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Review

Determinants of bone mass

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Abstract

According to WHO criteria, Osteoporosis is defined as bone mineral density (BMD) that is reduced by more than 2.5 standard deviations below the young adult mean. This leads to bone fragility and an increased risk of fractures. Bone mass rapidly accelerates in adolescents until peak bone mass (PBM) is achieved by the end of adolescence between the age of 20–25 and levels remain relatively static until the age of 45 when bone density starts to fall. Bone loss is positively associated with age and aging contributes to the development of osteoporosis. Genetic, hormonal and environmental factors such as low estrogen, low dietary calcium intake, vitamin D deficiency, current cigarette smoking, chronic alcohol consumption, and low physical activity or exercise also contribute to low bone density and skeletal fragility. Mechanical stress has long been recognized to have important effects on bone. Activities or exercises that are weight bearing or involve impact are most useful for increasing or maintaining bone mass. The development of PBM during the growing years is an important determinant for risk of osteoporosis in later life. Adequate nutrition and sufficient activity are critical factors in maximizing bone growth potential. Replacing milk intake by soft drinks appears to be detrimental to bone gain. The association between bone fractures and carbonated beverage consumption, in particular, is found in adolescences. Caffeine-containing beverage consumption has been reported to be associated with reduced bone mass due to its effects on a mild diuretic and short-term increased in urinary calcium excretion. This consumption with more milk may distribute effects of caffeine on body bone gain. Current greater cigarette consumption is at increased risk of hip fracture, and the decline in risk is observed until 10 years after smoking cessation. A small amount of alcohol has benefit to BMD, whereas high alcohol consumption increases risk of fractures that is relative to low body mass index.

Keywords: Bone mass, Peak bone mass, Exercise, Calcium intake, Beverages

INTRODUCTION

Osteoporosis is a disease that is characterized by low bone mass (or low bone mineral density; BMD) and structural deterioration of bone tissue leading to bone fragility and an increased susceptibility to fractures. According to recent World Health Organization (WHO) criteria, Osteoporosis is defined as BMD that is reduced by more than 2.5 standard deviations below the young adult mean (Kanis et al., 1994). The extensive epidemiologic data indicate that fracture risk increases two to three fold for every drop of 1 SD in BMD at any given site (Heaney, 1998). Therefore low BMD is such an important predictor of future fracture, and concerted

efforts have been undertaken to understand the factors that influence BMD. In the present, Osteoporotic fractures are a major public health concern, and a major cause of disability throughout the world. Identification of modifiable factors that can reduce fractures is important for healthy aging and reducing the social, medical and personal costs of fracture. Several studies have reported that the major processes, which are responsible for osteoporosis are acquisition of poor bone mass during adolescence (peak bone mass; PBM), and acceleration of bone loss during aging (especially during perimenopausal period in women). Both processes are regulated by genetic and environmental factors. Epidemiologic studies have revealed a number of environmental and lifestyle factors to be associated with reduced BMD, such as lean mass (body mass index), cigarette smoking, caffeinated soft drinks, nutritional deficiency, decreased physical activity, steroid use, and hormone deficiencies (during early menopause). As mentioned above, a high PBM is one of the most important factors in maintaining strong bones and determining risk of fractures in elderly. Diet and lifestyle behaviors operating during this period have therefore important consequences for PBM attainment and future fracture risk. Recent studies have revealed that consumption of carbonated soft drinks, particularly colas is extremely popular in affluent western populations, particularly during adolescence. This behavior is also associated with reduce bone mineral accrual and increase fracture risk in children and adolescence (Ma and Jones, 2004).

However, all of these determinants affecting bone mass will be revealed for the details in order to promote an attainment of bone mass and to prevent osteoporosis in later life. This article aims to review research studies and evidences that demonstrated determining factors affecting bone mass and their mechanisms. Physiology of bone is also important to remind to relate the factors to their effects. This knowledge can be further used to find out osteoporosis preventive strategy in all population-age, and reduced a number of patients with osteoporotic fracture.

Physiology of bone

Bone is a dynamic organ which has the ability to repair itself by a process called "*bone remodeling*" or *"bone turnover"*. Bone remodeling is essential for bone health to maintain the structural integrity of the skeleton and to fulfill its metabolic functions as a storehouse of calcium and phosphorus. A cycle of bone remodeling is carried out by a group of osteoclasts and osteoblasts called the basic multicellular unit (BMU). The remodeling cycle is composed of four sequential phases: activation, resorption, reversal and formation (Clarke, 2008). *The activation phase* is started by cells of the osteoblast lineage that secrete collagenase and other enzymes to digest proteins on the bone surface. Cells of the osteoblastic lineage are important for forming bone and for initiating bone resorption. Most of the hormonal factors that act on cells of the osteoblastic lineage are called *Receptor Activator of NF-Kappa B Ligand (RANKL),* or called Osteoclast Differentiating Factor (ODF) and Colony-Stimulating Factor 1 (CSF1). These factors are essential for osteoclastogenesis. RANKL can bind with the receptor called RANK that results in activation, migration, differentiation, and fusion of hematopoietic cells of the osteoclast lineage to begin the process of resorption (Troena, 2003). Moreover, osteoblasts also produce the additional factors that regulate bone resorption, including cytokines (IL-1 and IL-6), prostaglandins, and local growth factors, however PTH and $1,25(OH)_2D$ also stimulate production of RANKL in osteoblastic-lineage cells. *The resorption phase* begins with the migration of partially-differentiated mononuclear preosteoclasts to the bone surface to form the large multinucleated osteoclasts. A ruffled border is developed beneath the osteoclast. The osteoclasts secrete hydrogen ions into the resorbing compartment to lower the pH (<4.5) that helps mobilize bone mineral, and also secrete tartrate-resistant acid phosphatase, cathepsin K, matrix metalloproteinase 9, and gelatinase from cytoplasmic lysosomes to digest the organic matrix. This process causes a breakdown of the protein matrix of bone and release of calcium and other bone mineral constituents (Anderson, 2003). The *reversal phase* occurs in which mononuclear cells, possibly of monocyte/macrophage lineage, appear on the bone surface to prepare the surface for new osteoblasts to begin bone formation. A layer of glycoprotein-rich material is laid down on the resorbed surface, the so-called "cement line," to which the new osteoblasts can adhere. The *formation phase* follows, osteoblasts synthesize new collagenous organic matrix and regulate mineralization of matrix by releasing small, membrane-bound matrix vesicles that concentrate calcium and phosphate and enzymatically destroy mineralization inhibitors such as pyrophosphate or proteoglycans (Anderson, 2003). When this phase is complete, the surface is covered with flattened lining cells, and there is a prolonged resting period with little cellular activity on the bone surface until a new remodeling cycle begins. Stages of the remodeling cycle have different lengths. Resorption probably continues for about 2-4 weeks. The reversal phase may last up to 4-5 weeks, while formation can continue for 4-6 months until the new bone structural unit is fully formed. The end result of each bone remodeling cycle is production of a new osteon.

During lifespan however through the first ten years of human growth, bone remodeling is accelerated and formation is predominant. During the second to approximately the fourth decade of life, the processes of formation and resorption are in balance and PBM is reached. PBM is attained between the age of 20–25 and

levels remain relatively static until the age of 45 when bone density starts to fall (Ralston, 2006). Then, resorption slightly exceeds formation about beginning of the fifth decade of life, resulting in a net negative balance, consequently accelerating bone loss. Affecting of estrogen deficiency, menopause is the most prevalent cause of the accelerated bone loss (Frost, 1999). Remodeling can be activated by systemic and local factors, and changes in mechanical force. It is necessary to maintain skeletal strength and the serum levels of calcium. The systemic hormones affecting bone remodeling include calcium-regulating hormones [Parathyroid hormone (PTH), Vitamin D, and calcitonin], growth hormone, glucocorticoids, thyroid hormones, insulin, and gonadal hormones. *PTH* is the principal regulator of calcium homeostasis, and is a key factor in the control of bone emodeling. Elevated levels of PTH increase bone turnover, leading to either anabolic or catabolic effects on the skeleton depending upon the pattern and duration of elevation (Poole and Reeve, 2005). *Vitamin D* in form of $1,25(OH)_2D_3$ involves intestinal calcium and phosphorus absorption that is necessary for bone mineralization. *Calcitonin*, the endocrine hormone secreted from thyroid gland, inhibits bone resorption by acting directly on the osteoclast. The other systemic hormones, *Glucocorticoids* decrease calcium absorption in both the gut and the renal tubule and have the potential to induce osteoclastogenesis and bone resorption through increasing the expression of RANKL and CSF1 in osteoblasts (Hofbauer et al., 1999). *Thyroid hormones* affect bone remodeling by increasing bone resorption and turnover, and they have indirect effects on skeletal metabolism by suppressing the synthesis of thyroid-stimulating hormone (TSH), which can inhibit osteoclast formation and survival. The gonadal hormones, *Androgens* play a role in building the skeleton in young adults. They are indirectly mediated by regulation of cytokines and growth factors expressed locally in bone. They upregulate transforming growth factor (TGF) and insulin-like growth factors (IGFs), which stimulate bone formation, and downregulate interleukin (IL-6), which stimulates osteoclastogenesis (Clarke and Khosla, 2009). *Estrogen* acts to maintain the appropriate ratio between bone-forming osteoblasts and bone-resorbing osteoclasts by inducing apoptosis of pre-osteoclasts, modulating osteoclast formation, and inhibiting PTH-stimulated osteoclast-like cell formation (Oursler, 2003).

Bone mass and Bone Mineral Density (BMD)

Bone mass is the weight of bone substance per unit external volume. The bone mass of a given part of the skeleton is directly dependent upon both its volume or size and the density of the mineralized tissue contained within the periosteal envelope. Approximately 50-70% of bone content is minerals (mostly hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ (Clarke, 2008), therefore bone mass is also called bone density or bone mineral density (BMD). The bone density refers to the ratio of weight to the volume or area of the bones, and BMD refers to the amount of minerals (mostly calcium and phosphorous). The World Health Organization (WHO) definition of osteoporosis references the mean peak bone mass observed in women aged 20-45. Osteoporosis is defined by "T-score," which is the number of the standard deviations (SDs) from the mean BMD in young adult women. When T-score at any site of less than -2.5 or lowers is determined Osteoporosis, whereas Osteopenia is defined as a T-score between -1 and -2.5 (World Health Organization, 2011).

Change in bone mass and architecture over the lifespan can be characterized according to physiological changes of bone by a division into approximately five stages (stage I-V) (Sowers, 2000). *Stage I* begins at birth to approximately age 20 years that presents the formation and mineralization of bone during growth. *Stage II* shows optimizing and maintaining PBM during age 20-39 years. *Stage III* occurs with age 39-59 years that the menopausal transition represents a shift in ovarian hormone concentration and the eventual predominance of bone resorption. Risk of osteoporosis due to an excess of bone resorption demonstrates in *stage IV* during age 60-79 years. The final stage occurs at age more than 80 years that events of bone mineral loss, matrix deterioration (microfracture), increasing frailty, and presence of co-morbid conditions promote osteoporosis. However, in females, growth is characterized by increased estrogen levels and increased mass and strength of bone relative to that of muscle, whereas in men, increases in testosterone fuel large increases in muscle, resulting in muscle forces that coincide with a large growth in bone dimensions and strength (Lang, 2011). Bone mass continues to substantially accumulate at all skeletal sites in both males and females until the fourth decade, and reaches a peak maximum at 25-30 years. Therefore, it is important to understand the time course for attaining peak bone density if preventative lifestyle changes for osteoporosis are to be adequately implemented, and to understand how the origins of osteoporosis may begin during childhood and young adulthood.

Factors determining bone mass and osteoporosis

Osteoporosis is a common disease characterized by reduced bone mass and increased risk of fragility fractures. Many factors are known to influence bone mass accumulation during growth that are involved in maintenance of bone mass during adulthood, and in affecting bone loss in later life (Rizzoli et al., 2010). The determinants of bone mass include the most prominent

genetic factors, race, female sex, nutrients, endocrine factors, physical activity, body weight, and exposure to the risk factors (corticosteroids, coexisting diseases, smoking, excessive alcohol consumption). The factors that induce high bone turnover usually produce negative effects on BMD. Biochemical markers representing bone turnover are therefore used in several studies to predict bone loss and fracture risk as determinants of osteoporosis.

However, it have been reported that genetic factors play an important role in the pathogenesis of osteoporosis, as up to 75% of the variance in bone mass is genetically determined. Several studies have indicated age at menopause being genetically determined estrogen deficiency at menopause that is one of the most important determinants of bone loss in women (Rizzoli et al., 2001). The familial association in BMD was strongest among premenopausal daughters, and maternal BMD is an important independent predictor of BMD among daughters. A parental history of fracture particularly hip fracture conferred an increased risk of fracture that was independent of BMD (Kanisa et al., 2004). Moreover, a recent study revealed that sisters' fracture history was associated with 10-year fracture-free survival in perimenopausal women but not with BMD or its changes (Sirola et al., 2009). Although family and genetic studies have clearly established an important genetic influence on BMD, epidemiologic studies have revealed a number of environmental and lifestyle factors to be associated with reduced BMD that include less physical activity and exercise, high consumption of carbonated and caffeine contained beverages, alcohol drinks, and smoking. In addition, peak bone mass (PBM) which is a key determinant of the lifetime risk of osteoporosis, body weight (BW) and body mass index are also considered a strong predictor of BMD. While, evidences have showed that high calcium diets can recover the negative effects on bone resulting from those factors.

Peak Bone Mass

Peak bone mass (PBM) is defined as the amount of bony tissue present at the end of the skeletal maturation. It is an important determinant of osteoporotic fracture risk. The acquisition of PBM is normally considered to be completed by the end of the second decade of life, although a very small proportion of bone consolidation may occur during the third decade, particularly in males. At least 90% of PBM is acquired by age 18. Maximal peak bone mineral content (BMC) velocity is obtained at the ages of 14 years in boys and 12.5 years in girls, and over 25% of their future PBM acquired in adolescents is occurred during the 2 years of peak skeletal growth (Whiting et al., 2004). A population-based study (Löfman et al., 2000) found that PBM occurred in women in their early 20s at the proximal femur and at aged 28 and 31 years at the spine and forearm, respectively. However, the age of PBM is still unclear. It is achieved varies by sex, race and skeletal sites (Yang et al., 2011) have reported that BMD at the lumbar vertebrae, femoral neck and total hip of Chinese male (aged 17 to 23 years) reached a plateau at aged 22 years (2012) (Cheng et al., 2012) showed that the peak BMD values of the spine, femur, and total body are observed in Chinese females aged 30 to 39, 20 to 29, and 30 to 39 years, respectively, and in Chinese males aged 20 to 29 years at all sites. In Caucasian females, BMD of the spine, femoral neck, and trochanter region of the femur achieved peak values around aged 20 years, whereas that of the distal radius slightly increased between aged 20 and 47 years. In the femoral neck, BMD achieved the peak value around aged 20 years and showed a slight decrease during the following decades (Haapasalo et al., 1996). In Canadian women and men, lumbar spine PBM occurred at aged 33 to 40 years in women and aged 19 to 33 years in men. Total hip PBM occurred at aged 16 to 19 years in women and aged 19 to 21 years in men (Berger, 2010). However, these different reference values for PBM lead to different estimates of the prevalence of osteoporosis. In addition, previous studies have shown that bone mass and bone density in healthy adolescent males and females at skeletal maturity were inversely related to the timing of puberty (McKay et al., 1998). Delay of puberty and constitutional delay in adolescences resulted in decreased bone mineralization and lower PBM (Yinglinga and Khanejab, 2006). In particular, estrogen levels during growth are an important factor in the pathogenesis of bone fragility. Therefore, the acquisition of an optimal of PBM is a key factor for osteoporosis prevention.

As mentioned above, although PBM largely influenced by genetics, it can be modified by many factors including ethnicity, gender, diets (calcium and protein intake), physical activity, endocrine status such as sex hormones, vitamin D, growth hormone, and insulin-like growth factor (IGF-I), as well as exposure to risk factors such as excess cigarette smoking alcohol intake and carbonated beverage. These factors have been reported to be significant determinants of osteoporosis risk in later life. More important, it was indicated that behaviors to promote bone health are not being practiced by young people. The research studies of the present behaviors of 16–18 year olds in full-time education in the UK (Gurney and Simmonds, 2007) demonstrated that there was a low level of knowledge and awareness regarding the prevalence of osteoporosis and its associated risk factors. Targeted education programmes are therefore needed and should be aimed at both improving knowledge and affecting health beliefs in a manner appropriate and appealing to this age group.

Physical activity and exercise

It is now established that mechanical force through gravity or muscle pull is a major factor in maintaining or increasing bone mass and bone strength. Evidence showed that exercise can contribute to the prevention of osteoporosis by increasing PBM, reducing age-related bone loss, or restoring bone already lost in the elderly. Weight bearing physical activity (or resistance exercise) and increased mechanical loading had beneficial effects on bone mineralization and development in children and adolescents, particularly during periods of rapid bone growth (Hind and Burrows, 2007). They were the exercises that generated impact to the skeleton including aerobics, football and gymnastics; resistance training; plyometrics; circuit training; jumping exercises or a combination of exercises. Moreover, several reports have revealed that the effective effects of exercises on increasing bone strength, reducing fracture risk and a given peak BMD depend on the optimal intensity, frequency and duration of such exercise and the location of bone accrual (MK Karlsson, et al. (2001) found that exercise by playing competitive national-league soccer at more than 6 hours per week did not appear to confer any added BMD benefit to men. Whereas daily hopping exercises over 6 months could increase femoral neck BMD in premenopausal women but less frequent exercise had no significant effective (Bailey and Brooke-Wavell, 2010). The study in older women aged 75-85 years with low bone mass revealed that the agility training significantly increased cortical bone density at the tibial shaft, whilst the resistance training significantly increased cortical bone density at the radial shaft (Liu-Ambrose et al., 2004). Although both resistance exercise and aerobic exercise demonstrated protecting against the functional balance control that was strongly related to fall risk in Caucasian older women aged 60-95 years with 8 months (3 times per week) of resistance exercise, the resistance exercise was more effective than aerobic exercise for inducing favorable changes in BMD and muscle strength (Marques et al., 2011). Furthermore, dynamic loads were indicated to be determinants of PBM (Moisio et al., 2004). Results showed that external joint moments (during walking) enhanced predictability of proximal femoral BMD and BMC when compared to that of height and/or body mass. As much as 40% of the variance in peak BMD at the proximal hip could be explained by including dynamic loading information. In addition, participation in long-term (8 weeks) physical training program resulted in increased biomarkers of bone formation without substantial alterations in the markers of bone resorption (in young healthy women) (Lester et al., 2009). Whereas, strength training over 9 months (a 50-min strength training, twice weekly for 15 weeks) did not lead to significantly greater change in total body or regional BMD in premenopausal women aged 30-50 years (Singh et al., 2009). However, past physical activity significantly reduced the risk with a dose–response relationship, and recent physical activity decreased the risk to about two-thirds for both very active and active activities in elderly Thai women aged more than 51 (Boonyaratavej et al., 2001).

Besides protecting against osteoporosis, preventing falls and reducing fracture risk, the recent population-based study in adult men and women also reported that changes in physical activity were associated with changes in both BMD (increases) and in BMI (decreases), and increases in physical activity had the potential to decrease the obesity epidemic without increasing the risk of bone loss (Langsetmo et al., 2012).

High calcium diets

It has been now revealed that changes in bone mass and size during growth are dependent on both calcium (Ca) intake and exercise. The largest accumulation of bone is concentrated in children with high physical activity and high Ca intakes, because both behaviors can help in the prevention of osteoporosis in later life. Many studies showed that Ca was beneficial to the prevention of osteoporosis and related fractures (Heaney and Weaver, 2005). As reviewed above, approximately two-thirds of the weight of bone are mainly *crystalline hydroxyapatite*—$Ca_{10}(PO_4)_6(OH)_2$. The levels of extracellular Ca and phosphate (P) therefore reflect the roles of Ca and P in mineralization of bone. Ca balance influences a homeostatic state of bone turnover, and thus maintenance of bone mass. Serum Ca concentrations are normally tightly controlled within a narrow range, usually 8.5 to 10.5 mg/dL (2.1–2.6 mmol/L) (Taal et al., 2012). Low concentration of ionized Ca stimulates secretion of PTH that then affects on bone, intestine and renal to maintain the extracellular Ca level. Moreover, PTH functions in a positive feed-forward loop by stimulating production of 1,25-dihydroxyvitamin D (calcitriol) (Horner, 2006). For the action on bone, PTH can both increase bone formation and increase bone resorption according to whether PTH is administered continuously or intermittently (Melmed et al., 2011). When the secretion of PTH is stimulated continuously due to hypocalcemia, the effect of PTH on bone resorption dominates, and the net results are release of Ca from bone sequentially reduction of bone mass. These effects reflect the dietary factors affecting on Ca absorption and Ca excretion (such as excess intake of caffeine, alcohol and carbonated beverages, high dietary proteins) that determines extracellular Ca balance. However, it was reported that Ca also reduced bone remodeling rates through PTH suppression (Heaney and Weaver, 2005). Daily Ca requirement depends on age (Table 1), gender and race, and the intervention of vitamin D (cholecalciferol or vitamin D_3) (Table 2) is necessary to achieve an adequate Ca absorption

Table 1. Age dependence on Ca intake

Age (years)	Ca intake (mg/day)
< 1	400[1]– 600
1–5	800
6 – 10	800– 1200
11 –24	1200–1500
25– 65	1000–1500[2]
> 65	1500

[1] < 6-month-old.
[2]Postmenopausal women without estrogen replacement therapy, pregnant women, and during nursing.

Table 2. Age dependence of vitamin D intake

Age (years)	Vitamin D intake (IU/day)
19– 50	200
51– 70	400
> 70	600

(Rodrı´guez-Martı´nez and Garcı´a-Cohen, 2002). Ca intake of 1000–1500 mg/day is needed to ensure skeletal optimization across the population at all ages after childhood. However, in addition to promote bone health, high Ca consumption also demonstrates a protective effect regarding to those disruption of Ca balance.

Milk is recommended as an excellent Ca source for bone health, and is considered to affect bone metabolism directly. Active components in milk including milk whey protein can suppress osteoclast-mediated bone resorption and osteoclastic cell formation *in vitro*. Milk whey protein had no effect on Ca absorption, but it enhanced bone strength (in young ovariectomized rats) (Toba et al., 2000). The Ca content in natural soymilk is only 200 mg/L compared with cow's milk, which contains 1200 mg/L. However, Ca absorption is equivalent for Ca carbonate-fortified soymilk and cow's milk at similar Ca loads (Zhao et al., 2005). It is widely known that increasing milk or other dairy product intake promotes child and adolescent bone mineralization. Regular consumption of cow's milk during childhood and adolescence has been associated with higher bone density in adults and a lower risk of osteoporotic fracture in later life. Higher milk intake during adolescence is associated with greater total body, spine, and radial bone mineral measures during development of PBM, whereas current Ca intakes may influence spine bone mineral content. Frequent milk consumption before age 25 favorably influences hip bone mass in middle aged and older women (Murphy et al., 1994). Women with low milk intake (< 1 serving of milk/week) during childhood and adolescence have less bone mass in adulthood and greater risk of fracture, associated with a 2-fold greater risk of fracture (Kalkwarf et al., 2003). Supportive results showed that Ca deficit (< 500 mg/day) resulting from cow's milk allergy was associated with increased fracture risk in girls (Konstantynowicz et al., 2007). In postmenopausal women (average 6–7 years postmenopause), milk supplementation [50 g of high-calcium, low-fat, low-lactose milk powder every day (Anlene, New Zealand Dairy Board, Wellington, New Zealand)] helped prevent bone loss that could be sustained after 2 years (Lau et al., 2002). An adequate vitamin D intake was associated with a lower risk of osteoporotic hip fractures in postmenopausal women (Feskanich et al., 2003). However, to promote Ca retention in the elderly for osteoporosis prevention, improving vitamin D status and introducing foods high in bioavailable Ca are necessary.

Milk whey protein, especially milk basic protein (MBP) supplementation was effective in increasing BMD in young women (Uenishi et al., 2007), and preventing bone loss in menopausal women (Aoe et al.,2005). Moreover, fortified milk supplementation (delivered 1000 mg of extra Ca and 5 μg of $25(OH)D_3$ per day with or without added 80 μg per day phylloquinone for 16 weeks) affected a decrease in bone turnover significantly in premenopausal women (Kruger et al., 2006).

Carbonated beverages

Trend in food consumption in adolescences is currently moving towards a reduced milk intake coinciding with an increased consumption of soft drinks. The most popular are cola beverages, the carbonated soft drinks (Whiting

et al., 2001). This behavior contributes to low PBM attainment, increased fractures risk, and thus osteoporosis in later years. Carbonated soft drinks were defined as beverages that contained artificial sweeteners (e.g., aspartame) instead of added sugar. Why is the cola beverage especially? The major differences between cola and other carbonated beverages are caffeine, phosphoric acid (H_3PO_4), and cola extract. While, non-colas were defined as any drink using citric acid as the acidulant. With this property, caffeine and phosphoric acid may adversely affect bone. Several researchers found an inverse association between carbonated soft drink consumption, particularly cola beverages, and BMD. The cola beverages are highly associated with bone fractures among physically active girls. An observational study of boys and girls aged 12 and 15 years (McGartland et al., 2003) showed interested results that higher intakes of carbonated soft drinks (209.0±269.1 g/day and 251.3±341.4 g/day) were significantly associated with lower BMD at the heel than at the wrist, but only in girls. This was explained that the heel is composed mainly of trabecular bone, in contrast to the forearm, which is comprised mainly of cortical bone. D Ma and G Jones (2004) also reported that cola was associated with increased wrist and forearm fracture risk in children aged 9-16 years independent of other factors. Moreover, this effect was mediated by television watching and BMD but not by decreased milk intake (over 80% of subjects drink milk 6 or more times per week in the last year). On the other hand, a 10-day interventional study in young men with a low-Ca diet (Kristensen et al., 2005) reported that high intake of cola induced increased bone turnover that was significantly marked with serum cross-linked C-telopeptides (CTX) and urinary cross-linked N-telopeptides (NTX).

In addition, several studies have reported that effects of caffeine and phosphoric acid, the constituents of cola beverages, were associated on BMD. A study in adult women and men (Tucker et al., 2006) showed that regular cola intake (>3 serving per week) was associated with significantly lower at each hip site, but not the spine, in women but not in men. And this lower BMD was proposed to be contributed by the caffeine content of cola. It has been known that caffeine represents a diuretic effect which induces a negative Ca balance, hypocalcemia. This effect further increases bone resorption to maintain serum Ca. The supportive results showed that heavy cola soft drink consumption was associated with hypocalcemia both in clinical and experimental settings whereas consumption of carbonated beverages on a short-term basis was reported not to adversely affect serum or urinary Ca metabolism markers (Fernando et al., 2000). A conflicted result (Heaney and Rafferty, 2001) was revealed that a short-term consumption of caffeinated beverages, but not phosphoric acid contained beverages, associated with the excess calciuria in women. Another result was also presented that the net effect of carbonated beverage constituents on Ca economy is negligible, but the skeletal effects of that consumption are likely due primarily to milk displacement. On the other hand, previous studies have reported that consumption of the phosphoric acid-containing soft drinks also affect hypocalcemia. A case-control study presented that hypocalcemia was occurred in children who drank at least 1.5 liters/week of phosphoric acid-containing soft drinks, and there was a causal relationship between the ingestion of cola soft drink and hypocalcemia (Efrain et al., 1995). This result related to that the exogenous administration of phosphate can affect both hyperphosphatemia and hypocalcemia. In postmenopausal women study (Fernando et al., 1999), results showed that consumption of soft drinks with phosphoric acid was an independent risk factor for hypocalcemia, additionally increased serum levels of PTH (a compensatory secondary hyperparathyroidism) and hyperphosphaturia without significant differences in $1,25(OH)_2D_3$ and Ca excretion. Moreover, the secondary increased PTH possibly led to increased intestinal Ca absorption and perhaps to an improvement in the ionized Ca concentration, and acid loads may also adversely affect Ca and bone metabolism.

In addition to those direct effects of caffeine and phosphoric acid containing in beverages, intake of carbonated beverages may displace other nutriments such as milk from the diet, resulting in decreased Ca intake, which can lead to lower BMD. Thus, the amount as well as the nature of the carbonated beverage should be concerned an issue with respect to bone health of adolescents.

Caffeine contained beverages

Caffeine is contained in different foods and beverages (Table 3) (Bolignano et al., 2007). It is one of the main constituents of coffee that is the most widely consumed psychoactive beverages throughout the world. In fact, caffeine (1,3,7-methylxanthine) has the effects on bone metabolism that is recognized as a mild diuretic that affects short-term increases of urinary Ca and Na excretion. Urinary Ca had a negative influence, presumably by reducing Ca accretion into the skeleton, while Ca intake had a significant positive influence on BMC and BMD of the whole body and radius shaft (Matkovic et al., 1995).

Several epidemiological studies have reported the influence of caffeine on osteoporosis, but the effects of coffee on bone metabolism remain controversial because they are associated with nutrition, exercise, alcohol intake, smoking and lifestyle factors as well as amount of caffeine contained in the coffee. A review article (Bolignano et al., 2007) concluded that there is no evidence contraindicating the consumption of the equivalent of 3 to 4 cups of coffee per day in healthy or

Table 3. Amount of caffeine content in beverages.

Product	Size	Caffeine content (mg)
Roasted coffee	150 mL	85
Instant coffee	150 mL	60
Decaffeinated coffee	240 mL	5
Expresso	57 mL	100
Dark chocolate	43 g (1 bar)	31
Milk chocolate	43 g (1 bar)	10
Hot chocolate	150 mL	4
Cola classic	355 mL	34
Green tea	240 mL	15
Leaf or bag tea	240 mL	30

nephropathic subjects. Whilst, a cohort study in 70-year-old men and women reported that a daily consumption of three or more cups of coffee significantly related to a low bone mass in non-smoking women (Johansson et al., 1992). Another work indicated that administration of at least 300 mg of caffeine induced acute increased diuresis, and affected an increased Ca, Mg, and Na excretion (Maughan and Griffin, 2003). PB Rapuri, et al (2001) reported that intakes of caffeine in amounts >300 mg/day (~514 g, or 18 oz, brewed coffee) accelerated bone loss at the spine in elderly postmenopausal women, and women with the tt genetic variant of vitamin D receptor (VDR) appeared to be at a greater risk for this deleterious effect of caffeine on bone. Another work (Rico et al., 2002) revealed that intake of vitamin D, not of caffeine or other nutrients, influenced amplitude-dependent speed of sound (Ad-SOS) of phalangeal bone in healthy postmenopausal women, when comparing between the groups of low (≤100 mg/day) and high (>100 mg/day) caffeine intake, low (<800 mg/day) and normal (≥800 mg/day) calcium intake, and low (<400 IU/day) and normal (≥400 IU/day) vitamin D intake. Later in 2007, PB Rapuri, et al elucidated the mechanism whether 1,25-Dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) played a critical role in regulating bone metabolism. They found that caffeine dose dependently decreased VDR protein expression and alkaline phosphatase enzyme activity, a marker of osteoblast differentiation in osteoblast cells, and caffeine at concentrations of 1 and 10 mM decreased VDR expression by about 50–70%, respectively.

In addition, several previous works had reported effects of different amount of caffeine consumption and Ca intake on bone status and Ca excretion during childhood and young adults. Longitudinal investigation demonstrated that no significant differences of total body bone gain during ages 12-18 years among three groups with different amount of caffeine intake per day: low (<25 mg), moderate (25-50 mg), and high (>50 mg) (Lloyd et al., 1998). These results were explained that subjects with low caffeine intake consumed more milk and more fruit per day than did the other two groups, while the subjects with the highest caffeine intake consumed more sugar per day than did the other two groups. However, coffee consumption with more milk or more sugar may distribute effects of caffeine on body bone gain. The old report (Massey and Patsy, 1988) had showed that consumption of caffeine (3 mg/kg) with sucrose (3 g) in a soft drink increased the 3 hour total urinary Ca excretion when compared to a caffeine-free drink with a non-nutritive sweetener in adolescents aged 13-18 years. However, caffeine intake in the range consumed by young adult women was not an important risk factor for low BMD (Conlisk and Galuska, 2000). Their results showed the association between caffeine consumption and BMD at either site did not differ significantly between those who consumed low levels of calcium (≤836 mg/day) and those who consumed high levels of calcium (>836 mg/day).

Although the role of caffeine as a risk factor for bone loss and fracture is controversial, RP Heaney (2002) concluded that there was no evidence that caffeine had any harmful effect on bone status or on the Ca economy in individuals who ingested the currently recommended daily allowances of Ca. The negative effect of caffeine on Ca absorption was small enough to be fully offset by as little as 1–2 tablespoons of milk. The differences of peak caffeine absorption, time to peak absorption, and subjective effects of caffeine was determined by dose, time of day, added sweetener, environmental setting or contingencies.

However, mechanisms underlining the caffeine-induced bone loss and osteoporosis have been still published. M Focking et al. (2005) found that caffeine potentiated glucocorticoid receptor (GR) transcriptional activity, and the caffeine-mediated enhancement in glucocorticoid signalling was receptor- and DNA binding site-specific. It has been known GR is a major factor in the induction of osteoporosis, and it has the potential to decrease the number of functional osteoblasts via

promoting apoptotic pathways resulting in osteoblastic cell death. YH Tsuang, et al. (2006) concluded that caffeine had potential deleterious effect on the osteoblasts viability, which might enhance the rate of osteoblasts apoptosis. Data showed that the viability of the osteoblasts, the formation of ALP positive staining colonies and mineralization nodules formation in the osteoblasts cultures decreased significantly in the presence of 10 mM caffeine, moreover the intracellular LDH, ALP and PGE_2 content also decreased significantly. Yi Zhou, et al. (2009) proposed that estrogen may block cAMP-dependent PKA pathway which is shared by caffeine, to exhibit its antagonistic roles. This was due to caffeine, a known inhibitor of cAMP phosphodiesterase, activating cAMP-dependent protein kinase A (PKA) pathway. Moreover, they also mentioned unpublished studies which showed that the precursor cells of osteoblasts, bone marrow-derived mesenchymal stem cells (BMSCs), were more sensitive than osteoblasts when exposed to the same dose of caffeine.

Smoking and alcohol consumption

Cigarette smoking and alcohol consumption have been implicated as risk factors for low bone mass and thus osteoporosis. Smoking was positively and significantly associated with decreased hip BMD in older subjects, and the risk of hip fracture increased linearly with greater cigarette consumption. A meta-analysis showed that smokers had significantly reduced bone mass at all bone sites that were the greatest in men and elderly (Gerdhem and Obrant, 2002). Greater bone loss was found in current premenopausal women smokers, and bone density diminishing by about an additional 2% for every 10 year increase in age, with a difference of 6% at age 80 (Law and Hackshaw, 1997). A simple history of smoking duration was as good as to obtain more detailed smoking information, but that only 25% of the variation in BMD was explained by personal characteristics, family history and lifestyle factors (Grainge et al., 1998). BMD was more strongly related to the number of months spent smoking than to pack-years of smoking. There were significant reductions in BMD of habit smoking, but not for current smoking. However, there are the reports of pack-years of smoking being negatively associated with bone mass. Elderly smokers of at least 20 cigarettes per day had the lowest mean absorption fraction that less efficient Ca absorption may be one contributing factor (Krall and Dawson-Hughes, 1999). Smoking more than 1 pack/day significantly affected bone loss in elderly women aged 65–77 years, whereas smoking less than 1 pack/day did not show a significant deleterious effect (Rapuri et al., 2000). A recent study revealed that smoking status and duration of smoking (at least 20 cigarettes per day) were also deleterious factors on bone density of the lumbar spine, and this effect was cumulative with duration and quantity (Kuo et al., 2008). In addition, heavy smoking promoted an increase in bone resorption with a significant increase in bone remodeling markers, serum osteocalcin and urine N-telopeptide/creatinine (NTx/Cr) ratio. Loss of BMD in light and heavy elderly smokers is a result of decreased Ca absorption that is associated with secondary hyperparathyroidism (Rapuri et al., 2000).

Although many previous works have been demonstrated the negative effects of cigarette smoking on BMD, a recent review article concluded that smoking cessation helps reverse the impact of smoking (Wong et al., 2007). A previous study showed that smoking of more than 10 cigarettes daily before the operation and attempted fusion at two or more levels increased the risk of nonunion (Andersen et al., 2001), whilst a recent study revealed that smoking cessation increased fusion rates to near those of nonsmokers (Roberts and Falls, 2012). A research study in patients with posterior instrumented fusion at either L4–L5 or L4–S1 confirmed that postoperative smoking cessation can improve the negative bone effect of cigarette smoking after spinal fusion (Glassman et al., 2000). However, smoking cessation, even in later life, may be beneficial in slowing or halting bone loss and this benefit was not observed until 10 years after cessation. A current article (Wong et al., 2007) reviewed that smoking exerting a negative effect on bone mass at the major sites of osteoporotic fracture was independent of other risk factors, such as age, weight, sex and menopausal status. In addition, it was mentioned that nicotine, which is the principal active chemical in tobacco, compromised mechanical strength properties of bone during fracture healing. Nicotine smoking had a negative influence on bone mass independent of differences in weight and physical activity in elderly women (Gerdhem and Obrant, 2002).

Chronic alcoholism and the heaviest beer drinking have affected osteopenia and increased incidence of skeletal fractures, whereas lifetime and current of alcohol consumption have been indicated that do not have an independent association with BMD. A previous research (Hemenway et al., 1988) showed that middle-aged women who consumed alcohol drinking more than 15 g/day and whose relative BMI was less than 21 kg/m^2 were at increased risk of fractures, but these risk factors were not independent. Chronic alcohol consumption more than 40 g/day for at least 3 years induced low BMD in the femur Ward's triangle and trochanter that related to a slight decrease in osteocalcin and a slight increase in deoxypyridinoline (Kim et al., 2003). However, several studies have demonstrated moderate alcohol consumption positively correlated with the bone mass and BMD. At appropriate doses of alcohol is a powerful stimulant of calcitonin secretion that acts on both inhibition of bone resorption and stimulation of bone formation. Beer drinking promoted the greater bone density that might be a result of the phytoestrogen content, flavones which have a major estrogenic effect in

women, and beer drinking also stimulated calcitonin secretion (Pedrera-Zamorano et al., 2009). Evidence showed that a small amount of alcohol had benefit to BMD, whereas moderate alcohol intake (1–2 drinks/day) did not appear to have a detrimental effect on BMD, and even high intakes of alcohol did not appear to decrease BMD in men (May et al., 1995). A systematic review and meta-analysis from white European or American adults aged more than 50 years reported that persons who consumed 0.5 to 1.0 drink/day (7-14 g/day) have a lower risk of hip fracture, whereas consuming more than 2 drinks/day had higher risk when compared with abstainers. Moreover, the greater alcohol consumption (up to 2 drinks/day) is linearly associated with higher bone density (Berg et al., 2008). The present large-scale study of elderly Japanese men revealed that an alcohol intake of <55 g/day was positively correlated to BMD, whereas alcohol intake of ≥55 g/day was inversely correlated to BMD (Kouda et al., 2011). However, FMK Williams et al. (2005) concluded that moderate alcohol consumption was not harmful to bone health in women and may even be beneficial. This beneficial effect was proposed that did not appear to be mediated through an action on bone metabolism, because markers of bone turnover were not associated with alcohol or BMD.

The mechanisms of alcohol inducing bone loss have been studied in animal models. Prolonged moderate alcohol intake (3 weeks) impaired osteoblastic function and a reduction in osteoclast number. A recent animal study showed that cortical bone is more sensitive to alcohol dose effects than trabecular bone by inducing negative changes in cortical thickness and porosity [95]. Another study reported that chronic ethanol exposure in the rat inhibited direct bone formation during distraction osteogenesis (Wahl et al., 2006). More recently, DB Maurel et al. (2011) revealed the mechanisms underlying alcohol induced osteopenia that low BMD is associated with an excess in osteocyte apoptosis and with a fat accumulation in the bone marrow.

CONCLUSION

In addition to those promoting factors (exercise, high calcium diets, and peak bone mass), and risk factors (carbonated and caffeine contained beverages, chronic smoking and alcohol consumption. Body weight (BW) is also considered a strong predictor of BMD irrespective of age and gender. Body fat itself may be a risk factor for osteoporosis and bone fractures. High BW or high BMI is related to a high bone mass, and that decreases in BW might cause bone loss. Moreover, leptin, which is known to regulate appetite and energy expenditures, may also contribute to mediate the effects of fat mass on bone by modulating both osteoblast and osteoclast activities. However, Glucocorticoid-induced osteoporosis (GIO) is the most common cause of secondary osteoporosis and is the result of the profound effects of glucocorticoids (GCs) on the skeleton. Loss of bone mass is most rapid during the first year after initiation of GC therapy, which is followed by a slower but continual loss of bone mass (Silverman and Lane, 2009). Whereas using inhaled GCs in recommended doses reduces medication-related adverse effects, and GC-induced bone loss may be minimized through proper nutrition, weight-bearing exercise, calcium and vitamin D supplementation, and indicated bisphosphonate treatment (Romas, 2008; Boling, 2004).

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