caffeine consumption to bone health into question (see Table 4). For example, Massey<sup>40</sup> and Nawrot *et al.*<sup>41</sup> point out that caffeine consumption is often associated with confounding factors such as an increased level of smoking, age, low socio-economic status and a decreased Ca intake through the displacement of milk as a beverage. Several studies have found that after adjustment for such factors, relationships between caffeine consumption and low BMD become insignificant<sup>42–44</sup>.

Where relationships between caffeine consumption and bone health persist, the effects of caffeine may be mediated by various factors including age<sup>45</sup>, genotype<sup>46</sup> and Ca intake<sup>47–49</sup>. Furthermore, despite Ca being the foremost mineral in bone, several studies have shown little or no association between Ca intake and BMD in the elderly<sup>50–52</sup>. Yano *et al.*<sup>38</sup> also found that nutrients had a minimal effect upon bone status in the elderly compared with known confounding factors. However, other studies have shown relatively high Ca intake in early life to be related to high BMD and lower risk of fracture in later life<sup>6,53</sup>.

Yano *et al.*<sup>38</sup> also suggest that lifetime dietary habits may be more important for attaining a high peak bone mass than current dietary habits in the elderly. As many diet–BMD studies focus upon elderly cohorts, it is important to factor such information into study designs.

*Putative mechanisms for the effects of caffeine upon bone mineral density.* Several mechanisms for the effects of caffeine upon BMD have been suggested, including the alteration of the absorption efficiency of dietary Ca *in vivo*<sup>54</sup> and the alteration of sex steroid levels *in vivo*<sup>55</sup>. However, a further suggestion that has received considerable attention is based on the calciuretic effect of caffeine. For example, caffeine consumption was shown to increase the urinary excretion of Ca significantly in 168 premenopausal women to such an extent that a 50% increase over the group mean was predicted to produce a shift in Ca balance of  $-0.006 \text{ g/d}$ <sup>56</sup>. Yeh & Aloia<sup>57</sup> also found that increased dietary caffeine significantly promoted urinary Ca loss in both old and young rats (although intestinal absorption of Ca was increased in the young rats to compensate).

The relevance of the calciuretic effect to Ca balance is, however, questionable. For example, Chen & Whitford<sup>58</sup>

observed that whilst urinary Ca excretion was directly proportional (and faecal fluoride excretion was indirectly proportional) to caffeine intake in rats (fed over 6 weeks), the metabolic balance of fluoride and Ca were not significantly affected and could not be related to either an observed reduction in mineral content of the tibia or to a reduced ash content of the femur epiphysis in the group with the highest caffeine consumption. It was therefore concluded that in rats even high concentrations of caffeine had no measurable effect upon the metabolic balance or tissue concentration of fluoride, Ca or P. Similarly, in a study of the effects of carbonated beverages upon urinary Ca in women aged 20–40 years<sup>56</sup>, of several beverages tested only caffeine-containing beverages significantly increased urinary excretion of Ca. However, the increase in Ca excretion over the 5 h post-ingestion period measured was relatively small (4–14 mg). Furthermore, the authors noted that the calciuretic effect of caffeine was biphasic (i.e. calciuria was reduced later in the day, compensating for overall losses), leading Heaney & Rafferty<sup>56</sup> to conclude that the effects of carbonated beverages upon Ca balance were minor, and that epidemiological associations between consumption of carbonated beverages and low BMD were more likely to arise from the displacement of milk from the diet.

Other researchers have also observed the absence of a significant calciuretic effect when performing feeding studies with caffeine-containing beverages. For example, Sakamoto *et al.*<sup>59</sup> showed that the only difference between rats fed on a diet containing instant coffee (at 0.62 and 1.36%, equivalent to a human consumption of nine and twenty cups/d respectively) or a coffee-free diet was that urinary P excretion was increased in the former group (probably due to the P content of coffee). They showed that caffeine consumption did not lead to bone resorption, did not affect osteoclast levels or the production of bone-resorbing cytokines and did not affect levels of urinary deoxyypyridinoline or serum osteocalcin, both of which are indicators of bone resorption and formation.

It is also possible that caffeine-containing beverages may contain other components that affect bone health. In a study by García-Contreras *et al.*<sup>60</sup> ovariectomised rats given cola-based drinks for 2 months had significantly lower femoral BMD and Ca concentration (although LS and pelvic BMD was unaffected by cola-based drink consumption) than rats given drinking water. Paired eating experiments showed that the effect was independent of food intake. Whilst citing the caffeine content of beverages as one of several potential contributors, García-Contreras *et al.*<sup>60</sup> stated that the most likely cause of the observed effects may have been due to the high phosphoric acid content of cola-based drinks, as high phosphate consumption inhibits the absorption of Ca from the intestine, and renal tubular reabsorption.

*The significance of caffeine to bone mineral density: conclusions.* For a more detailed discussion of the effects of caffeine upon BMD, the reader is directed to a comprehensive review by Heaney<sup>61</sup>. As Massey<sup>62</sup>, Heaney<sup>61</sup> and Nawrot *et al.*<sup>41</sup> point out, the association between caffeine consumption and other confounding factors (smoking, age, Ca intake) makes it difficult to assess the significance of any impact of

**Table 4.** Summary of three selected groups of studies challenging current views on caffeine and bone mineral density (BMD)

Reference	Population	Results	Comment
Conlisk & Galuska (2000) <sup>44</sup>	Caucasian young adult women ( <i>n</i> 177)	Caffeine intake was associated with lower BMD. After correction for confounders (including smoking, alcohol and Ca intake), caffeine consumption (over the range consumed) was not a significant predictor of BMD	*
Johansson <i>et al.</i> (1992) <sup>42</sup>	Swedish men and women aged 70 years ( <i>n</i> 619)	Coffee drinking was associated with lower BMD, deteriorated dental state, high tobacco use and low socio-economic status. After correction for confounders the correlation became insignificant. Whilst high BMD was associated with a high socio-economic status, coffee drinking was an indicator of both low socio-economic status and tobacco use	*
Lloyd <i>et al.</i> (1997) <sup>43</sup>	Caucasian postmenopausal women aged 55–70 years ( <i>n</i> 138). Participants had never undergone HRT or had only used HRT for less than 1 year	No association between caffeine intake and bone density was found. Current and previous smoking and alcohol consumption habits were included in the exclusion criteria, with all participants being non-smokers consuming one or less alcoholic drink per d. No association between caffeine intake and either total bone density or femoral neck bone density was found, even for those in the lowest tertile of Ca intake	*
Cooper <i>et al.</i> (1992) <sup>45</sup>	Caucasian women, aged 40–80 years ( <i>n</i> 290)	Caffeine consumption had no significant correlation with bone mineral at five out of six sites tested. High caffeine consumption was associated with a high BMD at the femoral shaft in women under 60 years, but a slightly lower BMD in those aged 60 years and above	†
Rapuri <i>et al.</i> (2001) <sup>46</sup>	Postmenopausal women (489 in cross-sectional baseline study, ninety-six in longitudinal study) aged 65–77 years	After a 3-year follow-up (longitudinal study), BMD at the spine was lost at a higher rate in participants consuming more than 300 mg caffeine/d, compared with those with a lower caffeine consumption. Within 'high' caffeine consumers, women of the <i>tt</i> VDR genotype had significantly higher rates of spinal BMD loss than those of the <i>TT</i> genotype, yet the same relationship was not observed within the 'low' caffeine consumption group. The presence of the <i>tt</i> genotype may indicate an increased susceptibility to the effects of caffeine within postmenopausal elderly women. Massey (2001) <sup>62</sup> has pointed out that only five of the thirty-three women in the 'high' caffeine consumption group had the <i>tt</i> genotype, suggesting that a susceptibility to caffeine within a small segment of the population would be difficult to detect in studies where genotype is not considered	†
Bergman <i>et al.</i> (1987) <sup>47</sup>	Women aged 31–78 years ( <i>n</i> 37)	Various indicators of bone metabolism, serum mineral concentrations and urinary mineral excretion were measured during a 3 d period where caffeine was or was not permitted. All women reported habitual daily caffeine intake of $\geq 200$ mg. Participants with Ca intakes below 600 mg/d ( <i>n</i> 11) had significantly reduced levels of serum Ca and ultrafiltrable Ca, plus increased bone alkaline phosphatase during caffeine consumption. However, participants with Ca intakes above 600 mg/d ( <i>n</i> 26) did not exhibit similar changes	†
Harris & Dawson-Hughes (1994) <sup>48</sup>	Postmenopausal women ( <i>n</i> 205)	Mean caffeine and Ca intakes were 349 (SD/308) and 766 (SD/202) mg/d respectively. Rates of bone change did not differ significantly with caffeine consumption in women with a Ca intake above the median. However, higher caffeine intake was associated with greater bone loss in women with lower Ca intakes	†
Ilich <i>et al.</i> (2002) <sup>49</sup>	Caucasian postmenopausal women ( <i>n</i> 136)	A daily intake of 200–300 mg caffeine was associated with low BMD at the majority of sites tested. In participants with a Ca intake below the median of 750 mg/d, BMD values at the femoral neck and trochanter were significantly lower in caffeine consumers compared with non-consumers. However, there was no significant difference between BMD values for caffeine non-consumers in the lower Ca intake group, and values for caffeine consumers and non-consumers in the higher Ca intake group. Therefore, caffeine appeared to have less influence upon bone in those with higher Ca intakes	†
Glynn <i>et al.</i> (1995) <sup>50</sup>	Men over the age of 50 years ( <i>n</i> 523)	Whilst milk consumption was positively associated with BMD, the relationship was not always significant. Current Ca intake had no relationship to BMD of the hip. BMD and caffeine consumption were not associated	‡

Table 4. *Continued*

Reference	Population	Results	Comment
Rico <i>et al.</i> (2002) <sup>52</sup>	Postmenopausal women ( <i>n</i> 93)	Vitamin D (not caffeine) was the only dietary component significantly related to BMD	‡
Hannan <i>et al.</i> (2000) <sup>51</sup>	Elderly men and women ( <i>n</i> 800)	Whilst risk factors differed between the sexes, caffeine and Ca intake did not significantly affect bone mineral density measured at six separate sites at 4-year intervals	‡
Johnell <i>et al.</i> (1995) <sup>6</sup>	Women ( <i>n</i> 5618; 2086 hip fracture cases, 3532 controls) aged over 50 years from fourteen centres in six countries across southern Europe; MEDOS	Higher intake of Ca from the consumption of milk during childhood, adulthood and the recent past was significantly related to lower risk of hip fracture. The relationship was not linear, with increased risks being limited to the 10% with the lowest level of consumption	‡
New <i>et al.</i> (1997) <sup>53</sup>	Premenopausal women ( <i>n</i> 994)	Whilst current Ca intake was not associated with BMD, BMD was significantly related to reported milk intake in earlier life	‡

HRT, hormone replacement therapy; VDR, vitamin D receptor; MEDOS, Mediterranean Osteoporosis Study.

\* After adjustment for confounding factors, relationships between caffeine consumption and low BMD often become insignificant.

† Where relationships between caffeine consumption and bone health persist, the effects of caffeine may be mediated by various factors.

‡ It is likely that lifetime dietary habits have more effect upon BMD than current nutrient consumption.

caffeine consumption and bone status. Yet, several conclusions can be drawn. First, caffeine has various metabolic effects *in vivo* which can be measured, although the review by Heaney<sup>61</sup> suggested that the most likely prominent mechanism of effect is a moderation in Ca absorption efficiency rather than calciuresis. Heaney<sup>61</sup> also noted that whilst decreases in BMD are the most studied factor of skeletal fragility, fatigue and falls are also highly important and as yet no studies have considered whether caffeine has any effect upon repair of damaged bones. Nevertheless, when the evidence is considered, it can be concluded that the effect of caffeine upon BMD is likely to be mild for the majority of the population. Second, certain factors may increase the susceptibility of an individual to the effects of caffeine. Adequate Ca intake is one such factor; an opinion supported by Nawrot *et al.*<sup>41</sup>, who state that current evidence indicates that individuals consuming a minimum 800 mg Ca daily would not be significantly affected by daily caffeine intakes of below 400 mg. Considering the low caffeine content (per serving) of tea (Table 2), an individual with adequate Ca intake would have to habitually drink somewhere between or beyond eight to thirteen cups/d in order to be at any risk. Age is another important factor. Massey<sup>62</sup> stated that current evidence would suggest the elderly are less able than their younger counterparts to adapt their Ca absorption levels to counteract caffeine-induced urinary loss. The effects of caffeine *in vivo* are also likely to be complicated by other factors such as sex (women appear more susceptible than men), genetic predisposition, current physical status, diet and the amount ingested.

Table 5 summarises the tea components that may affect bone health.

### Fruit, vegetables and bone health

The consumption of fruit and vegetables has been associated (for example, Steinmetz & Potter<sup>63</sup>) with reduced risk of various oxidative stress-related diseases such as cancer and

heart disease. Such information led the WHO in 1990 to set a daily target for consumption of at least 400 g fruit and vegetables (including 30 g nuts, pulses and seeds), which has been interpreted by the regulatory authorities and health groups of many countries as the 'five portions of fruit and vegetables daily' advice. However, fruit and vegetable consumption has traditionally been associated with many other aspects of health, including that of bone formation and maintenance.

### Epidemiological studies

Several studies have noted favourable relationships between BMD and the consumption of fruit and vegetables across a wide range of age groups.

For example, a 'high' consumption of fruit and vegetables (three or more portions per d) was significantly related to higher bone area of the non-dominant wrist (8.3%) and the whole body (6%) compared with a 'low' consumption (less than three portions) in a group of fifty-six Caucasian early-pubertal girls<sup>64</sup>. Furthermore, those girls studied with higher fruit and vegetable consumption also had lower urinary excretion of Ca and parathyroid hormone. Similarly, the Saskatchewan Bone Mineral Accrual Study<sup>65</sup> showed that consumption of fruit and vegetables of less than five portions daily was associated with lower accumulation of bone mineral in adolescent girls, but not boys. Differences in Ca absorption efficiency as well as intake were suggested as possible explanations for sex-based differences in accumulation of bone mineral. Similar sex-based disparities were reported by McGartland *et al.*<sup>66</sup>, who observed (before adjustment) that heel BMD was significantly higher in 12-year-old (*n* 378) and 15-year-old (*n* 369) girls with a higher consumption of fruit compared with girls with moderate and lower intakes respectively. Whilst adjustment for various potential confounding factors such as body weight, height, smoking status and physical activity removed the significant association between heel BMD

**Table 5.** Tea components that may affect bone health, supporting epidemiological studies through *in vitro* and *in vivo* experimentation

Component	Effect
Catechins	There is <i>in vitro</i> and <i>in vivo</i> evidence for catechins having a positive effect on bone health
Flavonoids	Flavonoids in tea other than catechins may positively affect bone health through a number of different mechanisms
Fluoride	The fluoride content of green and black teas may have a positive effect on bone health
Fluoride	Excessive consumption of brick tea (consumed in Tibet) can lead to fluorosis because of high fluoride levels

and fruit intake in 15-year-old girls, the same relationship for 12-year-old girls was reinforced. No associations between fruit intake and BMD were found at the forearm in the girls, or at either site in boys aged 12 years ( $n$  324) and 15 years ( $n$  274).

In a group of 994 premenopausal women from Scotland, BMD was found to be significantly lower at the LS, FN, femoral trochanter and the femoral Ward's sites in those reporting a low intake of fruit in early adulthood compared with medium or high intakes at the same ages<sup>53</sup>. After adjustment for various confounders, the relationship remained significant for the LS and femoral trochanter sites. Chen *et al.*<sup>67</sup> report that bone health was related to fruit (but not vegetable) intake in a group of 668 Chinese women who had experienced early menopause. High BMD and BMC of the whole body, LS and left hip was associated with high levels of fruit consumption, after adjustment for age, years since menopause and BMI, remaining significant after adjustment for physical activity and intake of Ca, P and protein.

In studying the dietary habits of the elderly, Tucker *et al.*<sup>68</sup> found that BMD was highest in men with a greater consumption of fruit, vegetable and cereal and lowest in men with a greater consumption of high-sugar sweets compared with most other consumption groups. Women with high consumption of high-sugar sweets also had the lowest BMD compared with other groups; however, both those with a high fruit, vegetable and cereal consumption and those with high alcohol consumption were shown to have high BMD values. Whilst alcoholism is traditionally associated with poor bone health, any beneficial association between BMD and alcohol consumption might be explained in part by the flavonoid and phyto-oestrogen contents of wine and beer respectively. Fruit and vegetable intake was again significantly associated with BMD at three out of four sites tested in men and two out of four sites tested in women within the same cohort in an earlier study<sup>69</sup>. Furthermore, after a 4-year interval, high intakes of fruit and vegetables in the baseline diet were associated with significantly less bone loss at one site in men (although there was no similar association for women). However, K intake had a more pronounced relationship with BMD at baseline, with high K and Mg intakes being associated with significantly less bone loss at two sites in men.

Not all studies show such clear associations. In a study of 944 subjects aged 67–79 years (470 male, 474 female) fruit and vegetable consumption was not linked to rate of total hip BMD loss<sup>70</sup>. However, vitamin C intake was significantly linked to BMD conservation in women. Those in the highest consumption tertile (99–363 mg/d) lost BMD at a rate of 0.30% annually, compared with 0.65% annually for those in the lowest consumption tertile

(7–57 mg/d); almost twice the rate of bone mineral loss. Those in the middle consumption tertile (58–98 mg/d) lost bone mineral at a rate of 0.31% annually, suggesting that even small increases in vitamin C intake might benefit bone health.

Several other studies have linked the consumption of nutrients present within fruit and vegetables to bone health. For example, in studying a group of 891 women (aged 45–55 years at baseline) Macdonald *et al.*<sup>71</sup> found that Ca, vitamin C, Mg and K were associated with high FN BMD in women who were still menstruating at follow-up (5–7 years after baseline). Furthermore, Ca, vitamin C and Mg were associated with FN bone change in the 146-strong subgroup. In the study by New *et al.*<sup>53</sup> dietary intakes of vitamin C, Mg, K, Zn and fibre were positively related to BMD. After adjustment for confounders, Mg and vitamin C intake were significantly associated with BMD at the LS, whilst K intake was associated with BMD at the LS, FN, femoral trochanter and femoral Ward's sites. In a later study, New *et al.*<sup>72</sup> also showed that nutrients from fruit and vegetables (including K,  $\beta$ -carotene, vitamin C and Mg) were positively and significantly associated with bone health in a group of sixty-two healthy pre-, peri- and postmenopausal women, with FN BMD being significantly higher in those reporting a higher (one to four portions;  $\geq 5$  d/week) compared with medium or lower (one to four portions,  $\leq 2$  d per week) fruit consumption during childhood.

#### *Putative mechanisms of the effects of fruit and vegetables upon bone mineral density*

It has been suggested that bone demineralisation may be in part mediated by diets high in acid-forming components (amino acids, P, chlorine) and low in base-forming components (K, Ca, Mg, vitamin C)<sup>73</sup>, with the skeleton acting as a buffer, thus playing a vital role in pH balance. It is therefore possible that the protective effects of fruit and vegetables may be due to their content of base-forming components. A great deal of evidence surrounds and supports this theory (which is beyond the scope of the present review), and for a more detailed synopsis the reader is directed to a comprehensive review by New<sup>74</sup>.

It is entirely possible, however, that the protective effect of fruit and vegetables upon bone health is not exclusively dependent upon the ability of fruit and vegetable components to neutralise acid components of the diet, a prospect highlighted by the work of Mühlbauer *et al.*<sup>75</sup>. Here, the bone-related effects of adding various plant foods to the diets of rats were observed. Rats were pre-labelled with [<sup>3</sup>H]tetracycline through a series of subcutaneous injections (during the first 6 weeks of life). The urinary excretion of <sup>3</sup>H (determined by liquid scintillation counting)

was used as an indication of bone resorption. Rats were fed a diet either with or without animal protein (casein), with both diets being similar in terms of protein, Ca and phosphate content. Rats fed a vegetarian diet had urine that was high in pH, low in ammonium and had negative titratable acid. Upon conversion to the casein diet, urine became low in pH, comparatively high in ammonium and had higher titratable acid content. Addition of onion (7%) to the diet of casein-fed rats had the effect of mildly raising urinary pH and slightly lowering ammonium and titratable acid content. However, bone resorption was reduced by 18 (SE/2) %.

Taken alone, these results could support the acid–base theory. However, Mühlbauer *et al.*<sup>75</sup> also report that bone resorption was lowered (13 (SE/2) %) by the addition of onion to rats fed on the vegetarian diet. The more pronounced effect of onion upon rats fed the casein diet might have been mediated by an increase in base-forming components. However, supplementation of casein diets with potassium citrate (to buffer pH to levels similar to the vegetarian diet) slightly improved the effects of either onion or a mixture of fourteen vegetables, salads and herbs (previously observed to inhibit bone resorption in rats)<sup>76</sup> yet had no effect on bone resorption in the absence of these vegetables. The authors concluded that the base excess of the vegetarian diet was unlikely to mediate differences in response of the two diet types to the addition of onion or vegetable mix. Furthermore, the K content of test samples did not correlate with levels of bone resorption. In a follow-up study<sup>77</sup>, several other test samples including fennel, celeriac, prunes, oranges, French beans, farmed and wild mushrooms and red wine residue were also shown to significantly reduce bone resorption in rats. However, perhaps more importantly, seventeen other plant foods tested had no significant effect. In discussing all three studies, Mühlbauer *et al.*<sup>77</sup> noted inconsistencies between these results and the acid–base balance theory. Mühlbauer *et al.*<sup>77</sup> therefore suggest that the benefit accrued by prevention of bone resorption shown by some plant foods may be derived from ‘pharmacologically active compounds’.

#### *Role for polyphenols?*

Polyphenols, also present in significant concentrations within fruits and vegetables, may be candidates for one such group of compounds, with several having been shown to affect bone metabolism (for example, onions are an excellent source of various quercetin conjugates, such as rutin; see ‘Effects of flavonoids on bone health’ section). For instance, the daily consumption of 100 g dried plums (rich in anthocyanin flavonoids) in postmenopausal women was shown to increase significantly levels of insulin-like growth factor-1 and bone-specific alkaline phosphatase in serum after 3 months, indicating increased bone formation<sup>78</sup>. Chiba *et al.*<sup>79</sup> found that femoral bone loss caused by ovariectomy in 8-week-old female mice could be prevented by treatment with either hesperidin (a flavonoid common to citrus fruit) or  $\alpha$ -glucosylhesperidin. Treatment of bone samples with tartrate-resistant acid phosphatase showed that trabecular bone resorption was prevented through a reduction in the number of osteoclast cells. Hesperidin treatment also increased femoral Ca concentration to slightly above that for mice in the control group (sham-operated), whereas

femoral Ca concentration was lower 4 weeks after ovariectomy in mice not treated with hesperidin.

*Phyto-oestrogens and bone mineral density.* A large amount of work concerning the effects of isoflavones (Fig. 3) upon BMD has also been performed. *In vitro*, various isoflavones have been shown to inhibit bone resorption in bone culture through promoting the proliferation and activity of osteoblast cells and suppressing the proliferation and activity of osteoclast cells<sup>80</sup>. The structural and possible *in vivo* functional similarities between isoflavones and oestrogens have also highlighted the possibility that supplementation may benefit postmenopausal women, in terms of general as well as bone-specific health. The potentially harmful side effects of HRT such as swelling and bleeding of the uterus, CVD, gallbladder disease and increased risk of various cancers<sup>81</sup> mean that reliable dietary supplementation would be of considerable benefit. Therefore, much of the *in vivo* work in this field is focused upon ovariectomised rats and postmenopausal women. It has to be noted that in this area in particular, promising *in vitro* studies have proved difficult to confirm *in vivo* in human studies.

Twice-weekly intramuscular injections with either coumestrol (1.5  $\mu$ M) or zearalanol (3.1 mmol) inhibited bone loss in ovariectomised rats over a 6-week period<sup>82</sup>. However, incorporating a mixture of isoflavones extracted from clover (biochanin A, formononetin and genistein) into the diet of rats (131.25 mg/week) did not reduce bone loss compared with control rats following ovariectomy. In a similar study, Fanti *et al.*<sup>83</sup> showed that daily subcutaneous injections of 5 mg genistein/kg body weight inhibited reductions in BMD by over 50% (compared with control) 21 d after ovariectomy. Increasing the dose to 25 mg/kg body weight did not improve benefit, whilst doses of 1 mg/kg body weight had no effect upon bone loss. Though numbers of osteoblast cells were higher in ovariectomised compared with sham-operated animals, within the ovariectomised group osteoblast levels tended to be higher in rats treated with genistein. In a slightly longer study (16 weeks) Lee *et al.*<sup>84</sup> showed that inclusion of soya isoflavone extract (comprising various aglycone and conjugate forms of genistein, daidzein and glycitein) in the diet of ovariectomised rats (6.25 g/kg diet, equivalent to about 10.67 mg/d) significantly reduced losses in bone density and mineral content following ovariectomy. However, lower oral doses of isoflavones (approx 0.96 and 1.92 mg daily) over a 40 d period did not protect rats from ovariectomy-induced bone loss<sup>85</sup>.

Certain studies have reported dietary exposure to phyto-oestrogens (as food or via supplementation) to benefit BMD in human subjects. For example, in a long-term, double-blind, randomised, placebo-controlled trial performed by Atkinson *et al.*<sup>86</sup>, the effects of a daily isoflavone supplement (derived from red clover) containing biochanin A, daidzein, formononetin and genistein upon BMD and BMC of a group of women (aged 49–65 years) was assessed. In the 177 women that completed the trial, loss of BMC and BMD at the LS region was significantly lower in women receiving the isoflavone supplement compared with those receiving a placebo. Although urinary markers of bone resorption were not significantly different between groups, markers of bone

formation in plasma (bone-specific alkaline phosphatase and N-propeptide of collagen type I) were significantly higher in postmenopausal women receiving the supplement compared with placebo. A similar study by Chen *et al.*<sup>87</sup> observed changes in BMD with 175 (a sub-section of the 203 initially selected) postmenopausal Asian women (aged 48–62 years) before and after 12 months' treatment with soya. All participants consumed 500 mg Ca, 3.125 µg (125 IU) vitamin D plus differing quantities of soya isoflavones (predominantly daidzein, genistein and glycitein) on a daily basis. Women who received a high dose of soya (80 mg isoflavones) had significantly higher positive BMC change rate at total hip and trochanter compared with those receiving a medium dose (40 mg isoflavones) or a placebo. Furthermore, closer analysis showed that the significant benefit of soya isoflavone supplementation in the high-dose group originated from women with lower initial BMC. However, in a comparable study<sup>88</sup>, after 12 months' treatment with either a daily soya protein supplement (containing daidzein, genistein and glycitein) or a placebo there were no significant differences in BMD, markers of bone formation, or plasma levels of Ca and P between treatment groups in a cohort of 175 women aged between 60–75 years.

Another field of intense research concerns the use of the synthetic isoflavone ipriflavone. In a study of postmenopausal women (aged 50–60 years, 1–5 years after menopause) two groups (one observed at the distal radius the other at the LS) were given Ca (1 g/d) plus either ipriflavone (600 mg/d) or placebo for 2 years<sup>89</sup>. Whilst those receiving placebo experienced reduced BMD at both regions, those taking ipriflavone had increased BMD at the distal radius (seventy women) or no significant reduction in BMD at the LS (thirty women). In a similar study by Maugeri *et al.*<sup>90</sup>, eighty-four female patients over the age of 65 years with a history of osteoporosis and fracture were treated with ipriflavone or placebo as above for 2 years. Whilst those receiving placebo had decreased BMD at the distal radius (as well as increased pain and use of analgesics), those receiving ipriflavone had increased BMD, decreased pain and decreased requirement for analgesics. In both studies urinary excretion of hydroxyproline was significantly reduced, with all subjects tolerating ipriflavone well, leading to the suggestion that ipriflavone has considerable potential as a therapeutic agent.

The volume of data regarding the subject of phyto-oestrogens and bone health is considerable, and to attempt to tackle this field in its entirety is beyond the scope of the present review. For a more thorough account of the situation regarding phyto-oestrogens and bone health, the reader is directed to reviews by Yamaguchi<sup>91</sup>, Branca<sup>81</sup> and Setchell & Lydeking-Olsen<sup>80</sup>.

#### Role for other compounds?

Whilst much evidence supports the theory that polyphenols may benefit bone health, it should be pointed out that other fruit and vegetable components might also play a part. For example, in reviewing functional ingredients and BMD, Brouns & Vermeer<sup>92</sup> also suggest that vitamin K and non-digestible carbohydrates may have potential benefits to bone health. Carotenoids may also play a role.

For example, total and LS BMD was associated with high lycopene consumption in a cohort of sixty-eight men, and with high lycopene, lutein or zeaxanthin consumption in a cohort of 137 women, within which was also observed a correlation between high BMD and intake of β-carotene<sup>93</sup>. It is therefore suggested that the benefits of consuming fruit and vegetables with regard to BMD may be the result of several groups of components, with many different mechanisms of benefit, meaning that the scope for investigating the action of fruit and vegetable components upon bone metabolism may not yet be fully realised.

### Conclusions

Bone health is the combination of genetics, diet (Fig. 5) and lifestyle. Whilst genetic inheritance is difficult to control (at present), improvements in diet and lifestyle can reduce the risk of poor bone health. It has been suggested that whilst low BMD is often a problem of old age, it is the dietary habits of a lifetime that have the biggest effect upon BMD<sup>38</sup>. Therefore, emphasis should be placed on building a high BMD (through increasing the intake of beneficial components) over the long term rather than correcting a low BMD during old age. Several conclusions can be drawn regarding tea, caffeine, fruit and vegetables and their relationship to bone health.

First, tea is a mildly caffeinated beverage containing high levels of polyphenols which appears to potentially benefit bone health. Whilst the exact mechanism of these benefits is

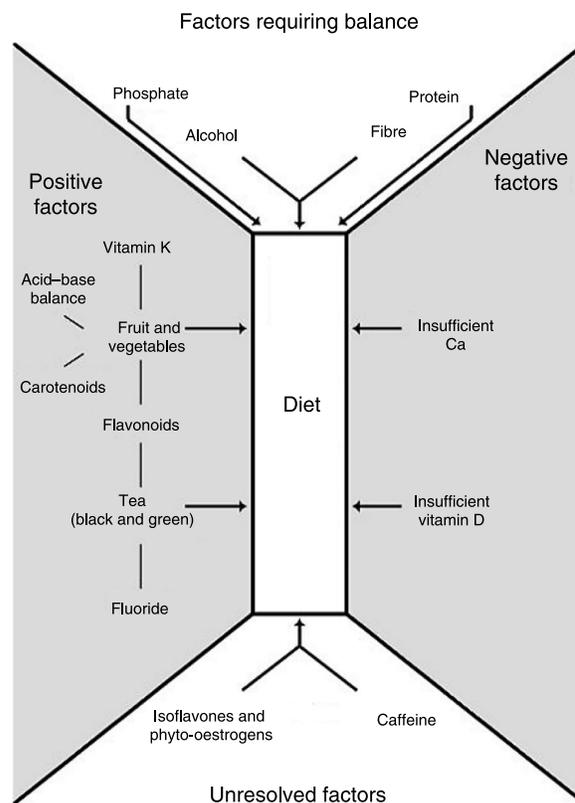


Fig. 5. Summary of some of the dietary factors that may affect bone health.

unclear, both the F content and the polyphenolic content of tea may contribute to higher BMD.

Second, although consumption of caffeine may affect Ca balance through several potential mechanisms *in vivo*, the current evidence would suggest that this might only prove detrimental to BMD in those already susceptible to the effects of caffeine (i.e. inadequate Ca intake, a predisposition toward osteoporosis or old age). Whilst the consumption of caffeinated beverages may have been previously viewed as detrimental to BMD, caffeine may not be as deleterious to bone health as is currently indicated in much dietary advice to those at risk. Recommendations to at-risk groups to avoid certain plant-based foods containing caffeine may, therefore, actually cause harm in depriving consumers of dietary sources of bioactives with beneficial consequences for bone health.

Finally, high intakes of fruit and vegetables have also been associated with high BMD, and whilst this may partly stem from base-forming components (K, Ca, Mg, vitamin C), it is likely that polyphenols (including flavonoids and possibly phyto-oestrogens) may also contribute to bone health. Several other fruit and vegetable components such as lycopene, lutein and zeaxanthin,  $\beta$ -carotene, vitamin K and non-digestible carbohydrates have also been reported as potential bone enhancers, suggesting that fruit and vegetables may benefit bone health through a combination of different mechanisms.

In summation, there appears to be the possibility of significant positive effects on bone health from fruit, vegetables and tea. It is suggested that future research in the area focus upon the following:

- (1) Human feeding studies. Epidemiology is a useful tool to highlight potential beneficial relationships between diet and bone health. However, more human *in vivo* studies (specifically double-blind, randomised, placebo-controlled trials) are required to confirm (or refute) the validity of such relationships (for foods and individual compounds). Development of reliable biomarkers of bone remodelling may help counter the large expense and timescales typical to the area.
- (2) Detailed mechanistic exploration. Several mechanisms of benefit to bone health have been suggested for plant-based foods. However, the significance of these mechanisms to the *in vivo* situation remains to be confirmed. Such work must also consider the varied metabolism of bioactives. Where *in vitro* research is performed (for example, cell culture), it is vital that appropriate metabolites at appropriate concentrations to the target area be assessed, rather than compounds native to the food or extract under scrutiny.
- (3) Investigate possibilities for synergy. Once mechanisms of benefit have been identified, it becomes possible to assess relationships between individual bioactives in either a given foodstuff or the diet in general. It may thus be possible to optimise any beneficial effects.

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