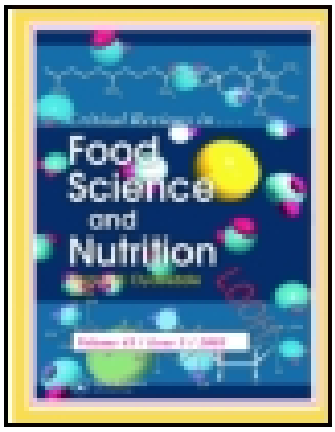

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Tea and Bone Health: Findings from Human Studies, Potential Mechanisms, and Identification of Knowledge Gaps

Leslie A. Nash[ac] & Wendy E. Ward[abc]

[a] Department of Health Science, Faculty of Applied Health Sciences, Brock University, 500 Glenridge Ave, St. Catharines, Ont., L2S 3A1 Canada

[b] Department of Kinesiology, Faculty of Applied Health Sciences, Brock University, 500 Glenridge Ave, St. Catharines, Ont., L2S 3A1 Canada

[c] Center for Bone and Muscle Health, Brock University, 500 Glenridge Ave, St. Catharines, Ont., L2S 3A1 Canada

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Tea and bone health: Findings from human studies, potential mechanisms, and identification of knowledge gaps

Leslie A. Nash[a,c], Wendy E. Ward[a,b,c]*

[a]Department of Health Science, Faculty of Applied Health Sciences, Brock University, 500 Glenridge Ave, St. Catharines, Ont., L2S 3A1 Canada

[b]Department of Kinesiology, Faculty of Applied Health Sciences, Brock University, 500 Glenridge Ave, St. Catharines, Ont., L2S 3A1 Canada

[c]Center for Bone and Muscle Health, Brock University, 500 Glenridge Ave, St. Catharines, Ont., L2S 3A1 Canada

* Corresponding author: wward@brocku.ca, 905-688-5550 x3024

Brock University, 500 Glenridge Ave, St. Catharines, Ont., L2S 3A1 Canada

Abstract

The population of the developed world is aging. With this aging population, strategies for prevention rather than treatment of chronic disease, such as osteoporosis, are essential for preserving quality of life and reducing health care costs. Tea is the second most consumed beverage in the world and is a rich source of flavonoids that may benefit bone health. There is strong evidence from human studies that habitual tea consumption is positively associated with

higher BMD at multiple skeletal sites, while the association with fracture risk is less clear. Fracture studies demonstrate a reduction or no difference in fragility fracture with tea consumption. There are key questions that need to be answered in future studies to clarify if higher consumption of tea not only supports a healthy BMD, but also reduces the risk of fragility fracture. And if the latter relationship is shown to exist, studies to elucidate mechanisms can be designed and executed. This review discusses findings from epidemiological studies as well as potential mechanisms by which flavonoids in tea may mediate an effect, and identifies key knowledge gaps in this research area.

Key words

Antioxidants, bone mineral density, flavonoids, fracture, osteoporosis, oxidant stress

1.0 Osteoporosis

The population of the developed world is aging. With the aging population, strategies for prevention rather than treatment of chronic disease, such as osteoporosis, are essential for preserving quality of life and reducing health care costs (Lin, et al., 2004; Burge, et al., 2007). Globally, it is estimated that 1 in 5 men and 1 in 3 women will experience a fragility fracture within their lifetime (Akesson, et al., 2013). A fragility fracture occurs every 3 seconds (Johnell, et al., 2006). Mortality is associated with all types of osteoporotic fractures and the highest risk of death due to fragility fracture is among men and women aged 60-69 years (Kanis, et al., 1994; Center, et al., 1999, Ioannidis, et al., 2009). Dietary approaches represent a potential preventive strategy that supports bone health. While calcium and vitamin D have been extensively studied, other foods and food components ï such as tea and its flavonoids ï are also of interest for their potential to promote and support bone health throughout an individualôs lifespan. This review will highlight the epidemiological evidence supporting an association between tea consumption and bone health, discuss potential mechanisms by which flavonoids in tea may mediate an effect, and identify key knowledge gaps in this research area.

2.0 Tea

Tea is the second most consumed beverage in the world and represents a global multi-billion dollar industry. Consumption of tea has increased more than 10% over the past 10 years and continues to rise (Tea Association of USA, 2013). In 2012, over 79 billion servings of tea were consumed in the United States, resulting in sales of over $2.25 billion dollars (Tea Association of USA, 2013). Tea is made from the plant *Camellia sinensis* – this includes black,

green, oolong and white tea. Herbal teas or tisanes are made from a variety of other plant sources with rooibos (*Aspalathus linearis*) being an increasingly popular choice (Joubert, et al., 2011). All these sources provide a rich source of flavonoids that are recognized for their biological activities. Moreover, some epidemiological studies suggest that regular tea intake is associated with higher BMD (Table 1) and reduced risk of fragility fracture (Table 2) with regular consumption of tea.

2.1 Tea and BMD

Findings from many, but not all cross-sectional, case-control and prospective studies have identified an association between consumption of tea and better bone health in a variety of populations throughout the world. Studies have been conducted in North America, Europe, Australia, and Asia. Postmenopausal women have been most thoroughly studied, with a few studies also including men, at a wide variety of ages, or premenopausal women. To date, the majority of these studies investigating the association between tea consumption and bone health use a measurement of bone mineral density (BMD) at one or multiple skeletal sites, commonly the hip and/or spine, using dual x-ray absorptiometry, as a measure of bone health and a surrogate measure for risk of fragility fracture.

2.1.1 Cross Sectional Studies

Cross sectional studies have identified positive correlations between habitual consumption of tea and BMD in postmenopausal women. A study of 62 postmenopausal women in Canada measured BMD at the lumbar spine and femoral neck and found a positive correlation between the intake of tea (reported as the number of 6 ounce cups/day, tea types not identified)

and BMD at both sites (Hoover, et al., 1996). Similarly, a study conducted in England examined the relationship between self-reported consumption of tea (grouped as 1-3, 4-6 or >6cups/day, tea types not identified) and BMD at three sites: lumbar spine, greater trochanter and Wardôs triangle in 1256 postmenopausal women. There was a positive association between tea consumption and BMD (Hegarty, et al., 2000). All women who consumed tea had approximately 5% greater BMD at all three sites. There was no difference in BMD between individuals who consumed 1-3 cups compared to 4 or more cups a day. Tea consumers were also categorized on whether they consumed milk in their tea, as milk provides a good source of calcium (approximately 300 mg of calcium per serving or 25% of the recommended intake for women over 50 years of age). However, milk added to tea did not influence the relationship (Hegarty, et al., 2000). Another positive correlation between tea and BMD was identified in postmenopausal women (n = 680) who habitually consumed oolong tea. Consuming at least 1 cup of oolong tea a day was associated with a higher BMD at the greater trochanter and Wardôs triangle (Wang, et al., 2014). These results were also reflected in a study of postmenopausal women in Japan (n = 632). Women who consumed green tea (Ó5 days/week) had higher BMD at the lumbar spine and better T-scores (-1.59±2.70 *vs* -2.17±2.08) than those who did not consume tea (Muraki, et al., 2007). Another study, from Taiwan, that included a wide age-range of women (28 to 70 years of age, n = 100) reported that tea consumers (reported as yes or no/rarely, tea types not identified) had higher hip BMD than non-consumers (Hsiao, et al., 2012) and a cross-sectional study using baseline measurements from the Danish Osteoporosis Prevention Study demonstrated a positive correlation between the number of cups of tea consumed a day (tea types not identified) and the BMD at the femoral neck of perimenopausal (n = 2016) women (Vestergaard, et al., 2001).

Unlike the previous studies, a study in Iran measured BMD at lumbar spine and hip in both males and female between the ages of 20-76 years. This study found that habitual consumption of tea (tea consumers were defined as consumption of more than 5 cups per day, tea types not identified) was associated with better bone health only in the female population. Women who habitually consumed tea had a higher BMD at the hip than non-consumers but when men were assessed, no relationship between tea consumption and BMD was identified (Hossein-Nezhad, et al., 2007). The lack of association among males was also observed in a study of young Greek men (age 18-30 years, *n* = 300) in the armed forces. Tea intake (reported as cups/day, tea types not identified) was not associated with BMC or BMD at the distal and ultradistal radius (Kyriazopoulos, et al., 2006). Another study of older males, ages 45-65 (*n* = 70), also reported no association between tea intake (reported as cups/day, tea types not identified) and BMD at the lumbar spine and femur (Saitoglu, et al., 2007). The potential sex-specific response may indicate that flavonoids in tea may be acting via an estrogen-mediated pathway and is discussed more in section 3.0.

2.1.2 Prospective Studies

Findings from prospective studies have also shown benefits to bone health among tea-consumers. A cross-sectional study in Australian (*n* = 1027) women showed that hip BMD was 2.8% higher in those who consumed tea (reported as cups/day) compared to those who did not (Devine, et al., 2007). Moreover, 164 of these women were followed for 4 years in which bone loss was measured at baseline (year 1) and at the end of the study (year 5). Those who drank tea ï without distinguishing the quantity or type of tea - had an average loss of 1.6% of their total

hip BMD over the four years, whereas non-tea consumers lost approximately 4.0%. Although benefits are found, this study did not include the consumption of herbal tea and therefore intake measurements could be substantially higher (Devine, et al., 2007).

Similar to the studies conducted in Australia and Denmark, a study in Taiwan which assessed consumption of black, green and oolong tea, found a positive association between long-term tea consumption and BMD (Wu, et al., 2002). The study enrolled both males and females (n =1037) from the prospective survey of chronic disease in Tainan, Taiwan and labeled habitual consumers as those who consumed tea at least once a week for a minimum of 6 months. Habitual tea consumers were further categorized by length of consumption: 1-5 years, 6-10 years and more than 10 years. A positive, linear correlation was identified between consumption of tea and BMD for the whole body, lumbar spine, femur neck and Ward triangle. Individuals who consumed tea for more than 10 years had the highest levels of BMD amongst the groups, while individuals who consumed tea for longer than 6 years had significantly greater lumbar spine BMD than non-tea consumers. No significant association was found between consumers and non-consumers when tea was consumed for less than 5 years (Wu, et al., 2002), suggesting benefits may only be observed with longer-term consumption.

As the benefits of tea consumption are typically identified more often in female populations, it is possible that flavonoids found in the tea are responsible for inducing the positive effects seen in bone health by acting as estrogen mimics. A study in a Scottish population investigated the diet of women between the ages of 45-54 years (n = 3226) and compared flavonoid intake and BMD at the lumbar spine and femoral neck (Hardcastle, et al.,

2011). The majority of an individualôs flavonoid intake (57%) was attributed to tea consumption (reported as mL/day, tea types not identified), in which there was an average intake of 596±504 mL of tea/day. Higher flavonoid intake, as measured using food frequency questionnaires, was positively correlated with femoral neck and lumbar spine BMD. Urinary free deoxypyridinoline, a marker of bone resorption, was also negatively correlated to flavonoid concentration, supporting the positive relationship between flavonoid intake and higher BMD (Hardcastle, et al., 2011). Findings from this study suggest that populations who habitually consume tea have higher levels of flavonoids in their systems and this may lower bone turnover and maintain BMD.

2.2 Tea and Fragility Fracture

While BMD is a good measure of bone health, it is known that BMD alone does not fully predict bone strength, and ultimately, an individualôs risk for fragility fracture (Marshall, et al., 1996). For that reason it is important to discuss studies that have measured the relationship between tea consumption and fragility fracture. Because prospective studies that use incidence of fracture as a primary outcome require substantially larger sample sizes and often require a follow-up time of several years, most studies are case-control studies or use BMD as a surrogate measure of fracture risk. Nonetheless there are a few prospective studies that have examined this relationship.

2.2.1 Case-control studies

A case-control study in India (43 male and 57 female cases matched to controls, mean age of 65 years) found that tea consumption (reported as ≤ 1 or >1 cup/day within the last 10

years, tea types not identified) was associated with a higher risk of fracture (Jha, et al., 2010). However, a study completed in Northern Italy in women ranging from age 19 to 74 years (*n* = 279) found no association between fracture and consumption of tea (self-reported as a tea drinker or not a tea drinker, tea types not identified) (Tavani, et al., 2005). Similarly, a smaller study completed in Toronto, Canada, which analyzed women (50-84yrs) with a wrist (*n* = 154) and hip (*n* = 102) fracture in comparison to 277 controls, found no association (< 3 cups/day or Ó3 cups/day, tea types not identified) (Kreiger, et al., 1992). Of note is that this study defined tea consumers as those who consume more than 3 cups a day while most other studies define tea consumption as 1 serving or more per day. Similarly, a study from China in which elderly individuals were studied (over age 90 years) showed no relationship among tea consumption (reported as yes or no, and as a current or former tea consumer, tea types not identified) and osteoporotic fractures in males or females (Du, et al., 2011). Another study from China, that assessed a population of men and women (*n* = 581, 55 to 80 years of age), demonstrated no relationship between individuals consuming Chinese herbal tea and the risk of hip fracture (Zeng, et al., 2013). Tea consumers were identified by consumption of at least 1 cup/week within the previous 6 months. Differences in defining what constitutes a tea-consumer versus a non-tea consumer is challenging when comparing study findings as many studies have varied definitions as to who is a tea consumer. For example, one study defines a tea consumer as an individual who drinks at least 5 cups a day (Hossein-Nezhad, et al., 2007) while another study indicates that tea consumers are those individuals that consume more than one cup a day (Jha, et al., 2010).

An individualôs risk of falling is another consideration when studying fracture risk. A cross-sectional study of older adults (*n* = 2398, Ó55 years) in Singapore demonstrated that tea

consumption (reported as Ó1 cup/week but less than 1 cup/day, 1-2 cups/day, 3-5 cups/day, 6-9 cups/day and 10 cups/day) was positively associated with better performance in balance, gait, instrumental activities such as using the phone, keeping track of money and medication; and basic activities such as using the stairs, dressing and bathing (Ng, et al., 2014). This is an important area for future study. Three main types of tea were considered: Ceylon/English tea, Chinese black/oolong tea and green tea.

2.2.2 Prospective Studies

Four prospective studies have investigated the relationship between tea consumption and risk of fragility fracture. The Womenôs Health Initiative in the United States has shown that tea consumption was positively associated with BMD at specific skeletal sites in post-menopausal women (n = 91,465) (Chen, et al., 2003). BMD of the spine, total hip and total body was measured. Habitual consumption of tea was categorized into the following categories: less than 1 cup/day, 1 cup/day, 2-3 cups/day or 4 or more cups/day; in which herbal and decaffeinated tea was not included for tea consumption. Individuals who consumed 4 or more cups a day had a higher total body BMD in comparison to the reference group who consumed less than 1 cup/day. Women consuming between 2-3 cups a day had significantly higher spine BMD in comparison to those who consumed less than 1 cup/day. Furthermore, there was a positive correlation between how many cups of tea were consumed per day and BMD. Although tea consumption was positively associated with BMD, there was no association between number of cups of tea consumed and the rate of fracture. Fractures included both those found in medical records and those that were self-reported over a 4-year period. As herbal and decaffeinated teas such as

rooibos were not included in tea consumption, the actual consumption of tea by the population could be under-estimated and not identify potential exposure to flavonoids in these teas and thus may alter their findings (Chen, et al., 2003).

Similar findings were reported in a Swedish population (Hallstrom, et al., 2006) that was investigating high consumption of caffeine and bone health. Because black tea has a high caffeine content relative to other types of tea, it was included in this study. This prospective study assessed the risk of fragility fracture in women aged 40-76 years (n =31,527) and how that related to their intake of black tea and coffee over a 10-year period. Fractures were identified by personal hospital records and included if they were considered typical areas of osteoporotic fractures such as the femur, hip, pelvis, spine, distal forearm and proximal humerus. Tea consumption was separated into the amount of liquid consumed on a daily basis (<1 cup/day, 1 cup/day, 2-3 cups/day, Ó4 cups/day) and compared against the rate of fractures. No significant difference was identified in the incidence of fragility fracture between tea consumers and non-consumers. No significant interaction was identified between bone fracture and increased intake of caffeinated tea (Hallstrom, et al., 2006).

In contrast, two shorter-term prospective studies (1 year in duration) reported a lower incidence of hip fracture. The MEDOS study, a 1-year prospective study completed in Europe, across six countries, measured the incidence of hip fracture in both men and women over the age of 50 (Kanis, et al., 1999). When assessing males (n = 1,762), the intake of tea was associated with a decrease in the risk of hip fracture, and tea type was not considered. Tea consumption was estimated to have prevented 14% of fractures in European men (Kanis, et al., 1999). Likewise,

European women (n = 5,618) who habitually consumed tea also had a lower rate of fragility fracture (Johnell, et al., 1995).

Understanding why the two shorter-term prospective studies (1 year) show reductions in fracture associated with tea consumption compared to longer term studies (4 or 10 years in duration) is an area for further investigation. It is noted that the longer term studies from the Swedish and American populations only study the effects of black tea, and black and green teas, respectively. Defining tea consumers as those who only consume black or black and green tea can underestimate tea consumption for those individuals who consume oolong and white tea that contain varying levels and type of flavonoids. Moreover, herbal infusions or tisanes (such as those containing rooibos) or decaffeinated tea also contribute to flavonoid intake. This underestimation could lead to a potential change in outcomes when comparing the number of fractures in consumers to non-consumers.

2.3 Summary of Studies Investigating Tea Consumption and BMD and/or Fragility Fracture

In summary, findings from cross-sectional and prospective studies suggest that tea consumption is associated with higher BMD at multiple sites and that this relationship is observed in women rather than men. There are currently very few studies that look at the relationship between tea consumption and BMD in the male populations. Current studies suggest that because of the lack of positive effect in BMD within male populations, the active components of tea may act as estrogen mimics. Unlike females after menopause, males produce testosterone throughout their lifetime, and therefore still have the ability to produce estrogen (via

aromatase) (Longcope, et al., 1969). Thus, the presence of estrogen would mean that flavonoids, present in much lower concentrations than estrogen, would have to compete with estrogen for binding to the estrogen receptors, in which estradiol has a higher affinity. Furthermore, many of the studies involving male populations look at younger populations and therefore the lack of changes in BMD may be a result of the study design. More studies that focus on the male population should therefore be studied to address this knowledge gap. While the relationship between tea consumption and BMD has been shown in many studies, the relationship with fragility fracture is much less clear. To date, the largest and longest prospective studies show no association between consumption of tea and fragility fracture. In contrast, two prospective studies of shorter duration ï 1 year ï report a lower incidence of hip fracture.

A potential confounder across all studies discussed is that many different types of tea (black, oolong, green, white) or herbal teas or tisanes may be consumed and vary considerably in their composition ï and not all types of tea are necessarily captured in the questionnaires. In particular, caffeine and flavonoid content is quite variable among different tea types. These differences can mediate different biological effects. Moreover, some studies simply classify individuals as tea consumers or non-consumers using cutoffs such as ñmore than 1 cup of tea per dayò and this may attenuate potential associations.

2.4 Composition of Tea

Tea from the leaves of *Camellia sinensis* (black, green, oolong and white tea) contains an abundance of diverse flavonoids that help provide antioxidant, anti-cancer, and anti-bacterial activity. Herbal teas or tisanes such as those made from *Aspalathus linearis* (rooibos) can also be

a rich source of flavonoids. Other main components that may modulate bone health include caffeine (but not in rooibos) and fluoride. Caffeine, fluoride and flavonoid content can vary due to differences in a growing season, the location, the age of the plant and how the plant is processed (Cabrera, et al., 2003; Lung, et al., 2003).

2.4.1 Caffeine

Several of the studies summarized in Table 1 and Table 2 were conducted because of the concern that high caffeine intake may be detrimental to bone health. Caffeine, a diuretic, is found in most tea, including green, oolong, and black tea. However, rooibos tea does not contain caffeine. Black tea in particular, contains the largest concentration of caffeine, approximately 61mg/8oz (Chin, et al., 2008), but this level is considerably less than a similar sized serving of coffee (McCusker, et al., 2003). High consumption (Ó4 cups/day of coffee (Hernandez-Avila, et al., 1991)) of caffeinated beverages can be a concern for bone health with its ability to cause higher excretion of urinary calcium (Massey, et al., 1993) and this has been associated with accelerated bone loss in elderly women (Rapuri, et al., 2001). A study in Sweden which followed women ages 40-76 over 10 years found that excessive caffeine intake equating to over 300 mg caffeine/day was associated with a significant increase in osteoporotic fracture, but attributed this to be partially due to inadequate calcium intake (Hallstrom, et al., 2006). In contrast, BMD assessed in 580 postmenopausal women at 5 skeletal sites of did not find any association between caffeine consumption (120-mg/day - 480mg/day, 25^{th} ï 75^{th} percentile) and BMD (Grainge, et al., 1998). More recently, a study that analyzed data from 38,984 women from the Swedish Mammography Cohort demonstrated that higher coffee consumption was not related to

an increased rate of fracture. However, high caffeine intake (Ó4 cups/day) was associated with significantly lower lumbar spine (4%), proximal femur (2%) and total body (2%) BMD compared to women who consumed < 1 coffee/day (Hallstrom, et al., 2013). These negative effects are believed to be in part due to lower than recommended intake of calcium. Women with calcium intakes of approximately 745 mg/day and high intakes of caffeine (Ó450 mg/day) have lower spine and total BMD. Yet, women who achieved calcium intake levels above 745 mg/day, did not see an effect of caffeine on spine and total BMD (Harris, et al., 1994).

In cell culture studies, caffeine decreased collagen formation, mineralization, osteocalcin levels and ALP in chick osteoblasts (Tassinari, et al., 1991). Similarly, rat bone marrow-derived mesenchymal stromal cells that differentiated into osteoblasts demonstrated a similar decrease in ALP expression, RUNX2 and collagen1 after exposure to caffeine (Zhou, et al., 2010). In rat osteoblasts, caffeine negatively affected OPG release, while enhancing the production of RANKL (Liu, et al., 2011). Caffeine exposure to two human osteoblast cell lines has also shown to negatively impact bone formation through reduced expression of 1,25-dihydroxy vitamin D3 receptors (Rapuri, et al., 2007), and enhanced expression of glucocorticoid receptors (Focking, et al., 2005). However, caffeine has demonstrated to be a positive modulator of osteoclast activity by increasing differentiation and maturation, as shown by increased levels of TRAP and cathepsin K (Choi, 2013).

2.4.2 Fluoride

Fluoride, another common element found in tea may modulate bone metabolism. Fluoride largely exists in tea due to the water used to boil tea leaves and therefore can vary

largely among geographical areas. In comparison to the placebo group, women who consumed 75 mg/day of fluoride with 1500 mg/day of calcium had higher BMD at sites with high ratios of trabecular bone to cortical. But, BMD was reduced in sites that contained a higher proportion of cortical bone (Riggs, et al., 1990). It is suggested that because of the increase in matrix production in the trabecular bone, mineral from the cortical bone is rearranged and deposited in the trabecular bone, resulting in lower cortical BMD. In addition, new mineralization sites at the cortical bone may have the hydroxyapatite replaced by a fluoride analogue, thereby reducing the strength of the bone (Riggs, et al., 1990). This was evident by the significant increase in non-vertebral fractures in the fluoride group, suggesting that while fluoride may result in higher BMD, it is not beneficial for bone structure (Riggs, et al., 1990). Although rare, there have been several reports published on individual cases where higher consumption of tea has resulted in fluoride bone disorders. This occurs due to fluoride intake levels being orders of magnitude greater than the average individual (Johnson, et al., 2007) and above the recommended intake guidelines (Fawell, et al., 2006). A high intake of fluoride leads to the formation of fluorapatite crystals rather than hydroxyapatite in bone. Fluorapatite is detrimental to bone health as it results in bone with lost elasticity and tensile strength (DePaula, et al., 2002), thus increasing the likelihood of fracture. According to the World Health Organization, skeletal fluorosis arises at levels greater than 10mg/L and therefore the acceptable limit for fluoride in drinking water is 1.5mg/L (Fawell, et al., 2006).

2.4.3 Flavonoids

The positive relationship between consumption of tea and BMD that predominates in many studies (as summarized in Table 1) may be due to the presence of flavonoids that mimic the activity of estrogen. Thus, flavonoids may also regulate inflammation and cellular stress via antioxidant activity. These effects include decreased apoptosis in osteoblasts that enhance the activity of osteoblasts relative to osteoclast activity. In addition, flavonoids may enhance the production of mineral and regulate bone markers by interacting with osteoblasts, while inhibiting activity and/or increasing apoptosis in osteoclasts (Cooper, et al., 2005; Marnewick, et al., 2000).

Estrogen (Figure 1 a) is a key modulator of bone health and its receptors (ERŭ, ERŏ) are found on various cell types in order to mediate a biological effect. The loss of estrogen production at menopause is strongly associated with a significant reduction in BMD and bone strength, and an increased the risk of a fragility fracture (Lee, et al., 2003). When a substrate, such as a hormone binds to an estrogen receptor, the two will form a complex that results in a translocation of the receptor to the nucleus. This new complex will give access for binding of response elements using its activator protein 1 (AP1) site. Binding of the estrogen response elements allow for recruitment of proteins that may activate or repress gene expression (Deroo, et al., 2006). Identifying potential dietary components ï such as flavonoids - that can bind to or influence these receptors may be beneficial in supporting bone health.

Flavonoids are abundant in tea as well as fruits and vegetables, and represent a class of chemical compounds with multiple cyclic rings (Figure 1) that are responsible for adding taste, smell and color to food and beverages. These compounds can be further broken down into classes such as: flavones, flavonols, flavanones, isoflavones, catechins, anthocyanidins and

chalones, which are dependent on the functional groups that surround their ring systems (Hollman, et al., 1999). Flavonoids have become increasingly important because of their structural similarity to hormones such as estrogen, the dominant hormone that mediates bone turnover (Falahati-Nini, et al., 2000; Morishima, et al., 1995). Because different types of tea contain diverse combinations and levels of flavonoids, it is useful to review what is known about each type of tea and its corresponding flavonoids.

2.5 Green tea

Originating from China, the popularity of green tea ï made from the leaves of *Camellia sinensis* - has increased over the past decade and is widely consumed across North America. Green tea is present in a wide range of beverages, supplements and in various foods. This non-fermented tea contains caffeine and is largely recognized for its high polyphenol content and antioxidant capacity (Chen, et al., 1995; Gadow, et al., 1997), while also being suggested as a cure for many diseases (Choi, et al., 2001; Lee, et al., 2011; Rezai-Zadeh, et al., 2005; Yang, et al., 2010).

Green tea flavonoids such as epigallocatechin gallate (EGCG, Figure 1 c) have been associated with higher BMD (Muraki, et al., 2007; Shen, et al., 2008) and a reduction in osteoclast activity (Morinobu, et al., 2008). Green tea polyphenols (0.5% in water) given to rats with chronic inflammation for 12 weeks demonstrated a significant increase in BMD at the femur, and a significant decrease in tartrate-resistant acid phosphatase and 8-hydroxy-2ô-deoxyguanosine, both markers of bone resorption (Shen, et al., 2010). A six-month intervention with green tea polyphenol and Tai Chi exercise proved beneficial to a group of 171

postmenopausal women with osteopenia (Shen, et al., 2012). Ingestion of green tea polyphenols significantly increased muscle strength (at 6 months), alkaline phosphatase activity and also the ratio of alkaline phosphatase activity to tartrate-resistant acid phosphatase activity (at 3 months), demonstrating a positive effect on bone turnover. When participants consumed green tea polyphenols in addition to participating in Tai Chi, they were able to maintain a significantly higher ratio of alkaline phosphatase to tartrate-resistant acid phosphatase activity for the entire 6 months, in addition to elevated muscle strength (Shen, et al., 2012).

2.6 Black tea

Black tea also originates from the leaves of *Camellia sinensis*, and makes up the largest market in tea sales for North America (Tea Association of USA, 2013). It is primarily recognized for its antioxidant capacity (Chen, et al., 1995; Gadow, et al., 1997) and caffeine content (Chin, et al., 2008). Due to the oxidation that takes place to produce the tea, black tea contains a variety of different flavonoids such as theaflavins (Figure 1 d). When theaflavins were compared to green tea catechins such as epicatechin gallate (ECG) and EGCG, theaflavins were shown to be equally effective as antioxidants (Leung, et al., 2001). Therefore black tea may be effective in mitigating bone loss and promoting healthy bone turnover.

Studies have shown that administering black tea extract to 3 month old, ovariectomized rats for 28 days demonstrated higher BMD levels at the right femur, thoracic rib and vertebra in comparison to the ovariectomized group (Das, et al., 2004). Furthermore, rats that were given the black tea extract had higher levels of calcium and phosphate within their bones in comparison to ovariectomized rats. This was complemented by the lower levels of calcium, phosphate,

creatinine and hydroxyproline in the urine (Das, et al., 2004). Intervention with a black tea extract (1 ml/100g body weight) has also been shown to enhance intestinal absorption of calcium in female ovariectomized Wistar rats (Das, et al., 2013). These rats demonstrated a significantly higher rate of Ca^{2+}-activated ATPase within the duodenum, jejunum and ileum. Moreover, rats given black tea extract had higher levels of alkaline phosphatase activity and calcium transferase than controls. When ovariectomized rats were assessed for hormonal levels, rats given black tea extract had almost tripled the levels (41.52 pg/ml *versus* 14.81 pg/ml) of 17ϐ-estradiol in comparison to control, with no significant differences in parathyroid hormone. This may due to the flavonoids interacting with receptors to up-regulate the production of 17ϐ-estradiol (Das, et al., 2013). However, these results are contradictory to a study which assessed 109 Polish women and compared their tea consumption to their 17ϐ-estradiol levels. Women who consumed above the mean daily intake of black tea (473 mL) had significantly lower 17ϐ-estradiol levels than women below the mean (Kapiszekska, et al., 2006). Furthermore, black tea contains both theaflavins and catechins, both of which have been shown to have inhibitory effects on CYP19, an aromatase responsible for the conversion of androgens into estrogens (Goodin, et al., 2003; Way, et al., 2004). Most interestingly, rats supplemented with estradiol (10 µg/kg body weight) that were ovariectomized, had no difference in urinary bone markers (calcium, phosphate, hydroxyproline, creatinine) or serum parameters (alkaline phosphatase, tartrate alkaline phosphatase activity) when compared to rats supplemented with black tea (Das, et al., 2013). As black tea extract is largely concentrated polyphenols, and it has the potential to increase estrogen levels found within ovariectomized rats, it is likely that the flavonoids are able to act as both antioxidants and estrogen mimics and therefore promote bone health.

2.7 Rooibos tea

Rooibos (*Aspalathus linearis*), found in the mountainous areas of Western Cape, South Africa, also provides a rich source of flavonoids. Rooibos is an herbal tea or tisane rather than traditional tea that is made from *Camellia sinensis.* The plant contains very thin, needle-like leaves that can be used for fermented (green rooibos) or unfermented (red rooibos) rooibos tea (McKay, et al., 2007, Van Wyk, et al., 2011). Potential health benefits of rooibos tea are of particular interest because of its high level of flavonoids, potent antioxidant activity, and lack of caffeine that could potentially compromise bone health.

Flavonoids in rooibos tea have been shown to neutralize free radicals (Joubert, et al., 1996), making them desirable for biological systems. The antioxidant activity of rooibos was demonstrated using a rat model. Rats given rooibos tea had reduced lipid peroxidation in the presence of fumonisin, a free radical agent that promotes liver cancer (Marnewick, et al., 2009). This strong antioxidant potential has been reinforced by a human intervention study. Among forty volunteers who consumed 6 cups of rooibos tea daily for 6 weeks, total plasma flavonoid concentrations of the subjects increased by approximately 13% and lipid peroxidation decreased by 58.5 nmol/mL. Additionally, the ratio of reduced glutathione to oxidized glutathione increased with consumption of rooibos tea and was maintained after completion of the study (Marnewick, et al., 2011). This finding suggests that rooibos tea may have the potential to positively influence bone health through its antioxidant behavior.

As the benefits may be mediated through flavonoid interactions, it is important to note that higher grades are associated with higher flavonoid concentrations (Joubert, et al., 2012).

When assessing different grades of rooibos for flavonoid content, higher levels of the following flavonoids: aspalathin, isoquercetin, rutin, hyperoside, quercetin-3-O-robinobioside, were observed with higher quality rooibos (Joubert, et al., 2012). Flavonoid content can also be altered through fermentation. An analysis of rooibos compared flavonoid concentrations from 100g of fermented and non-fermented rooibos leaves. Quantifiable flavonoids from rooibos in both fermented and unfermented conditions are listed in Table 3 (Joubert, et al., 2011). Several of the main flavonoids in rooibos are not present in other types of tea.

3.0 Potential Mechanisms of Flavonoids

Anti-cancer (Casagrande, et al., 2001; Cheng, et al., 2013; Post, et al., 1992), anti-bacterial (Battikh, et al., 2013; Boyanova, 2014), anti-viral (Cantatore, et al., 2013; Song, et al., 2005) activities as well as potential effects on bone metabolism are all important characteristics of tea flavonoids. Bioavailability is also a characteristic for consideration as flavonoids must enter plasma to produce an effect on the surrounding environment. As rooibos contains a variety of flavonoids that are largely unique compared to other teas, the bioavailability of rooibos flavonoids were assessed by having participants fast overnight and consume 500 mL of rooibos tea. Immediate analysis of plasma indicated that a large variety of flavonoids can enter plasma after consumption of rooibos (summary of findings in Table 3, Breiter, et al., 2011). Bioavailability of flavonoids from black tea, green tea and a green tea extract have also been analyzed after individuals consumed either 4 bags of black tea or 3 bags of green tea, steeped in 426 mL, to match the EGCG content of the green tea extract. Epigallocatechin (EGC), epicatechin (EC), EGCG and ECG were detected in plasma after consumption of green tea, black

tea and green tea extract. Green tea extract provided the highest levels of absorption for all catechins (Henning, et al., 2004). Another study compared the bioavailability of green tea catechins after consumption of either a green tea extract (contains 618mg of ECGC, 168 mg of epigallo catechin and 166 epicatechin and 77 mg of epicatechin gallate) or 580 mg of purified EGCG. Using high performance liquid chromatography, green tea catechins were detected in plasma at a maximum concentration of 3.2 µM after consumption of the tea extract. Consumption of the purified EGCG was detected in plasma at 0.5 µM (Henning, et al., 2005). Tea flavonoids such as green tea catechins and black tea theaflavins have also been detected in the small intestine, liver, and prostate tissue after participants consumed 284 mL of tea (black, green) 5 times/day for 5 days (Henning, et al., 2006). Through their ability to enter plasma, flavonoids from tea may modulate bone health through estrogen-like activity ï acting as antioxidants and/or having direct effects on bone forming osteoblasts or bone resorbing osteoclasts.

3.1 Action as antioxidants:

Reactive oxygen species are a marker of oxidative stress and are released by osteoclasts to aid in the digestion of mineral, to increase bone resorption. In aging male Wistar rats, markers of oxidative stress increased and antioxidant defense systems such as superoxide dismutase decreased during aging. This increase in oxidative stress was negatively correlated to BMD at the femur (Zhang, et al., 2011). Oxidant stress in post-menopausal women (n = 135) was also shown to be negatively associated to BMD at the lumbar spine, total hip, femoral neck, trochanter and type 1 collagen C-telopeptide (Baek, et al., 2010). Antioxidants such as vitamin E

have been shown to help alleviate oxidative stress in an environment and limit bone resorption, resulting in optimized bone growth (Seifert, et al., 1997). It is currently understood that free radical production can up-regulate NF-əƃ that activates bone resorption (Sheweita, et al., 2007). When a biomarker for oxidative stress (8-iso-PGF2a) was measured in 101 men and women and compared to BMD, a negative, linear association was discovered between 8-iso-PGF2a and total BMD, as well as 8-iso-PGF2a and BMD at the lumbar spine (Basu, et al., 2001). Moreover, free radicals have been shown to inhibit osteoblast differentiation; however these effects could then be counteracted by treatment with antioxidants (Mody, et al., 2001).

Flavonol levels are positively correlated to antioxidant status, with green tea containing the highest antioxidant levels compared to regular black and decaffeinated black teas (Henning, et al., 2003). Consumption of 400 mL of green tea in humans significantly increased antioxidant capacity as early as 20 minutes after ingestion, relative to control (Benzie, et al., 1999). Despite differences in flavonoid content, both fermented and unfermented rooibos tea have high levels of antioxidant activity. Fermented tea resulted in a 6.6% higher antioxidant capacity within human plasma while unfermented tea increased antioxidant capacity by 2.9%, compared to control (Villano, et al., 2010). Antioxidant status was improved in participants who consumed fermented rooibos tea for six weeks, shown by a significant increase in reduced levels of glutathione (Marnewick, et al., 2011). Comparison of antioxidant activity in green, oolong, black, unfermented rooibos, semi-fermented rooibos and fermented rooibos teas, demonstrated that green tea and unfermented rooibos tea had similar potential at scavenging free radicals, with an inhibition of 91% and 87%, respectively (Gadow, et al., 1997). In contrast, another study indicated that black tea contained not only a higher concentration of flavonoids (105mg/g *versus*

68.4mg/g), but also out-competed rooibos tea, (fermented and unfermented) in antioxidant activity (Bramati, et al., 2003). A more recent study suggested that 1 µg/ml of rooibos tea extract was better at protecting cardiomyocytes from cellular stress in comparison to 50 µg/ml of vitamin E, a powerful antioxidant (Dludla, et al., 2014). Therefore, rooibos tea may have benefits in protecting individuals from oxidative stress related bone loss. As tea represents a good source for flavonoids, and flavonoids have been shown to be strong antioxidants, tea may provide a natural and dietary approach to mitigating bone loss.

3.2 Action on osteoblasts

Osteoblast development and activity is largely reliant on the Wnt pathway. The Wnt canonical pathway consists of Wnt, a glycoprotein, binding to the frizzled family of receptors and then to a co-receptor known as the low density lipoprotein receptor-related proteins 5 or 6 (LRP-5, LRP-6) which are required to facilitate the signaling cascade (Manolagas, et al., 2007). Upon activation of the conical pathway, a destruction complex is inactivated, preventing the degradation of ϐ-catenin. ϐ-catenin will translocate from the cytoplasm to the nucleus where it can interact with T-cell binding transcription factors to regulate gene production that promote osteoblast activity (ie. alkaline phosphatase activity, RUNX2) (Baron, et al., 2013; Glass, et al., 2005). When levels of reactive oxygen species are elevated, free ϐ-catenin levels are significantly reduced as it binds to transcriptional factor forkhead box O (FOX-O) to help counteract the oxidative stress. The binding of ϐ-catenin to FOX-O helps to increase the production of antioxidant enzymes such as superoxide dismutase, but subsequently reduces the expression of genes involved in osteoblast differentiation and proliferation (Almedia, et al.,

2007). Sclerostin (Li, et al., 2005), and Dickkopf (Fujita, et al., 2007), both inhibitors of the Wnt pathway, can bind to the lipoprotein receptor-related proteins to inactivate them and prevent the Wnt signaling pathway. This leads to a degradation of ϐ-catenin in the cytoplasm, essential for maintaining appropriate BMD.

Bone morphogenetic proteins (BMP) are also critical for the development of bone. Through binding of BMP to BMP receptors they can initiate activation of SMAD proteins. These SMAD proteins will bind to co-activators in the nucleus to increase the expression of osteoblast markers (Chen, et al., 2012). Therefore, an increase in BMP expression has shown to increase favorable osteoblast markers such as alkaline phosphatase activity, osteocalcin and RUNX2 (Chen, et al., 2012).

Rutin (Srivastava, et al., 2013), quercetin (Srivastava, et al., 2013; Wong, et al., 2008), hesperetin (Trzeciakiewicz, et al., 2010), and EGCG (Vali, et al., 2007), are just a few of the flavonoids found in tea that have demonstrated their ability to positively influence osteoblast behavior through increasing mineralization and alkaline phosphatase activity. These flavonoids have been shown to increase expression of transcriptional factors (RUNX2, osterix) important for the differentiation and maturation of osteoblasts. Rooibos flavonoids orientin and luteolin have also shown the ability to increase mineralization in human osteoblasts, by increasing ALP activity and cell mitochondrial activity, while suppressing the production of mineral inhibitors sclerostin and osteopontin (Nash, et al., 2014)

3.3 Action on osteoclasts

Estrogen deficiency results in increased levels of inflammatory markers that are able to promote the differentiation of osteoclasts. This increase in inflammatory markers is also followed by an increase in lifespan and number of active osteoclasts (Hughes, et al., 1996). RANKL and macrophage colony stimulating factor (M-CSF) are proteins that promote osteoclast precursors to differentiate and become active. Estrogen is believed to reduce osteoclast lifespan by inducing apoptosis and preventing the release of these stimulating proteins (Hughes, et al., 1996). Introduction of estrogen to bone marrow of murine cells resulted in a reduction of osteoclast precursors by preventing the action of M-CSF and RANKL on osteoclast formation. This is believed to have occurred by estrogen hindering the signaling of RANKL that was seen by a significant reduction in expression of the RANKL target c-Jun (involved in differentiation and proliferation of osteoclasts) and subsequent transcription of AP-1 (Shevde, et al., 2000). When cells were not introduced to estrogen, osteoclast formation proceeded. Furthermore, osteoclast formation increased when RANKL was added to cell culture. The addition of RANKL also led to a significant increase in c-Jun and AP-1 gene expression (Shevde, et al., 2000).

Activation of nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) is required for the expression of osteoclast genes such as tartrate-resistant acid phosphatase. Tartrate-resistant phosphatase has been positively correlated with osteoclastic differentiation and activity (Alatalo, et al., 2000; Halleen, et al., 2000; Minkin, 1982), and thus a decrease in these genes results in a significant reduction in osteoclastogenesis and bone resorption. Fisetin, a flavonoid found in a variety of plants, fruits and vegetables have demonstrated an ability to inhibit osteoclast activity through the down regulation of NFATc1 (Choi, et al., 2012). Similar to fisetin, luteolin, a

flavonoid found in rooibos tea, was able to inhibit osteoclast activity through down regulation of NFATc1, in addition to inhibiting osteoclast differentiation by RANKL (Lee, et al., 2009).

Flavonoids quercetin (Woo, et al., 2004), EGCG (Morinobu, et al., 2008), naringenin (La, et al., 2009) and kaempherol (Lee, et al., 2014) have also demonstrated the ability to down-regulate tartrate-resistant acid phosphatase, pro-inflammatory markers such as IL-1 and prevent or slow bone resorption. This suggests flavonoids have the potential to mimic the ability of estrogen to positively influence bone turnover by inhibiting the differentiation and activity of osteoclasts. Therefore habitual consumption of tea may provide a healthy, natural approach to better bone health.

4.0 Knowledge Gaps and Future Research

There is strong evidence from human studies that habitual tea consumption is positively associated with higher BMD at multiple skeletal sites while the association with fracture risk is less clear. Fracture studies demonstrate a reduction or no difference in fragility fracture with tea consumption. There are key questions that need to be answered in future studies to clarify if higher consumption of tea not only supports a healthy BMD but also reduces the risk of fragility fracture. And if the latter relationship is shown to exist, studies to elucidate mechanisms can be designed and executed. Key gaps in knowledge are the following:

Type of tea consumed: Most studies to date have not specifically assessed the type(s) of tea consumed. This is important information given the different flavonoids that are present in specific teas and also the variable levels of flavonoids in teas. Given the unique flavonoids

present in rooibos tea, and the fact that it contains no caffeine, there are exciting opportunities to specifically investigate this tea because of its distinct differences from green and black teas.

Quantity of tea consumed: The quantity of tea consumed among studies is not consistently reported in detail, i.e. 1-2 cups per day, 3-4 cups per day, 1 cup per week. Many studies use cut-offs above or below a certain intake that is arbitrarily set by the investigators. Thus, it is difficult to have a consistent definition of the tea intake of a ôhabitualô tea consumer in many of the studies discussed.

Life stages: Many studies include a wide range of ages. Determining when an individual experiences the greatest potential benefit would be useful. Furthermore, hormone levels are vastly different in individuals who are at menopause compared to young females. This may represent an issue if flavonoids are imitating hormones that are currently present in the body, as they do not have the affinity nor the levels to compete with estrogen to produce a biological affect.

Sex: Potential sex-based responses need to confirmed and understood. Studies to date suggest that, in general, females receive benefit pertaining to their BMD from habitual consumption of tea. When men were incorporated into the studies this positive effect was largely lost. However it is noted that compared to non-consumers, European men demonstrated a lower rate of fracture when they habitually consumed tea (Kanis, et al., 1999). With very few studies focused on the male population, more research is required to elucidate and confirm any effect of tea drinking in males.

Bioactivity of flavonoids: Identifying the biologically active components in tea which contribute to better bone health (i.e. higher BMD, lower rate of fracture) is important to better understand the influence that dietary nutrients can have on a skeletal system. It is important to assess how small changes in a structure can positively influence osteoblast activity. Understanding these interactions may be useful for supplement and drug related discoveries as it can isolate key areas of the structure that are vital for eliciting biological changes.

Mechanisms: There is a need to identify which pathways the active components of tea are influencing. Identifying such pathways is important for increasing our understanding of bone physiology and how potential dietary components can influence them.

Diet: More information is required to understand how consumption of a meal with tea can influence bioavailability and bioactivity of tea flavonoids in comparison to studies that assess flavonoid bioavailability and activity after fasting. Consumption of food with tea can influence the absorption of flavonoids potentially through acts of synergism that may help to increase bioavailability, or if food components may inhibit absorption. Identifying these changes are important as consumption of food with a beverage is a more realistic situation for the average individual and many studies incorporate fasting to isolate flavonoid bioavailability attributed to tea alone.

5.0 Conclusion

Habitual consumption of tea in postmenopausal women is generally associated with better bone health, as indicated by higher BMD than non-consumers. How tea consumption translates to risk of a fragility fracture requires further investigation. Tea contains high

concentrations of estrogen mimics known as flavonoids, and as such, these flavonoids may act as antioxidants, increase osteoblast activity and inhibit bone resorption by osteoclasts.

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Table 1. Summary of Cross-Sectional and Prospective Studies Investigating Tea Consumption and BMD

Study	Country of Study, Sample Size, Sex, Age	Tea Consumed*	Type of Study	Main Findings
Hoover, et al., 1996	Canada *n* = 62 F +57yrs	Tea consumers: Number of 6 ounce servings of tea/day	Cross-sectional	Tea consumption was positively correlated to lumbar spine ($p < 0.01$) and femoral neck ($p < 0.01$) BMD.
Hegarty, et al., 2000	England *n* = 1,256 F +65yrs	Tea consumers: 1-3 cups of tea/day, 4-6 cups of tea/day or 4+ cups of tea/day	Cross-sectional	Tea consumers had 5% greater BMD at lumbar spine ($p = 0.03$), greater trochanter ($p = 0.004$) and Wardôs triangle ($p = 0.02$) than non-consumers. No difference in BMD when amount of tea consumed was compared (1-3 cups/day and ≥4 cups/day). Addition of milk did not result in any differences
Wang, et al., 2014	China *n* = 680 F 62yrsÿ	Oolong tea consumers: <1 cup/day Ó1 cup/day	Cross-sectional	Tea consumers had a higher BMD at the greater trochanteric ($p = 0.013$) and a higher Wardôs triangle ($p = 0.013$) BMD in comparison to non-consumers
Muraki, et al., 2007	Japan *n* = 632 F +60yrs	Green tea consumers: > 5 days/week	Cross-sectional	Consumers of green tea had higher BMD ($p < 0.05$) and better T-scores (-1.59±2.70 *versus* -2.17±2.08, $p < 0.05$) at the lumbar spine than those who did not consume tea.

Hsiao, et al., 2012	Taiwan *n* = 100 F 28-70yrs	Tea consumers	Cross-sectional	Tea consumers had a higher hip BMD in comparison to non-consumers ($p < 0.01$)
Vestergaard, et al., 2001	Denmark *n* = 2,016 F 45-58yrs	Tea consumers	Cross-sectional	Tea consumption was positively associated with T scores above -0.75 for BMD at the femoral neck, but not at the lumbar spine.
Hossein-Nezhad, et al., 2007	Iran *n* = 830 M, F +20yrs	Tea consumers: Ó5 cups of tea/day	Cross-sectional	Women who habitually consumed tea in had higher BMD at the hip ($p = 0.01$) but not spine ($p < 0.07$). Among males, no differences in BMD of consumers and non-consumers.
Kyriazopoulos, et al., 2006	Greece *n* = 300 M 18-30yrs	Tea consumers	Cross-sectional	No significant relationship between tea intake and BMD or tea intake and bone mineral content of the distal and ultra-distal radius ($p > 0.05$).
Saitoglu, et al., 2007	Turkey *n* = 70 M 45-65yrs	Tea consumers	Cross-sectional	No significant relationship between tea intake and BMD at the lumbar spine and femur ($p > 0.05$).
Devine, et al., 2007	Australia *n* = 1,027 F +70yrs	Tea consumers *Herbal tea was not included for tea consumption.*	Cross-sectional	Tea consumers had a significantly higher BMD in comparison to non-consumers for total hip ($p < 0.05$) and trochanter ($p < 0.01$) BMD.
Devine, et al., 2007	Australia *n* = 164 F +70yrs	Tea consumers *Herbal tea was not included for tea*	Prospective (4 years)	Tea consumers lost 1.4% of total hip BMD *versus* non-consumers who lost 4.7% in the fully adjusted model (*p*

		consumption.		< 0.01). Tea consumers lost 2.0 % of greater trochanter BMD *versus* non-consumers who lost 6.3% ($p < 0.01$). Tea consumers lost 1.0% of intertrochanter BMD *versus* non-consumers who lost 4.5% ($p < 0.01$).
Wu, et al., 2002	Taiwan n = 1,037 M, F +30yrs	Habitual versus non-habitual tea consumption (at least 6 months) Categories: 1-5 years, 6-10 years >10 years	Prospective (2-4 years in duration)	Tea consumers (for 6 or more years) had greater lumbar spine BMD ($p < 0.01$), hip BMD (at the neck and Ward triangle, $p < .05$) and total body BMD ($p < 0.001$) than non-consumers. There was no significant relationship between BMD at any site when tea was consumed less than 5yrs ($p > 0.05$). There was no significant relationship between BMD (lumbar spine, hip and total body) of those who consumed green and oolong tea *versus* those who consumed black tea.
Hardcastle, et al., 2011	Scotland n = 3,226 F 45-54yrs	Flavonoid consumption	Prospective (5-9 years in duration)	57% of flavonoid intake was attributed to consumption of tea. Total flavonoid levels were correlated with femoral neck ($p < 0.01$) and lumbar spine ($p < 0.05$) BMD. Catechins and procyanidin levels were correlated with % annual change in BMD at both the femoral neck and lumbar spine ($p < 0.05$). Total flavonoids were negatively correlated to free

				deoxypyridinolines (a marker of bone resorption) ($p <$ 0.05). A higher flavonoid intake relates to a decrease in bone resorption ($p = 0.001$).

*Tea consumed includes consumption of all tea (green, black, oolong, herbal tea) unless specified and tea consumer indicates no quantitative measure used if no quantity is specified.

M = male, F = female

ÿ indicates average age.

The Womenôs Health Initiative in the United States measured both BMD and fracture, and therefore can be found in Table 2

Table 2. Summary of Case-Control, Cross-Sectional and Prospective Studies Investigating Tea Consumption and Fracture

Study	Country of Study, Sample Size, Sex, Age	Tea Consumed*	Type of Study	Main Findings
Jha, et al. 2010	India *n* = 100 M F 65yrsÿ	Ò1 cup/day, > 1 cup/day	Case-control	Tea consumers had an increased risk of fracture ($p <$ 0.001)
Tavani, et al., 1995	Northern Italy *n* = 279 F 19-74 yrs	Tea consumers or non-consumers	Case-control	No significant association between tea consumption and fracture
Kreiger, et al., 1992	Canada *n* = 102 hip fractures *n* = 154 wrist fractures 50-84 yrs	< 3 cups/day Ó3 cups/day	Case-control	No significant association between tea consumption and fracture
Du, et al., 2011	China *n* = 703 M F 90+yrs	Tea consumers or non- consumers	Case-control	No significant association between tea consumption and fracture
Zeng, et al., 2013	China *n* = 581 M F 55-80 yrs	Tea consumers: at least 1 cup/week within the previous 6 months	Case-control	No significant association between traditional dietary patterns (includes consumption of Chinese herbal tea) and risk of hip fracture.
Chen, et al., 2003	United States *n* = 91,465 F +50yrs	<1 cup/day, 1 cup/day, 2-3 cups/day, >4 cups/day *Herbal and decaffeinated tea was not included for tea consumption.*	Prospective (4.1 years in duration)	Tea consumption was positively correlated to total body BMD (p = 0.03). Individuals who consumed at least 4 cups/day had greater total body BMD than those who consumed less than 1 cup/day (p <0.05) and individuals who consumed between 2-3 cups/day had

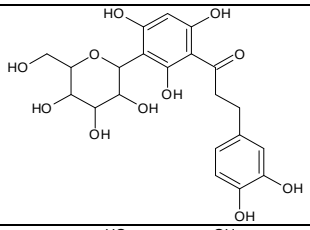
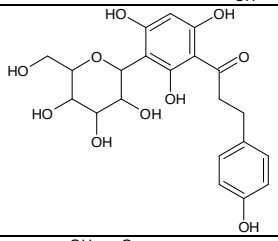
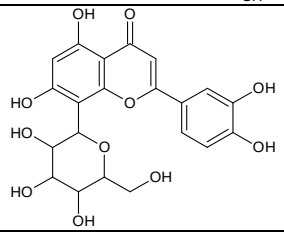
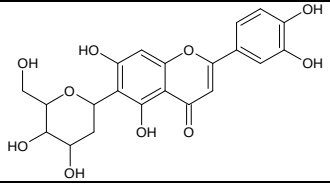
				higher BMD at the lumbar spine than those who consumed less than 1 cup/day (p<0.05). No significant difference in incidence of fragility fracture between consumers and non-consumers. Fractures were listed as hip fractures, forearm or wrist fractures and other fractures.
Hallstrom, et al., 2006	Sweden n = 31,527 F +40yrs	Black tea <1 cup/day, 1 cup/day, 2-3 cups/day, Ó4 cups/day	Prospective (10.3 years in duration)	No significant difference in incidence of fragility fracture between consumers and non-consumers. Osteoporotic fractures were identified as a first incident fracture at the proximal femur, hip, pelvis, spine, distal forearm and proximal humerus.
Kanis, et al., 1999	Europe n = 1,762 M +50yrs	Tea consumers or non- consumers	Prospective (1 year in duration)	Tea consumers had a lower incidence of hip fracture (p = 0.0035)
Johnell, et al., 1995	Europe n = 5,618 F +50 yrs	Tea consumers or non- consumers	Prospective (1 year in duration)	Tea consumers had a lower incidence of hip fracture (p < 0.001)

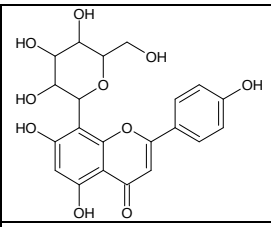
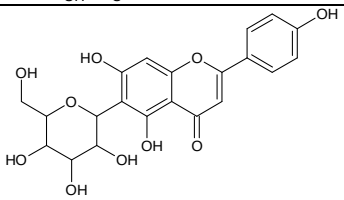
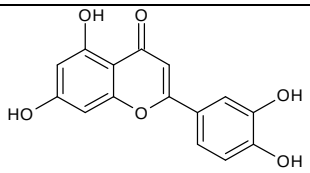
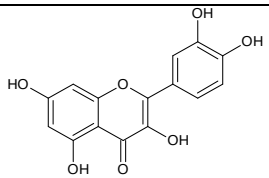
*Tea consumed includes consumption of all tea (green, black, oolong, herbal tea) unless specified and tea consumer indicates no quantitative measure used if no quantity is specified.

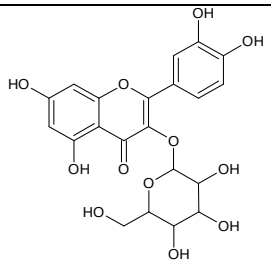
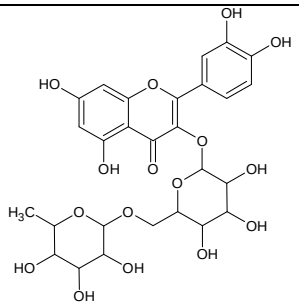
M = male, F = female

ÿ indicates average age

Table 3. Flavonoids in rooibos tea, food and botanicals. Flavonoid content of fermented and unfermented rooibos tea (De Beer and Joubert 2011), in addition to flavonoids quantified in rooibos tea found in plasma after ingestion (Breiter, et al., 2011)

Compound	Structure	Fermented Rooibos tea (g/100g)	Unfermented Rooibos tea (g/100g)	Average Weight (mg/500ml)	Plasma Range (nmol)	Food or Botanicals other than Rooibos Tea (mg/100g) (Rothwell, et al., 2013)
Aspalathin		2.559 ± 0.699	0.421 ± 0.017	287	0.0-1.9719	None
Nothofagin		0.251 ± 0.230	0.040 ± 0.022	34.4	-	None
Orientin		0.263 ± 0.087	0.202 ± 0.026	17	0.0-0.7243	Black olives (0.22mg)
Iso-orientin		0.450 ± 0.163	0.329 ± 0.049	26	0.0-0.1212	None

Vitexin		0.042 ± 0.020	0.035 ± 0.009	3.1	0.0-0.2892	Buckwheat (0.90mg)
Iso-vitexin		0.049 ± 0.029	0.035 ± 0.012	2.8	0.0-0.0611	None
Luteolin		0.007 ± 0.006	0.010 ± 0.005	NA	NA	Mexican oregano (56.33mg), Artichoke heads (42.10mg), Thyme (39.50mg), Sage (33.40mg), Black Olives (3.43mg)
Quercetin		0.001 ± 0.001	0.010 ± 0.001	NA	NA	Mexican oregano (42.00mg), Black Elderberry (42.00mg). Capers (32.82mg), Cloves (28.40mg), Chocolate (25.00mg

						)
Hyperoside		0.021 ± 0.012	0.016 ± 0.015	0.8	NA	Black Chokeberry (46.46mg), Lingonberry (13.22mg), American cranberry (10.81mg), Black tea (4.17mg)
Rutin		0.245 ± 0.141	0.173 ± 0.016	7.9	0.0-0.4686	Buckwheat whole grain flour (37.27mg), Capers (332.29mg), Olives (45.36mg), Asparagus (23.19mg), Black raspberries (19.00mg)

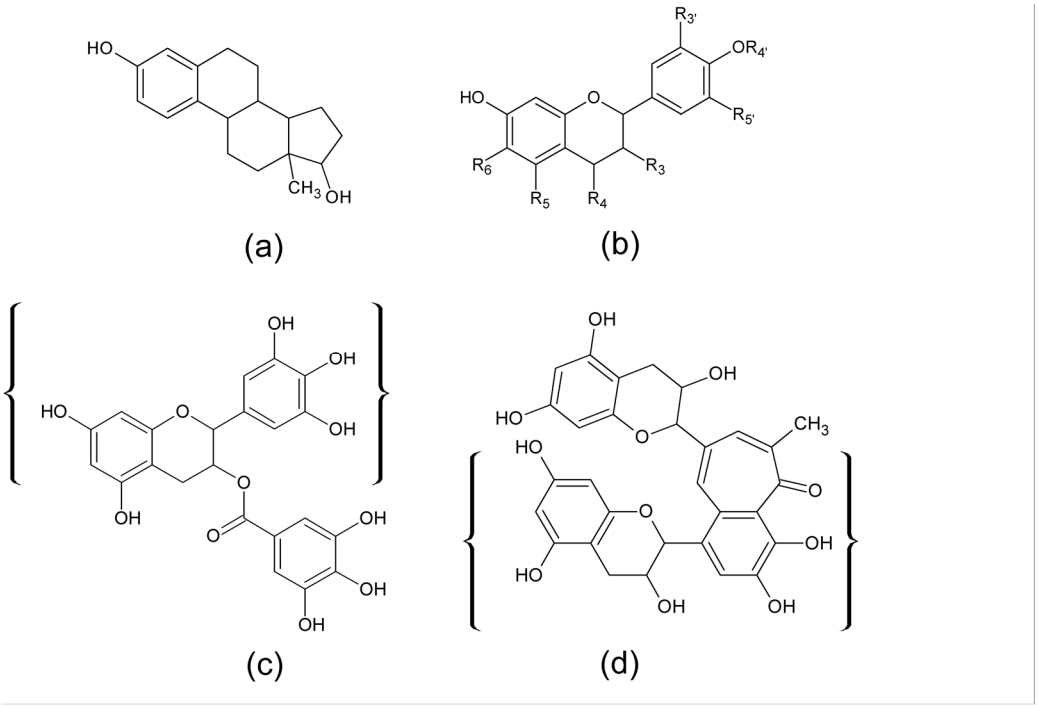


Figure 1. The structure of (a) 17β-estradiol, (b) the general flavonoid backbone, (c) epigallocatechin gallate (EGCG) from green tea, and (d) theaflavin from black tea. The bracketed portion marks the flavonoid backbone. R represents replacement with different chemical moieties and Rôare editions on to the backbone based on the subclass of flavonoid.