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Review article

Chocolate and chocolate constituents influence bone health and osteoporosis risk

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ABSTRACT

Bone loss resulting in increased risk for osteoporosis is a major health issue worldwide. Chocolate is a rich source of antioxidant and antiinflammatory flavonoids and dietary minerals with the potential to benefit bone health. However, other chocolate constituents such as cocoa butter, sugar, and methylxanthines may be detrimental to bone. Human studies investigating the role of chocolate consumption on serum bone markers and bone mineral density (BMD) have been inconsistent. A contributing factor is likely the different composition and thereby the nutrient and bioactive content among chocolate types. White and milk chocolate are high in sugar and low in flavonoids and most minerals. Dark chocolate (45–85% cocoa solids) is high in flavonoids, most minerals, and low in sugar with ≥70% cocoa solids resulting in higher fat and methylxanthine content. The aim of this review was to examine the relationship between chocolate consumption and its constiuents, including flavonoid content, on bone health and osteoporosis risk. Studies showed postmenopausal women had no bone effects at moderate chocolate intakes, whereas adolescents consuming chocolate had greater longitudinal bone growth. Based on flavonoid and mineral content, unsweetened cocoa powder appeared to be the best option followed by dark chocolate with higher cocoa content in terms of supporting and preserving bone health. Determining dietary recommendations for chocolate consumption relative to bone health is important because of the growing popularity of chocolate, particularly dark chocolate, and an expected increase in consumption owing to suggestions of health benefits against various degenerative diseases.

Introduction

Media and consumer interest in chocolate and its constituents as a functional food, a nutraceutical, or both, is due to studies indicating a positive relationship between chocolate consumption and reduced risk for cardiovascular disease [1–3]. The role of chocolate as a functional food has focused primarily on its polyphenol content. However, it should be noted that chocolate and cocoa-based beverages contain other nutritive constituents such as carbohydrates, lipids, and dietary minerals, as well as non-nutritive constituents, oxalates, and methylxanthines, [4] with potential to influence diet-related chronic diseases.

Osteoporosis is a degenerative skeletal disease caused by an imbalance between bone formation and resorption, resulting in bone mineral loss and microstructural deterioration that places bones at increased risk for fracture [5]. An estimated 200 million people worldwide have osteopenia, reduced bone mineral density (BMD) or osteoporosis (BMD > 2.5 SD below the mean for young adults). This represents about one in three women and one in five men ≥50 y of age [6]. In the United States, osteoporosis-related morbidity and mortality is expected to increase 2.4-fold in women and 3.1-fold in men by 2050. The authors attributed the greater projected increase of osteoporosis in men to their improved life expectancy [7]. Treatments for osteoporosis include medications and hormone therapy [8]. However, the high cost of drugs, inconvenient dosing regimens, and potential side effects (e.g., esophagitis, gastrointestinal symptoms) often result in poor compliance [9]. Nutrients important to bone health such as vitamin D, calcium, phosphorus, magnesium, and trace minerals (e.g., copper, zinc, and iron) are readily available in the diet or as dietary supplements [10,11]. Diets that promote bone health have mainly focused on increasing calcium and vitamin D consumption [12], but there is growing interest in phytochemicals.

Dietary polyphenols consist of a large group of plant-derived secondary metabolites divided into four different classes, one of

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which is the flavonoids (diphenylpropanes C_6-C_3-C_6). Flavonoids contain several classes of bioactive compounds [13] (Fig. 1). Evidence has demonstrated that intake of specific flavonoids may promote bone health. These include soybean isoflavones (e.g., genistein and daidzein), flavonols (e.g., aglycone quercetin) found in plums, and flavonones (e.g., hesperidin) found in citrus pulp and juice [14–16]. Another food source noted for its flavonoid content is chocolate [17]. Cocoa, a major constituent in chocolate, has the highest flavanol content of all foods on a per-weight basis and contributes to greater total dietary intake of flavonoids than tea, fruits, and vegetables [18,19].

Western Europe and the United States are among the countries with the highest chocolate consumption with a maximum of 9 kg/y (~209 regular size 43 g or 1.5 oz chocolate bars per person) in Switzerland and 4.3 kg/y in the United States [20]. Consumption patterns indicate that ~15% of women 40 to 49 y of age consume chocolate at least once every 3 d [21]. This represents a significant proportion of the population at risk for developing osteoporosis because bone is lost at a rate of ~0.2% to 0.5% per year after the age of 40 to 45 y, with bone loss in women accelerated by 2% to 5% immediately before menopause [22,23]. The aim of this study was to review evidence of a potential role of consuming chocolate, its flavonoid content, and other chocolate constituents in bone health and osteoporosis risk.

Biological plausibility

In a cross-sectional study conducted in Scotland on postmenopausal women (N = 5119, 45–54 y of age), flavonoid intake was associated with higher hip ($r = 0.054$, $P \leq 0.05$) and lumbar spine ($r = 0.036$, $P \leq 0.05$) BMD [24]. Flavonoid intake was mainly attributed to tea drinking (57%) and the flavonol; catechin was identified as the main flavonoid consumed. Another rich source of flavonoids are cocoa beans, ~37% of which are comprised of catechins [25]. (−)–epicatechin accounts for ~35% of the total phenolic content and ranged from 43.3 in raw to 34.6 mg/g in processed cocoa beans [25]. As a cocoa bean–containing product, chocolate is also a rich source of flavonoids with flavanols represented as enantiomers of (+)–catechin and (−)–epicatechin and their derivatives (Fig. 1). Halvorsen et al. [26] ranked 50 food products consumed in the United States for their antioxidant content and reported that 5 were chocolate-based foods. The antioxidant properties of chocolate have implications for bone health because chronic bone loss often is accompanied by oxidative stress and inflammation [27].

Both animal models and human clinical trials have reported an inverse association between reactive oxygen species (ROS) and bone health [28,29]. ROS can affect bone cells in various ways, including stimulation of osteoblast apoptosis and senescence and by upregulation of receptor activator of nuclear factor κ-B ligand (RANKL) to activate osteoclast differentiation and bone resorption [14]. The potential for catechins to act as antioxidant and antiinflammatory therapeutic agents for preserving bone health has been demonstrated in vitro. Primary human osteoblasts cultured in ROS-inducing cigarette smoke medium treated with catechin doses of 50, 100, and 200 μg/mL significantly reduced ROS formation [30]. Proinflammatory cytokines, interleukin (IL)-1, IL-6, and tumor nuclear factor (TNF)-α are among the important regulators of bone resorption and have been suggested to play a role in age and estrogen deficiency-related bone loss [31]. In an in vitro study, addition of TNF-α to osteoblastic MC3T3-E1 cells stimulated inflammatory cytokine IL-6, thereby promoting osteoblast apoptosis [32]. Treatment with 10^{-5} M (+)–catechin reduced cytoplasmic TNF-α concentration by 32%, increased osteoblast survival by 43%, and increased alkaline phosphatase, a marker of osteoblast activity. The authors concluded that catechins preserved bone-forming osteoblasts by exerting antiinflammatory actions. In a crossover study involving normal and hypercholesterolemic individuals, consumption of cocoa powder mixed in milk 15 g (1 tbsp) twice daily for 4 wk significantly decreased serum inflammatory biomarkers for IL-1 compared with milk alone [33]. Based on these studies, antiinflammatory and antioxidant properties of flavonoids indicate the potential for chocolate to promote bone health.

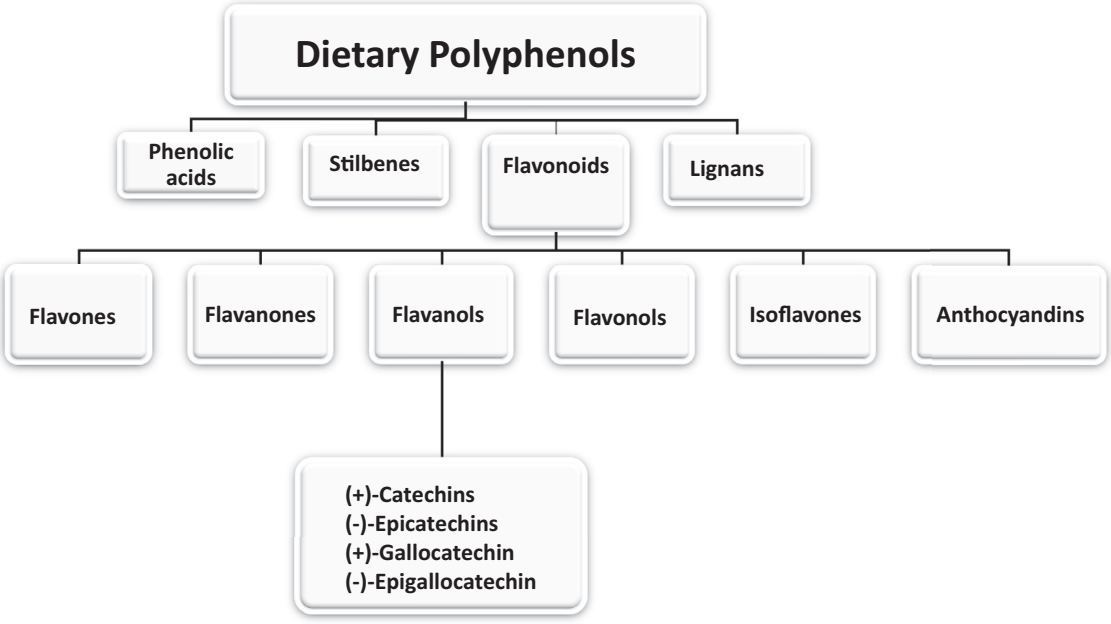

Fig. 1. Polyphenol classification. Dietary polyphenols consist of a large group of plant-derived secondary metabolites divided into four different classes, one of which is the flavonoids. Within each class of flavonoids there are various compounds.

Studies on chocolate consumption and bone health

Although studies have investigated the consumption of flavored milk such as chocolate milk as a strategy to increase milk and calcium consumption in children [34], these studies did not include an assessment of bone status. This is important because bone mineral accrual during childhood and adolescence is a key determinant of future risk for osteoporosis [10]. In a randomized double-blind, placebo-controlled study, prepubertal (n = 149, 6.6–9.4 y of age) girls consumed two calcium-supplemented food products daily to achieve a calcium dose of 850 mg/d [35]. Results showed consuming calcium-supplemented chocolate bars, cakes, or cocoa beverages for 48 wk significantly increased height and bone mass acquisition in the radius and femur. Dietary intervention to increase calcium intake using these foods resulted in high compliance (75%), and higher bone mass was maintained 1 y past termination of the intervention [35]. Based on the study results, chocolate can play a role in promoting bone mass in prepubertal girls (Table 1). However, potential mechanisms to explain greater bone mass and stature were not investigated.

A preclinical study that included a potential mechanism of negative effects on bone growth was conducted using Balb/c mice [36]. Feeding murine dams a chow diet supplemented with 400 mg unsweetened chocolate during pregnancy and lactation resulted in progeny with significantly shortened forefeet and hind limbs. Vascular endothelial growth factor, which plays a role in ontogenesis and longitudinal bone growth related to angiogenesis in the epiphyseal growth plate, was significantly reduced in the femora of immature (4 wk of age) mice pups exposed perinatally to chocolate [36]. A follow-up study investigating the relationship of the chocolate constituent, catechins on angiogenesis and bone mineralization in the progeny of murine dams fed chocolate showed a negative correlation ($r = -0.62$, $P < 0.05$) between embryo tissue epigallocatechin concentration and mean number of newly formed blood vessels [37]. Crystallinity of compact bone of diaphyses was 17% greater, and femoral epiphyseal cancellous bone was 30% greater in pups 4 wk of age exposed perinatally to chocolate compared with pups from control dams. The authors suggested that the antiangiogenic activity of chocolate catechins disturbed the processes of bone elongation and mineralization [37]. However, it should be noted chocolate intake by pregnant mice in both studies was 400 mg, which is the equivalent to daily consumption of 8.7 average-sized 46 g (1.6 oz) chocolate bars.

Lau and Ooi [38] investigated the effect of a chocolate malt drink on bone markers in healthy young men (N = 40, age 19–25 y) undertaking circuit training or remaining sedentary. Results showed a daily dose of 45 g of chocolate malt powder in water for 6 wk had no significant effect on the sedentary group. Men who trained and consumed the chocolate malt exhibited reduced ($P < 0.05$) bone resorption marker serum C-telopeptide of type I collagen (1-CTP). In contrast, a study of healthy young women (N = 44, age 19–25 y) undertaking aerobic dance training and provided daily chocolate malt powder in water for 8 weeks had no significant effects on 1-CTP but increased ($P < 0.01$) bone formation marker serum alkaline phosphatase [39]. Differences in effects of chocolate consumption on bone turnover markers between the studies were suggested to be due to sex differences. Also, the women consumed the chocolate malt drink for a longer duration. The chocolate malt powder in both studies contained milk and cocoa, which is a good source of flavonoids and minerals. Another study focused on a specific chocolate constituent [40]. Healthy, overweight, or obese premenopausal women (N = 50, age 30.3–42.2 y) following an energy-restricted diet and consuming a high flavanol cocoa beverage (280 mg flavanols/d; 60 kcals/d) and two dark chocolate snacks (Hershey's Extra Dark, 60% cacao; 90 kcals/d; 240 mg/flavanols/d) for 18 wk showed a significant increase in hip and forearm BMD ($P < 0.01$) and significant decreases in serum inflammatory cytokine TNF-α but no effect on oxidative stress. However, changes were not significantly different compared with women provided an energy-restricted diet without additional flavanols. Results indicated that the cocoa beverage and dark chocolate did not provide benefits to biomarkers of bone turnover, BMD, oxidative stress, and inflammation above that provided by weight loss [40]. These studies involved young individuals, whereas chronic bone loss owing to oxidative stress is associated with aging.

A 5-y prospective, randomized controlled trial involving older western Australian women (N = 1001, age 70–85 y) investigated

Table 1
Studies investigating effect of chocolate consumption on bone health

Participants	Chocolate dose	Results	References
Prepubertal girls Age 6.6–9.4 y N = 149	Calcium (850 mg/d) + chocolate bar, cakes, or cocoa beverage 48 wk	↑ height ↑ femur bone mass ↑ radius bone mass	Bonjour et al., 1997 [35]
Young men Age 19–25 y N = 44	45 g/d chocolate malt beverage 6 wk	NS serum alkaline phosphatase NS serum osteocalcin NS serum total calcium NS C-telopeptide 1 collagen	Lau and Ooi, 2014 [38]
Premenopausal women Age 19–25 y N = 44	45 g/d chocolate malt beverage 8 wk	↑ serum alkaline phosphatase NS serum osteocalcin NS serum total calcium NS C-telopeptide 1 collagen	Atiqah et al., 2015 [39]
Premenopausal women Age 30–48 y N = 60	cocoa beverage 60 kcals/d + 2 dark chocolate bars 60% cocoa/d; 90 kcals/d 18 wk	↑ hip BMD ↑ forearm BMD ↓ TNF-α	Piehowski, 2011 [40]
Postmenopausal women Age 70–85 y N = 1001	Chocolate + cocoa beverage <1 cup/wk 1–6 cups/wk ≥1 cup/d	NS heel BMD ≥1 cup/d ↓ tibia BMD ↓ total hip BMD ↓ femoral neck BMD ↓ trochanter BMD ↓ intertrochanter BMD ↓ whole body BMD	Hodgson et al., 2008 [41]

BMD, bone mineral density; NS, non-significant; TNF, tumor necrosis factor.

the effects of calcium supplementation on bone density and strength [41]. Australia is ranked seventh among countries with high chocolate consumption [20]. The women reported their estimated weekly chocolate or cocoa beverage intake using a food frequency questionnaire. Chocolate and cocoa beverage intakes were categorized as daily at ≥1 cup/d, moderate at 1 to 6 cups/wk, or rarely at <1 cup/wk. Measurement of heel bone density and strength using calcaneal quantitative ultrasonography showed no differences among individuals who consumed chocolate daily, moderately, or rarely. However, tibia bone density and strength determined by peripheral quantitative computed tomography were significantly lower in postmenopausal women categorized as having daily chocolate consumption than in those whose consumption was rare [41]. BMD measurements using dual-energy x-ray absorptiometry showed an inverse association between chocolate consumption for total hip, femoral neck, trochanter, intertrochanter, and whole body BMD. Based on these results, the authors concluded that daily (≥1 cup/d) chocolate consumption increased the risk for lower bone density and strength in older postmenopausal women. Study participants who reported consuming chocolate daily had higher socioeconomic status and exhibited lower body weights and body mass index values despite greater total caloric, saturated fat, total carbohydrate, and sugar intake associated with chocolate consumption. Other lifestyle factors such as physical activity levels that contribute to weight and bone status were not monitored during the 5 y intervention. Limitations of this study included reliance on self-reporting, estimates of dietary intakes, and not specifying the type of chocolate consumed. The type of chocolate consumed is an important dietary factor to consider because the ingredients and processing methods used in the manufacture of different chocolates results in varying polyphenol contents [42].

Chocolate polyphenol content

Cacao (Theobroma cacao) refers to the raw unprocessed bean from the cacao fruit tree. Cocoa bean is the term used after the cacao bean has undergone processing to obtain the ingredient used in chocolate manufacturing [43]. Variety and genetics of the cacao bean influences its polyphenol content. Even within a cacao species, polyphenol content is variable owing to factors such as the growing region, climatic conditions during growth, maturity at harvest, and storage time. Oracz et al. [44] has reviewed the influence of these factors on polyphenol content. The polyphenol content of cacao bean is ~10% dry weight making cacao bean and cocoa-containing products a rich source of dietary polyphenols [45]. Lee et al. [18] reported cocoa contains higher total flavonoids per serving than red wine or black and green tea and demonstrated the highest antioxidant activity in in vitro assays. Cocoa is rich in flavonoids mainly consisting of the flavanols (−)−epicatechin and (+)−catechins (Table 2). However, polyphenols are also responsible for the bitter and astringent taste of cacao beans. To produce cocoa and cocoa products that are more palatable to consumers, cacao beans undergo processing [46].

Immediately after harvest, cacao beans undergo fermentation for flavor development [47]. Cacao beans generally require 2-7 d of fermentation for flavor development [44]. De Brito et al. [48] reported a 24% decrease in total polyphenol content after 60 h of fermentation and a 58% decrease after 8 d of fermentation. After fermentation, cocoa beans are dried to decrease microbial growth. Fermented dried cocoa beans are then roasted at 100°C to 150°C either whole or as nibs (deshelled broken pieces). Roasting plays an important role in the formation of characteristic aromatic compounds [49]. Roasted cocoa beans or nibs are then ground to cocoa liquor for use in the manufacture of chocolate. Semisweet or bittersweet chocolate, often referred to as dark chocolate, contain <35% by weight of cocoa liquor [4]. Milk chocolate contains 10% to 12% cocoa liquor with the addition of condensed or powdered milk and sugar. White chocolate contains only cocoa butter (20% by weight) combined with sweeteners and milk ingredients. To make cocoa powder, cocoa liquor is mechanically pressed to expel cocoa butter leaving a solid cake, which is then ground into cocoa powder for use in chocolate syrups, beverages, and baked goods [50].

Both cocoa powder and cocoa liquor can be further modified by alkali treatment or “Dutching,” which darkens the cocoa ingredients and reduces bitterness [51]. Payne et al. [52] compared the effect of cacao bean processing on flavonoids and the major flavanol enantiomers, epicatechin, and catechin. Drying had a minimal effect on the epicatechin and catechin content, whereas fermentation of cocoa beans resulted in a >80% reduction of catechin and epicatechin content. Roasting cocoa beans at temperatures >70°C resulted in the loss of epicatechin. However, catechin content increased when roasting temperatures reached 120°C owing to increased epimerization of epicatechin to catechin. The processing step that produced the greatest reduction of flavanols was alkalization, which decreased catechin content by 80% and epicatechin content by up to 98%. Miller et al. [50] determined total polyphenol content, flavanol, and antioxidant activity in five unprocessed and 15 commercially available alkalized cocoa powders. Flavanol content in unprocessed cocoa powder was 34.6 ± 6.8 mg/g compared with 13.8 ± 7.3 mg/g in lightly alkalized cocoas, 7.8 ± 4 mg/g in medium alkalized powders and 3.9 ± 1.8 mg/g in heavily alkalized powders. Similarly, total polyphenol content was higher in the less

Table 2
Flavonoid content of different types of chocolate and cocoa powders from the USDA National Nutrient Database for Standard Reference

Food description	Flavonoids	Number of samples	Mean ± SEM (mg/100g)
Cacao beans	(−)−Epicatechin	3	99.18 ± NA
	(+)−Catechin	3	88.45 ± NA
Cocoa, dry powder, unsweetened	(−)−Epicatechin	13	196.43 ± 45.38
	(+)−Catechin	13	64.82 ± 14.53
	Quercetin	11	10.00 ± 2.36
Cocoa, dry powder, unsweetened, processed with alkali	(−)−Epicatechin	12	56.60 ± 15.76
	(+)−Catechin	12	36.71 ± 9.91
	Quercetin	10	3.37 ± NA
Baking chocolate, unsweetened	(−)−Epicatechin	6	141.83 ± 23.58
	(+)−Catechin	6	64.33 ± 15.49
Dark chocolate	(−)−Epicatechin	5	84.40 ± 13.54
	(+)−Catechin	5	24.20 ± 5.70
Milk chocolate	(−)−Epicatechin	9	10.88 ± 2.68
	(+)−Catechin	9	4.16 ± 1.21

NA, not available.

processed and less alkalized cocoa powders. Unprocessed cocoa powder had the highest antioxidant capacity as determined by oxygen radical absorbance capacity score, whereas heavily alkalized powder had the lowest oxygen radical absorbance capacity score. Collectively, results indicated that alkalization decreased the amount of flavanols, total polyphenol content, and antioxidant activity [50]. However, alkalized cocoa powders are typically used in non-confectionery applications, and alkalized cocoa liquors are not commonly used as major ingredients in the manufacture of chocolate confections [53].

Depending on the processing method, chocolate can contain large amounts of flavonoids, particularly catechins and epicatechins [54]. According to the US Department of Agriculture (USDA) [55], (+)–catechin content in descending order was cacao beans, cocoa powder, baking chocolate, alkalized cocoa powder, and chocolate confection (Table 2). In the US diet, chocolate is a major contributor of antioxidants after fruits and vegetables [45]. Vinson et al. [56] reported a positive relationship between non-fat cocoa solids and antioxidant activity. Miller et al. [57] reported non-fat cocoa solids were highest in cocoa powder (72–87%), followed by dark chocolate (20–30%), semisweet chocolate (15–19%), and milk chocolate (5–7%). Using 40 g (1.4 oz) of chocolate as the reference, milk chocolate provides 394 mg polyphenol antioxidants, whereas a similar serving of dark chocolate provides 951 mg polyphenol antioxidants. According to the USDA [55] data, dark chocolate is higher in both the flavanols (−)–epicatechin and (+)–catechin than milk chocolate (Table 2). However, the most popular of type of chocolate consumed in the United States is milk chocolate [53,56]. Therefore, identifying the type of chocolate consumed in studies is important.

Other chocolate constituents

In addition to flavonoid content, chocolate is a rich source of other bioactive compounds with the potential to exert either positive or negative osteogenic effects. Although chocolate is low in vitamins important for bone health, cocoa powder is used to flavor milk to encourage consumption of milk as a good source of vitamins A and D as noted by the 2015–2020 Dietary Guidelines for Americans. Recently, Kuhn et al. [58] suggested that food composition databases should be updated to show chocolate is a source of vitamin D: highest to lowest values were dark chocolate (3.95 ± 1.58 μg/100 g) then milk chocolate (1.96 ± 0.37 μg/100 g) then white chocolate (1.35 ± 0.568 μg/100 g).

Dietary mineral content

Calcium is required for appropriate mineralization of the skeleton [59]. In a study by Hodgson et al. [41], women categorized as having daily (≥1 cup/d) compared to rare (<1 cup/d) chocolate consumption showed no significant differences in calcium intake. However, calcium content can range from 14 to 281 mg/100 g depending on the type of chocolate. As shown in Table 3, among the different types of chocolates analyzed, white chocolate contained the highest amount of calcium. Sodium content was also highest in white chocolate, which may influence calcium bioavailability if part of a high-sodium diet. In a randomized crossover trial, postmenopausal women (n = 11, age 59–73 y) who were provided a high calcium (1,284 mg/d) and high sodium (11.2 g/d) diet had increased urinary calcium excretion ($P = 0.0008$) and negative bone calcium balance ($P < 0.02$) compared with postmenopausal women consuming a low calcium (518 mg/d) and low sodium (3.9 g/d) diet [60].

Another important mineral, magnesium is key for bone crystal growth and stabilization and playing a role in the vitamin D parathyroid hormone axis [59]. A meta-analysis pooling 12 studies found significant positive correlations between magnesium intake and femoral neck BMD ($r = 0.14$; 95% confidence interval [CI], 0.001–0.02) and total hip BMD ($r = 1.92$; 95% CI, 0.18–4.55) [61]. Chocolate provides a significant amount of magnesium with a typical serving (44 g; 1.55 oz) of dark chocolate providing 15% of the magnesium recommended dietary allowance (RDA) for adults [62]. Unexpectedly, in the Hodgson study [41], postmenopausal women categorized as having moderate chocolate intake (1–6 cups/wk) showed significantly lower magnesium intake than those categorized with rare (<1 cup/d) chocolate intake. However, magnesium content differs depending on the type of chocolate. As shown in Table 3, cocoa powder had the highest magnesium content, then dark chocolate with higher percent cocoa solids, then milk chocolate, followed by white chocolate with the lowest magnesium content.

Trace elements copper and zinc also are essential for normal skeletal development [63]. Copper inhibits bone resorption through its action as a cofactor for superoxide dismutase. In addition, copper-dependent lysyl oxidase is required for the formation of lysine-derived crosslinks in collagen and elastin in bone [64]. A study of male and female participants from the National Health and Nutrition Examination Survey (2011–2014; N = 722, age 44.9–68 y) demonstrated that lower serum copper levels (<98.5 μg/dL) were associated with lower femoral neck ($P = 0.02$) and total femur BMD ($P = 0.007$) [65]. It has been estimated that chocolate contributes 9.4% of the daily copper intake for Americans [66]. Per serving (mg/100 g), cocoa powder had the highest copper content, followed by dark chocolate with higher percent cocoa solids, then milk chocolate, and white chocolate provides the lowest amount of copper (Table 3). Cocoa powder (15 g; 1 tbsp) provides up to 82% of the RDA for copper, dark chocolate (28.3 g; 1.9 tbsp) containing ≥70 cocoa solids provides up to 70%, milk chocolate

Table 3
Mineral content of different types of chocolate and cocoa powder

Chocolate Source*	Number of samples	Total Minerals (g/100 mg)	Na	Ca	P	Mg mg/100 g	Cu	Zn	Fe
Cocoa powder, unsweetened	3–9	5.80 ± 0.55	21 ± 3	128 ± 8	743 ± 19	499 ± 11	3.79 ± 0.15	6.81 ± 0.25	13.86 ± 1.61
Dark chocolate (70–85% cocoa)	2	2.32 ± 0.49	20 ± 2	73 ± 4	308 ± 16	228 ± 14	1.77 ± 0.00	3.31 ± 0.20	11.90 ± 1.1
Dark chocolate (60–69% cocoa)	6	1.90 ± 0.01	10 ± 1	62 ± 3	206 ± 1	176 ± 3	1.25 ± 0.12	2.65 ± 0.01	6.32 ± 0.07
Dark chocolate (45–59% cocoa)	5	1.70 ± 0.01	24 ± 4	56 ± 4	206 ± 2	146 ± 2	1.03 ± 0.01	2.01 ± 0.02	8.02 ± 0.06
Milk chocolate	27	1.78 ± 0.03	79 ± 8	189 ± 0	208 ± 6	63 ± 3	0.49 ± 0.08	2.30 ± 1.00	2.35 ± 1.00
White chocolate	3	1.50 ± NA	90 ± NA	199 ± NA	176 ± NA	12 ± NA	0.06 ± NA	0.74 ± NA	0.24 ± NA
RDA			1200–1500	1000–1300	700–1250	240–420	0.7–0.9	8–11	8–18

Ca, calcium; Cu, copper; Fe, iron; Mg, magnesium; NA, not available; P, phosphorus; RDA, recommended dietary allowance; Na, sodium; Zn, zinc.
*Values are the mean ± SEM based on the USDA National Nutrient Database for Standard Reference and Canadian Nutrient Files.
https://ndb.nal.usda.gov/ndb/search/list, Canadian Nutrient File https://food-nutrition.canada.ca/cnf-fce/index-eng.jsp.

(44 g; 1.55 oz bar) provides up to 31%, whereas white chocolate (85 g; 3 oz bar) provides only 7% of the RDA for copper [67,68].

The bone matrix consists of zinc-containing hydroxyapatite crystals that play a structural role. Zinc also directly activates aminoacyl-tRNA synthetase in osteoblastic cells, stimulates cellular protein synthesis for osteoblastic activity, and promotes bone mineralization by acting as a cofactor for alkaline phosphatase [69]. A randomized double-blind, placebo-controlled trial consisting of healthy premenarchal girls (N = 174, aged 9–11 y) consuming a daily oral zinc tablet (9 mg) for 4 wk significantly increased serum procollagen type 1 amino acid terminal propeptide (a bone formation marker) compared with placebo [70]. In a study investigating the effects of zinc supplementation (50 mg/d) in healthy men (N = 20, age 24–29 y), bone-specific alkaline phosphatase (a marker of bone formation) was increased by 11% after 6 wk [71]. Among the different types of cocoa products, cocoa powder had the highest zinc content. Dark chocolate and milk chocolate provided similar amounts of zinc, whereas white chocolate provided the least (Table 3). Cocoa powder (15 g; 1 tbsp) provides up to 13%, dark chocolate (28.3 g; 1.9 tbsp) containing ≥70 cocoa solids provides up to 11%, milk chocolate (44 g; 1.55 oz bar) provides up to 12.5%, and white chocolate (85 g; 3 oz bar) provides <1% of the RDA for zinc [67].

Iron plays an important role in collagen synthesis and osteoblast function [72]. Harris et al. [73] reported that increased iron intake by healthy postmenopausal women (N = 242, age 50–60.4 y) was associated with greater ($P \leq 0.01$) BMD at all skeletal sites. Cocoa powder (6 g; 0.4 tbsp) can provide 0.9 to 2.4 mg iron, a nutritionally significant amount. As shown in Table 3, cocoa powder had the highest amount of iron, followed by dark chocolate with higher cocoa solids, then milk chocolate, whereas white chocolate provided the lowest iron content. The RDA for iron intake is 8 to 18 mg/d depending on life stage. Cocoa powder (15 g; 1 tbsp) provides 12% to 26%, dark chocolate (28.3 g; 1.9 tbsp) containing ≥70% cocoa solids provides 18% to 42%, milk chocolate (44 g; 1.55 oz bar) provides 22% to 48%, and white chocolate (85 g; 3 oz bar) provides <1% of the RDA for iron [67]. Determination of iron bioavailability in cocoa powder using a rodent model reported moderate bioavailability [74].

Collectively, studies showed that chocolate, particularly cocoa powder and dark chocolate with higher percent cocoa solids can provide a source of several dietary minerals important for bone health. However, oxalates are known to bind and reduce mineral absorption. Noonan and Savage [75] reported oxalate content in chocolate is high, ranging between 500 and 900 mg/100 g. Oxalate content in cocoa powder ranged between 721 and 737 mg/100 g, whereas total oxalate content for dark chocolates ranged from 155 to 485 mg/100 g; other types of chocolate were not analyzed. Additionally, mineral absorption and excretion can be influenced by other chocolate components.

Fat content

The lipid content of chocolate is due to the cocoa butter content in the cocoa bean and added milk powder and vegetable oils [76]. Among different types of chocolates, total fat content was highest in dark chocolate and white chocolate (Fig. 2A). The major contributor to the fat content in chocolate is cocoa butter with one-third of the lipid in cocoa butter comprised of the saturated fatty acid (SFA) stearic acid (18:0) [62]. Cocoa butter is also rich in palmitic acid (16:0), followed by monounsaturated fatty acids (MUFAs), mainly oleic acid (18:1ω-9), whereas polyunsaturated fatty acids (PUFAs), mainly as α-linolenic acid (18:3ω-3), comprise only a small amount (Fig. 3A). A meta-analysis of observational studies showed a significant positive association between SFA and hip fracture risk (1.79; 95% CI, 1.05–3.03; P=0.03). The authors suggested mechanisms for negative bone effects of SFA intake included reduced calcium absorption, increased urinary calcium excretion, increased osteoclast survival, and inhibition of differentiation of marrow mesenchymal cells into osteoblasts [77].

Hodgson et al. [41] reported that fat intake was higher ($P < 0.001$) in postmenopausal women with daily (≥1 cup/d) or moderate (1–6 cups/wk) compared with rare (<1 cup/wk) chocolate consumption. Despite varying fat content and fatty acid composition, the type of chocolate was not specified. Among different types of chocolate, dark chocolate containing 70% to 85% cocoa solids has the highest amount of SFA (Fig. 3B) and MUFA (Fig. 3C), which decreased with lower percentage cocoa solids content. A meta-analysis of human studies concluded no significant association of MUFA intake and risk for bone fractures [76]. On the other hand, the Woman's Health Initiative, consisting of a cohort of postmenopausal women (N = 137 465, age 50–79 y), reported consuming PUFA α-linoleic acid (0.84 g/d) resulted in a significant reduction in total bone fracture risk [78]. Potential beneficial bone effects of PUFA intake include improving calcium balance, reducing proinflammatory prostaglandin synthesis, and promoting osteoblast formation [79]. White chocolate had the highest α-linoleic acid content among different types of chocolates (Fig. 3D). Because lipid content and fatty acid composition differ, it is important that future studies identify the type of chocolate consumed.

Sugar content

Postmenopausal women categorized as having high chocolate consumption (≥1 cup/d) had higher sugar intake than women categorized as having moderate (1–6 cups/wk) or rare (<1 cup/wk) chocolate consumption [41]. Sugar is added to reduce the bitter taste of cocoa [53]. As expected, unsweetened cocoa powder contains the lowest total sugar. For dark chocolate, total sugar content increased as percent cocoa solids decreased. Among different types of chocolates, total sugar content was highest in white chocolate followed by milk chocolate (Fig. 2B). The addition of sugar can also effect bioavailability of other chocolate constituents. Interestingly, human and animal studies reported that consumption of cocoa with sugar increases the bioavailability of flavanols [80,81].

To our knowledge, few human studies have investigated sugar intake on bone health. A review of preclinical evidence concluded that high sugar intake generally resulted in negative bone effects [82]. A potential mechanism is that high sugar intake induces hyperinsulinemia. Prolonged hyperinsulinemia increased calcium excretion owing to decreased calcium reabsorption by renal cells and in turn, this may lead to bone loss over time [83]. A cross-sectional population-based study of healthy adults (N = 1153, age 18–69 y) showed daily consumption of 100 g (3.5 oz) of chocolate reduced insulin resistance (IR), regardless of sex and age [84]. The authors suggested flavanols and related polyphenolic antioxidants in chocolate counteracted IR by increasing nitric oxide bioavailability [84]. Type of chocolate consumed in the study was not available to determine whether sugar content was a factor. IR is symptomatic of type 2 diabetes and lower sugar intake is recommended. A study conducted on a large cohort of postmenopausal US women (N = 92 678, age 56–71.2 y) reported moderate consumption of 28 g (1 oz) chocolate between 1.5 and 3 times per month significantly reduced diabetes risk, but not at the higher chocolate intakes of more than three times per week [85]. Type of chocolate consumed was unavailable to assess whether loss of protective effects at higher intake may be due to higher sugar intake.

Brand-Miller et al. [86] reported that adding cocoa powder to different foods produced greater insulinemia, regardless of the food

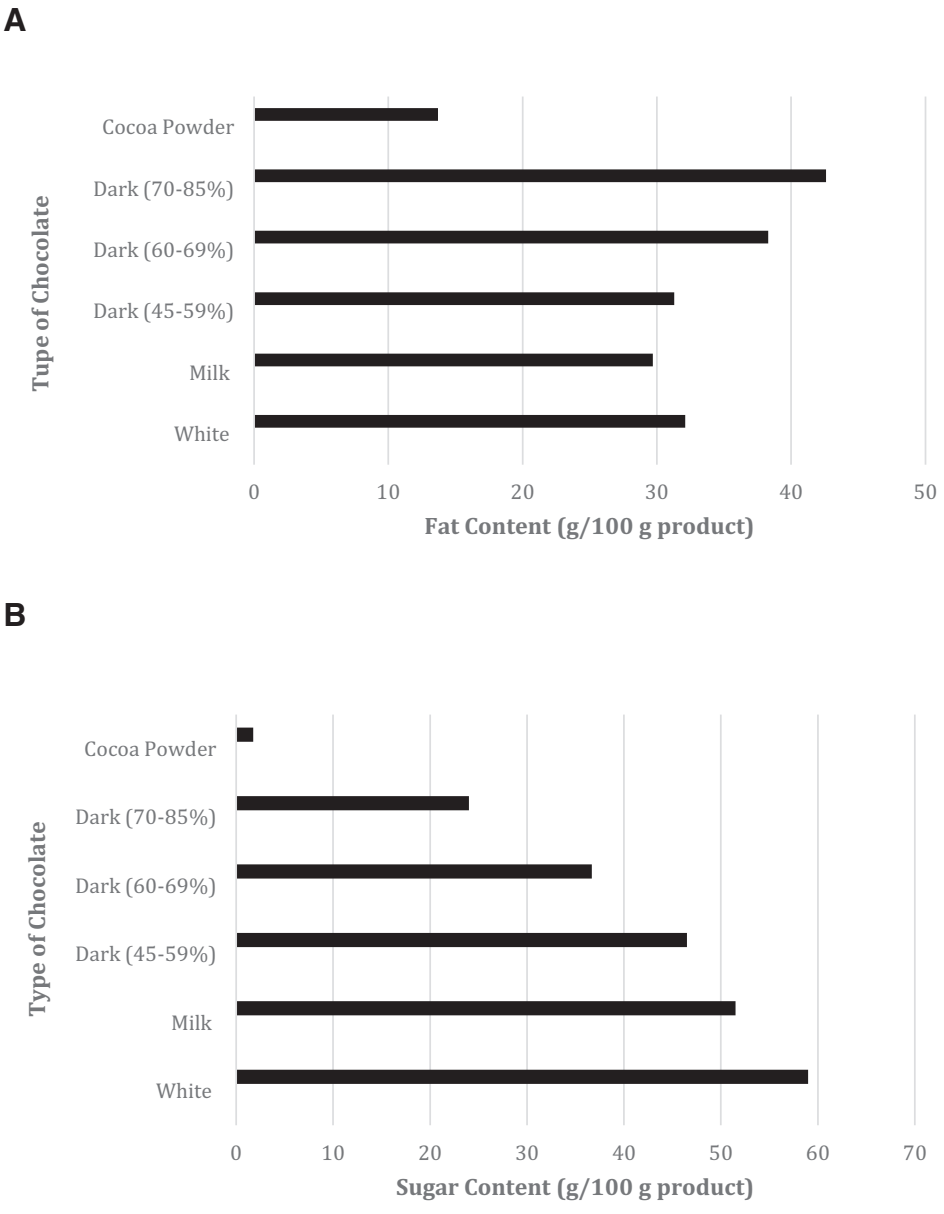

Fig. 2. Macronutrient content of different types of chocolates: (**A**) total fat content, (**B**) total sugar content.

source or the overall composition of the food (e.g., sugar, fat, protein, fiber, or energy density). In a crossover trial of healthy men (N = 19, age 37–49 y) receiving either dark chocolate (100 g; 3.5 oz/d) containing 88 mg flavanols or flavanol-free white chocolate (90 g; 3.1 oz/d) for 15 d (chocolates were adjusted to provide 480 kcal energy and similar amounts of macronutrients, cocoa butter, fiber, and minerals), dark chocolate consumption reduced IR indicating flavanols was the beneficial chocolate ingredient [87]. Collectively, studies suggested moderate dark chocolate consumption, whether owing to its high antioxidant flavonoids or lower sugar content, potentially exerts metabolic effects that can benefit bone health.

Methylxanthine content

A major effect of methylxanthines is the regulation of calcium concentration within cells by stimulating release of stored calcium from the body [88]. Because 99% of the calcium in the body is stored in bone, high consumption of methylxanthines may potentially lead to bone mineral loss. Postmenopausal women with daily chocolate consumption (≥1 cup/d) reported deleterious bone effects. The authors suggested methylxanthines may have been a contributing factor, but this was not analyzed in the study [41].

Methylxanthines comprise caffeine, theobromine, and theophylline [88]. Caffeine has been shown to reduce calcium absorption, increase calcium excretion in urine, and decrease vitamin D-stimulated osteoblast alkaline phosphatase activity [89,90]. Studies comparing different chocolates reported the highest caffeine content in dark chocolate (87.5–62.5 mg/100 g) followed by cocoa powder (48.9 mg/100 g), then milk chocolate (5.6 mg/100 g) [91,92]. Sanchez [93] measured caffeine content in different brands of white, milk, and dark chocolate with 50% to 85% cocoa

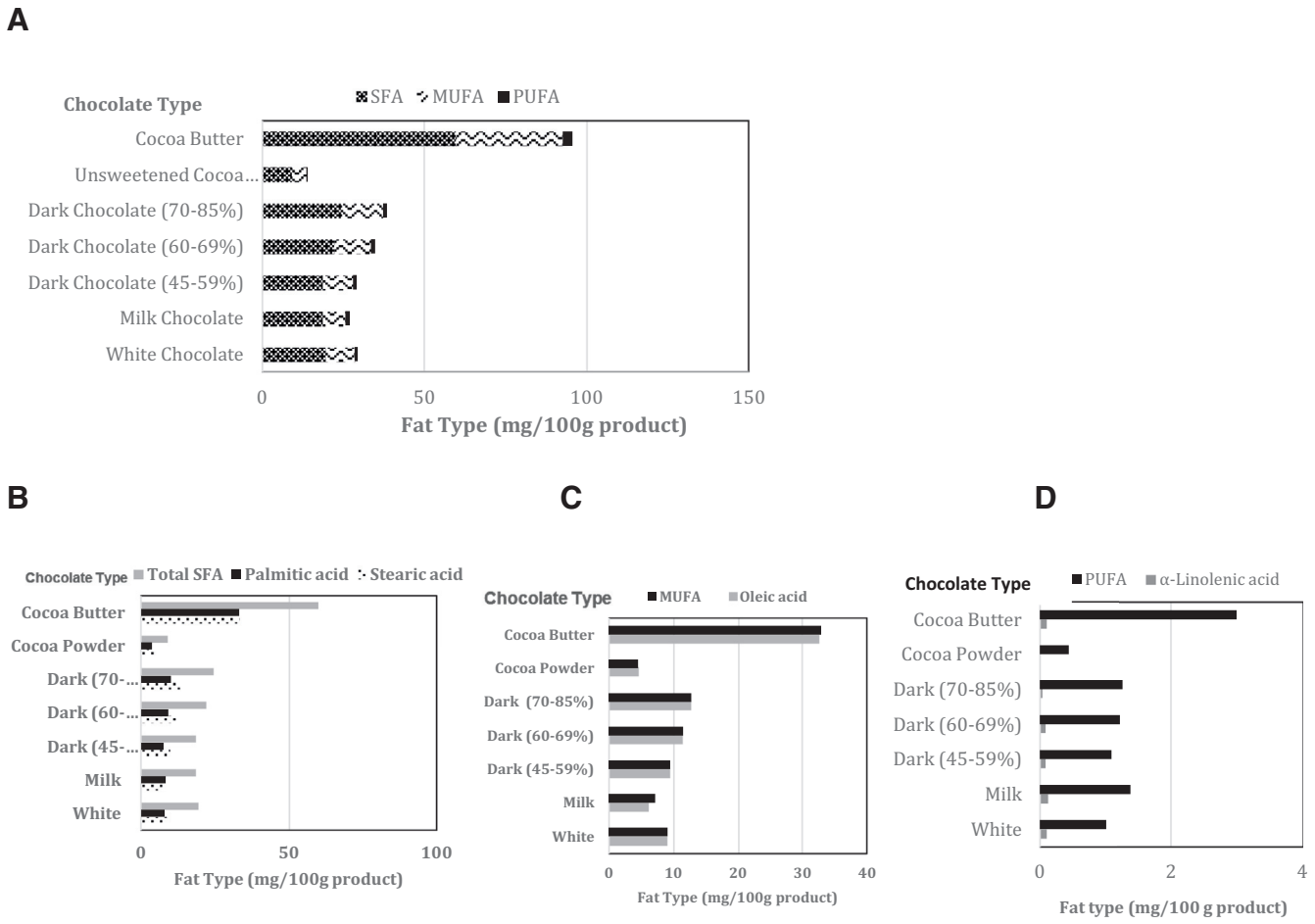

Fig. 3. Fat and fatty acid composition of different types of chocolates: (**A**) total SFAs, MUFAs, and PUFAs, (**B**) types of SFAs, (**C**) types of MUFAs, and (**D**) types of PUFAs. MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

commercially available in Spain. Caffeine content increased as the percentage of cocoa increased from 5 to <150 mg/100 g. According to the USDA database [56], caffeine was highest in cocoa powder followed by dark chocolate containing 70% to 85% cocoa solids with half the amount in dark chocolate containing 60% to 69% cocoa solids and the lowest amount in milk chocolate, whereas white chocolate had no detectable amounts (Fig. 4A). However, chocolate contributes comparatively little caffeine to the estimated mean daily caffeine intake of 280 mg for a 70 kg individual in the United States [94]. Standard US values reported that chocolate milk (6 oz serving) provides 4 mg caffeine and chocolate confections (0.3 g; 1 oz serving) provide <1.5 to 6 mg caffeine compared with ground roasted coffee (5 oz serving), which provides 85 mg caffeine; tea (5 oz serving), which provides 30 mg caffeine for leaf/bag; or soft drinks (6 oz serving), which provide 18 mg caffeine [94].

Theophylline was not detectable in cacao bean, cocoa bean, or cocoa products such as chocolate [90,95]. On the other hand, 2% to 3% of cocoa bean weight is theobromine, which has high bioavailability and resists degradation during cocoa processing [88]. Theobromine content in chocolate is higher than in coffee, tea, and carbonated beverages [91]. Among different types of chocolate, theobromine content was reported to be highest in dark chocolate (500–750 mg/ 100 g), then cocoa powder (462.1 mg/ 100 g), and lowest in milk chocolate (100.4 mg/100 g) [91,92]. Meng et al. [96] also reported theobromine was highest in dark chocolate (883.11 ± 3.54 mg/100 g) followed by milk chocolate (125.15 ± 0.98 mg/100 g), whereas levels in white chocolate were below the detection level of 0.5 mg/100 g. In chocolate commercially available in Spain, theobromine content ranged from 150 to 750 mg with higher amounts in dark chocolate (50–85%) than in milk chocolate, whereas white chocolate had the lowest amount [93]. Similarly, the USDA database [55] values for theobromine per 100 g serving was highest in cocoa powder then dark chocolates, with higher cocoa solids having higher theobromine content followed by milk chocolate, whereas there was no detectable theobromine content in white chocolate (Fig. 4B).

In an in vitro study, treating human bone marrow mesenchymal stem cells with theobromine (25–100 μM) promoted differentiation into osteoblasts and mineralization of differentiated osteoblasts and increased alkaline phosphatase and osteoprotegerin (osteoclastogenesis inhibitory factor) activity [97]. In vivo, rats exposed to 10 mg/kg total body weight theobromine in utero daily until postnatal day 50 exhibited increased ($P = 0.03$) femoral length, cortical thickness, and trabecular thickness and number, but had no significant difference in femoral strength compared with rats fed chow [97]. To our knowledge, no human studies have investigated the effect of theobromine consumption on skeletal health. Theobromine is generally recognized as safe for human consumption and toxicity would require consuming 18 g/d of theobromine or 151 chocolate bars (29.8 g or 1.05 oz) per day [98]. The potential effects of theobromine on bone health merits further study because of high concentration in chocolate.

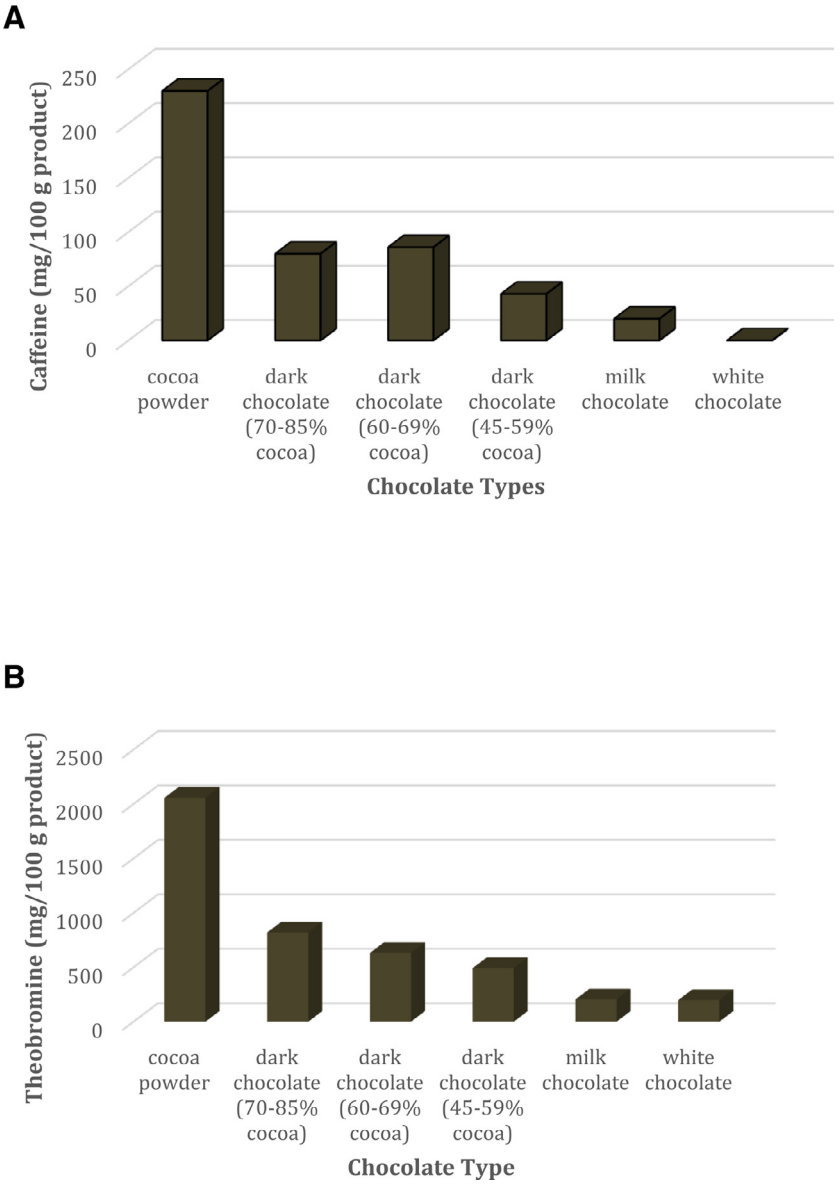

Fig. 4. Methylxanthine content of different types of chocolates: (**A**) caffeine, (**B**) theobromine.

Summary and conclusions

Chocolate and cocoa powder provide a dietary source of flavonoids and other constituents with the potential to affect bone. However, the beneficial antioxidant and anti-inflammatory effects of flavonoids and mineral content of chocolate on bone health may be outweighed by other components in chocolate such as oxalate, added cocoa butter, sugar, and methylxanthines with potential to exert adverse effects on bone health. Hodgson et al. [41] reported an inverse association between chocolate consumption and BMD in postmenopausal women. However, studies showed postmenopausal women had no bone effects at moderate chocolate intakes, whereas adolescents consuming chocolate had greater longitudinal bone growth. As demonstrated by the preponderance of evidence cited in this review, the osteogenic effects of chocolate ingredients varied depending on the type of chocolate. Based on flavonoid and mineral content, unsweetened cocoa powder appeared to be the best option, followed by dark chocolate with higher cocoa content, in terms of supporting and preserving bone health. However, more basic research and human studies are warranted that include specifying the type of chocolate consumed in studies. The 2015–2020 Dietary Guidelines for Americans list chocolate milk as a source of calcium, potassium, and vitamin D. Moreover, both the U.S. Dietary Guidelines and the 2019 Canada's Food Guide advise that due to its high sugar and fat content, chocolate bars should be at the top of the nutritional pyramid under fats, oils, and sweets that are to be used sparingly. Determining dietary recommendations for chocolate consumption and its implications for bone health and osteoporosis risk is a topic of importance given the widespread and growing popularity of chocolate consumption.

References

[1] Gianfredi V, Salvatori T, Nucci D, Villarini M, Moretti M. Can chocolate consumption reduce cardiocerebrovascular risk? A systematic review and meta-analysis. Nutrition 2018;46:103–14.
[2] Araujo QR, Gattward JN, Almoosawi S, Parada Costa Silva MD, Dantas PA, Araujo Júnior QR. Cocoa and human health: from head to foot–a review. Crit Rev Food Sci Nutr 2016;56:1–12.

[3] Ren Y, Liu Y, Sun XZ, Wang BY, Zhao DD, Liu XJ, et al. Chocolate consumption and risk of cardiovascular diseases: a meta-analysis of prospective studies [Epub ahead of print]. Heart 2018.
[4] Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. Antioxid Redox Signal 2011;15:2779–811.
[5] National Institutes of Health. National Institute of Health Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA 2001;285:785–95.
[6] Cooper C, Campion G, Melton 3rd LJ. Hip fractures in the elderly: a worldwide projection. Osteoporosis Int 1992;2:285–9.
[7] Gullberg B, Johnell O, Kanis JA. Worldwide projections for hip fracture. Osteoporosis Int 1997;7:407–13.
[8] Nardone V, D'Asta F, Brandi ML. Pharmacological management of osteogenesis. Clinics (Sao Paulo) 2014;69:438–46.
[9] Rossini M, Bianchi G, Munno OD, Giannini S, Minisola S, Sinigaglia L. Treatment of Osteoporosis in clinical Practice (TOP) Study Group. Determinants of adherence to osteoporosis treatment in clinical practice. Osteoporos Int 2006;17:914–21.
[10] Rizzoli R. Nutritional aspects of bone health. Best Pract Res Clin Endocrinol Metab 2014;28:795–808.
[11] Levis S, Lagari VS. The role of diet in osteoporosis prevention and management. Curr Osteoporos Rep 2012;10:296–302.
[12] Sahni S, Mangano KM, McLean RR, Hannan MT, Kiel DP. Dietary approaches for bone health: lessons from the Framingham Osteoporosis Study. Curr Osteoporos Rep 2015;13:245–55.
[13] Beecher GR. Overview of dietary flavonoids: nomenclature, occurrence and intake. J Nutr 2003;133:3248S–54S.
[14] Weaver CM, Alekel DL, Ward WE, Ronis MJ. Flavonoid intake and bone health. J Nutr Gerontol Geriatr 2012;31:239–53.
[15] Zhang Z, He L, Liu Y, Liu J, Su Y, Chen Y. Association between dietary intake of flavonoid and bone mineral density in middle aged and elderly Chinese women and men. Osteoporos Int 2014;25:2417–25.
[16] Welch AA, Hardcastle AC. The effects of flavonoids on bone. Curr Osteoporos Rep 2014;12:205–10.
[17] Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004;79:727–47.
[18] Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem 2003;51:7292–5.
[19] Vinson JA, Proch J, Zubik L. Phenol antioxidant quantity and quality in foods: cocoa, dark chocolate, and milk chocolate. J Agric Food Chem 1999;47:4821–4.
[20] Wickramasuriya AM, Dunwell JM. Cacao biotechnology:current status and future prospectives. Plant Biotechnol J 2018;16:4–17.
[21] Seligson FH, Krummel DA, Apgar JL. Patterns of chocolate consumption. Am J Clin Nutr 1994;60(suppl):1060S–4S.
[22] Seeman E. Age- and menopause-related bone loss compromise cortical and trabecular microstructure. J Gerontol A Biol Sci Med Sci 2013;68:1218–25.
[23] Riggs BL, Khosla S, Melton LJ. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 2002;23:279–302.
[24] Hardcastle AC, Aucott L, Reid DM, Macdonald HM. Associations between dietary flavonoid intakes and bone health in a Scottish population. Bone Miner Res 2011;26:941–7.
[25] Wollgast J, Anklam E. Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantication. Food Res Intl 2000;33:423–47.
[26] Halvorsen BL, Carlsen MH, Phillips KM, Bøhn SK, Holte K, Jr Jacobs DR, et al. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. Am J Clin Nutr 2006;84:95–135.
[27] Mundy GR. Osteoporosis and inflammation. Nutr Rev 2007;65:S147–51.
[28] Muthusami S, Ramachandran I, Muthusamy B, Vasudevan G, Prabhu V, Subramaniam V, et al. Ovariectomy induces oxidative stress and impairs bone antioxidant system in adult rats. Clin Chim Acta 2005;360:81–6.
[29] Baek KH, Oh KW, Lee WY, Lee SS, Kim MK, Kwon HS, et al. Association of oxidative stress with postmenopausal osteoporosis and the effects of hydrogen peroxide on osteoclast formation in human bone marrow cell cultures. Calcif Tissue Int 2010;87:226–35.
[30] Holzer N, Braun KF, Ehnert S, Egaña JT, Schenck TL, Buchholz A, et al. Green tea protects human osteoblasts from cigarette smoke-induced injury: possible clinical implications. Langenbecks Arch Surg 2012;397:467–74.
[31] McLean RR. Proinflammatory cytokines and osteoporosis. Curr Osteoporos Rep 2009;7:134–9.
[32] Choi EM, Hwang JK. Effects of (+)–catechin on the function of osteoblastic cells. Biol Pharm Bull 2003;26:523–6.
[33] Sarria B, Martinez-Lopez S, Sierra-Cinos JL, Garcia-Diz L, Mateos R, Bravo L. Regular consumption of a cocoa product improves the cardiometabolic profile in healthy and moderately hypercholesterolaemic adults. Br J Nutr 2004;111:122–34.
[34] Fayet-Moore F. Effect of flavored milk vs plain milk on total milk intake and nutrient provision in children. Nutr Rev 2016;74:1–17.
[35] Bonjour JP, Carrie AL, Ferrari S, Clavien H, Slosman D, Theintz G, et al. Calcium-enriched foods and bone growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. J Clin Invest 1997;99:1287–94.
[36] Skopiński P, Skopińska-Różewska E, Sommer E, Chorostowska-Wynimko J, Rogala E, Cendrowska I, et al. Chocolate feeding of pregnant mice influences length of limbs of their progeny. Pol J Vet Sci 2003;6:S57–9.
[37] Skopiński P, Skopińska-Różewska E, Kamiński A, Dziedzic-Gocławska A, Sommer E, Chorostowska-Wynimko J, et al. Chocolate feeding of pregnant mice resulted in epigallocatechin-related embryonic angiogenesis suppression and bone mineralization disorder. Pol J Vet Sci 2004;7(suppl):S131–3.
[38] Lau SJ, Ooi FK. Changes in blood bone turnover markers following combination circuit training programme and chocolate malt drink supplementation in young males. Int J Applied Health Sci 2014;1:30–8.
[39] Atiqah WAN, Ooi FK, Chen CK, Nudri WDW. Effects of chocolate malt drink consumption combined with aerobic dance exercise on blood bone metabolism markers, antioxidant enzymes and aerobic capacity in young females. Sport Exerc Med 2012;1:71–80.
[40] Piehowski KE. Effects of incorporating dark chocolate and a cocoa beverage into a weight loss diet on inflammatory status and bone turnover 2011. Unpublished doctoral Pennsylvania State University, State College.
[41] Hodgson JM, Devine A, Burke V, Dick IM, Prince RL. Chocolate consumption and bone density in older women. Am J Clin Nutr 2008;87:175–80.
[42] Hu S, Kim BY, Baik MY. Physicochemical properties and antioxidant capacity of raw, roasted and puffed cacao beans. Food Chem 2016;194:1089–94.
[43] Lim TK. Theobroma cacao. In: Lim TK, ed. Edible Medicinal and Non-Medicinal Plants, Vol. 3. Fruits, Springer; 2012:208–51.
[44] Oracz J, Zyzelewicz D, Nebesny E. The content of polyphenolic compounds in cocoa beans (Theobroma cacao L.), depending on variety, growing region, and processing operations: a review. Crit Rev Food Sci Nutr 2015;55:1176–92.
[45] Rusconi M, Conti A. Theobroma cacao L, the food of the gods: a scientific approach beyond myths and claims. Pharmacol Res 2010;61:5–13.
[46] Bonvehi JS, Coll FV. Evaluation of bitterness and astringency of polyphenolic compounds in cocoa powder. Food Chem 1997;60:365–70.
[47] Hannum SM, Erdman Jr JW. Emerging health benefits from cocoa and chocolate. J Med Food 1997;3:73–80.
[48] De Brito EX, Pezoa Garcia NH, Gallao MI, Al Crtelazzo, Feveriro PS, Brage MR. Structural and chemical changes in cocoa (*Theobroma cacao L)* during fermentation, drying and roasting. J Sci Food Agric 2001;81:281–8.
[49] Counet C, Ouwerx C, Rosoux D, Collin S. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. J Agric Food Chem 2004;52:6243–9.
[50] Miller KB, Hurst WJ, Payne MJ, Stuart DA, Apgar J, Sweigart DS, Ou B. Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. J Agric Food Chem 2008;56:8527–33.
[51] Ellam S, Williamson G. Cocoa and human health. Annu Rev Nutr 2013;33:105–28.
[52] Payne MJ, Hurst WJ, Miller KB, Rank C, Stuart DA. Impact of fermentation, drying, roasting, and Dutch processing on epicatechin and catechin content of cacao beans and cocoa ingredients. J Agric Food Chem 2010;58:10518–27.
[53] Beckett ST. The Science of Chocolate. Piccadilly. London: The Royal Society of Chemistry 2000. p. 8–30.
[54] Ariefdjohan MW, Savaiano DA. Chocolate and cardiovascular health: is it too good to be true? Nutr Rev 2005;63:427–30.
[55] United States Department of Agriculture Agricultural Research Service. United States Department of Agriculture Agricultural Food Composition Databases. Available at: https://ndb.nal.usda.gov/ndb/search/list. Accessed March 18, 2019.
[56] Vinson JA, Proch J, Bose P, Muchler S, Taffera P, Shuta D, et al. Chocolate is a powerful ex vivo and in vivo antioxidant, an antiatherosclerotic agent in an animal model, and a significant contributor to antioxidants in the European and American diets. J Agric Food Chem 2006;54:8071–6.
[57] Miller KB, Stuart DA, Smith NL, Lee CY, Mchale NL, Flanagan JA, et al. Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. J Agric Food Chem 2006;54:4062–8.
[58] Kuhn J, Schroter A, Hartmann BM, Stangl GI. Cocoa and chocolate are sources of vitamin D_2. Food Chem 2015;269:318–20.
[59] Prentice A. Diet, nutrition and the prevention of osteoporosis. Public Health Nutr 2004;7:227–43.
[60] Teucher B, Dainty JR, Spinks CA, Majsak-Newman G, Berry DJ, Hoogewerff JA, et al. Sodium and bone health: impact of moderately high and low salt intakes on calcium metabolism in postmenopausal women. J Bone Miner Res 2008;23:1477–85.
[61] Farsinejad-Marj M, Saneei P, Esmaillzadeh A. Dietary magnesium intake, bone mineral density and risk of fracture: a systematic review and meta-analysis. Osteoporosis Int 2016;27:1389–99.
[62] Steinberg FM, Bearden MM, Leem CL. Cocoa and chocolate flavonoids: Implications for cardiovascular health. J Am Diet Assoc 2003;103:213–23.
[63] Aaseth J, Boivin G, Andersen O. Osteoporosis and trace elements—an overview. J Trace Elem Med Biol 2012;26:127–30.
[64] Rucker RB, Kosonen T, Clegg MS, Mitchell AE, Rucker BR, Uriu-Hare JY, et al. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. Am J Clin Nutr 1998;67(suppl):996S–1002S.
[65] Qu X, He Z, Qiao H, Zhai Z, Mao Z, Yu Z, Dai K. Serum copper levels are associated with bone mineral density and total fracture. J Orthop Translat 2018;14:34–44.

[66] Joo S, Kies C, Schnepf M. Chocolate and chocolate-like products: impact of copper status of humans. J Appl Nutr 1995;47:67–77.
[67] NDL/FNIC Food Composition Database Home Page. Available at: http://ndb.nal.usda.gov/. Accessed October 21, 2018.
[68] DRI Tables and Application Reports | Food and Nutrition Information Center. Available at: https://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables-and-application-reports. Accessed October 21, 2018.
[69] Yamaguchi M, Gao YH. Potent effect of zinc acexamate on bone components in the femoral-metaphyseal tissues of elderly female rats. Gen Pharmacol 1998;30:423–7.
[70] Berger PK, Pollock NK, Laing EM, Chertin V, Bernard PJ, Grider A, et al. Zinc supplementation increases procollagen type 1 amino-terminal propeptide in premenarcheal girls: a randomized control trial. J Nutr 2015;145:2699–704.
[71] Peretz A, Papadopoulos T, Willems D, Hotimsky A, Michiels N, Siderova V, et al. Zinc supplementation increases bone alkaline phosphatase in healthy men. J Trace Elem Med Biol 2001;15:175–8.
[72] Parelman M, Stoecker B, Baker A, Medeiros D. Iron restriction negatively affects bone in female rats and mineralization of hFOB osteoblast cells. Exp Biol Med 2006;231:378–86.
[73] Harris MM, Houtkooper LB, Stanford VA, Parkhill C, Weber JL, Flint-Wagner H, et al. Dietary iron is associated with bone mineral density in healthy postmenopausal women. J Nutr 2003;133:3598–602.
[74] Yokoi K, Konomi A, Otagi M. Iron bioavailability of cocoa powder as determined by the Hb regeneration efficiency method. Br J Nutr 2009;102:215–20.
[75] Noonan SC, Savage GP. Oxalate content of foods and its effect on humans. Asia Pac J Clin Nutr 1999;8:64–74.
[76] McShea A, Ramiro-Puig E, Munro SB, Casadesus G, Castell M, Smith MA. Clinical benefit and preservation of flavanols in dark chocolate manufacturing. Nutr Rev 2008;66:630–41.
[77] Mozaffari H, Djafarian K, Mofrad MD, Shab-Bidar S. Dietary fat, saturated fatty acid, and monounsaturated fatty acid intakes and risk of bone fracture: a systematic review and meta-analysis of observational studies. Osteoporosis Int 2018;29:1949–61.
[78] Orchard TS, Cauley JA, Frank GC, Neuhouser ML, Robinson JG, Snetselaar L, et al. Fatty acid consumption and risk of fracture in the Women's Health Initiative. Am J Clin Nutr 2010;92:1452–60.
[79] Wauquier F, Léotoing L, Philippe C, Spilmont M, Coxam V, Wittrant Y. Pros and cons of fatty acids in bone biology. Prog Lipid Res 2015;58:121–45.
[80] Schramm DD, Karim M, Schrader HR, Holt RR, Kirkpatrick NJ, Polagruto JA, et al. Food effects on the absorption and pharmacokinetics of cocoa flavanols. Life Sci 2003;4:857–69.
[81] Neilson AP, George JC, Janle EM, Mattes RD, Rudolph R, Matusheski NV, et al. Influence of chocolate matrix composition on cocoa flavan-3-ol bioaccessibility in vitro and bioavailability in humans. J Agric Food Chem 2009;57:9418–26.
[82] Tsanzi E, Fitch CW, Tou JC. Effect of consuming different caloric sweeteners on bone health and possible mechanisms. Nutr Rev 2008;66:301–9.
[83] Kishnani PS, Austin SL, Abdenur JE, Arn P, Bali DS, Boney A, et al. Diagnosis and management of glycogen storage disease type I: a practice guideline of the American College of Medical Genetics and Genomics. Genet Med 2014;16:e1–29.
[84] Alkerwi A, Sauvageor N, Crichton GE, Elias M, Stranges S. Dietary chocolate consumption is inversely associated with insulin resistance and liver enzymes in the observation of cardiovascular risk factors Luxembourg study. Br J Nutr 2016;115:1161–8.
[85] Greenberg JA, Manson JE, Tinker L, Neuhouser ML, Garcia L, Vitolins MZ, et al. Chocolate intake and diabetes risk in postmenopausal American women. Eur J Clin Nutr 2017;71:1068–93.
[86] Brand-Miller J, Holt, de Jong SH, Petocz V. Cocoa powder increases postprandial insulinemia in lean young adults. J Nutr 2003;133:3149–52.
[87] Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, et al. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension 2005;46:398–405.
[88] Franco R, Oñatibia-Astibia A, Martínez-Pinilla E. Health benefits of methylxanthines in cacao and chocolate. Nutrients 2013;5:4159–73.
[89] Heaney RP. Effects of caffeine on bone and the calcium economy. Food Chem Toxicol 2002;40:1263–70.
[90] Rapuri PB, Gallagher JC, Nawaz Z. Caffeine decreases vitamin D receptor protein expression and 1,25(OH)2D3 stimulated alkaline phosphatase activity in human osteoblast cells. J Steroid Biochem Mol 2007;103:368–71.
[91] Srdjenovic B, Djordjevic-Milic V, Grujic N, Injac R, Lepojevic Z. Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks, and herbal products. J Chromatogr Sci 2008;46:144–9.
[92] Bruinsma K, Taren DL. Chocolate: food or drug? J Am Diet Assoc 1999;99:1249–56.
[93] Sanchez JM. Methylxanthine content in commonly consumed foods in Spain and determination of its intake during consumption. Foods 2017;102:1–13.
[94] Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol 1996;34:119–29.
[95] Risner CH. Simultaneous determination of theobromine, (+)–catechin, caffeine, and (−)–epicatechin in standard reference material baking chocolate 2384, cocoa, cocoa beans, and cocoa butter. J Chromatogr Sci 2008;46:892–9.
[96] Meng CC, Jalil AM, Ismail A. Phenolic and theobromine contents of commercial dark, milk and white chocolates on the Malaysian market. Molecules 2009;14:200–9.
[97] Clough BH, Ylostalo J, Browder E, McNeill EP, Bartosh TJ, Rawls HR, et al. Theobromine upregulates osteogenesis by human mesenchymal stem cell in vitro and accelerates bone development in rats. Calcif Tissue Int 2017;100:298–310.
[98] Stavric B. Methylxanthines: toxicity to humans. Theobromine, paraxanthine and the combined effects of methylxanthines. Food Chem Toxicol 1988;26:725–33.