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Role of obesity, alcohol and smoking on bone health

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1. ABSTRACT

The burden of osteoporosis is increasing in all societies. In comparison with other organs or apparatuses fewer studies have focused on incorrect lifestyles and bone. This article reviews clinical and experimental studies on the effects of obesity, alcohol abuse and smoking on bone. Overweight and obesity protect bone, thus reducing the fracture risk and the development of osteoporosis in older adults. However, extreme obesity (body mass index > 40 kg/m²) seems to be a risk factor for osteoporosis. Moderate alcohol consumption may have a protective effect, whereas excessive consumption is an important risk factor. Cytokines are the main mediators of the detrimental effects of obesity and alcohol. Smoking contributes to bone loss and fracture probably by interfering with estrogens, calcium and vitamin D. Health information campaigns against these harmful lifestyles should be strengthened by using available scientific information to increase awareness about their consequences on the bone.

2. INTRODUCTION

Lifestyle may be defined as a pattern of individual practices and personal behaviors that are related to increased or reduced health risk and over which people may have more or less control. Harmful lifestyles represent avoidable risk factors that negatively impact health and wellbeing, average life expectancy and the onset of many diseases (1). In particular, among these risk factors, overweight and obesity, alcoholism and tobacco smoking are the most common cause of adverse effects on health and consequently constitute an increasing economic burden (2, 3, 4,5).

Overweight, defined as a body mass index (BMI) equal to or more than 25, and obesity, defined as a BMI equal to or more than 30, result from a chronic imbalance between energy intake and energy expenditure. They may impair health thus reducing life expectancy and/or increasing chronic diseases such as cardiovascular disease,

diabetes, musculoskeletal disorders – especially osteoarthritis, and cancers (uterus, colorectal, breast, kidney and gallbladder) (2, 3, 4, 5). The World Health Organization further projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese (6).

Alcoholism is a global health problem facing many countries, involving about 140 million people suffering from alcohol dependence (7). The lifetime risk of developing alcohol-use disorders for men is more than 20%, with a risk of about 15% for alcohol abuse and 10% for alcohol dependence (8). The link between alcohol consumption and consequences depends on a) the two main dimensions of alcohol consumption: average volume of consumption and patterns of drinking; and b) the mediating mechanisms: biochemical effects, intoxication, and dependence (9). Referring to alcohol consumption volume, moderate drinkers tend to have better health and live longer than those who are either abstainers or heavy drinkers (10). The adverse health effects of alcohol have been shown for many disorders, including liver cirrhosis, pancreatitis, mental illness, several types of cancers (mouth, gullet, liver, colon and breast), cardiovascular disease, and damage to the fetus among pregnant women (7).

Finally, the damage and disease risks connected to tobacco smoking, proportional to the amount and duration of smoking, affect the duration of life and cause many diseases such as cardiovascular diseases, chronic obstructive pulmonary disease, renal failure and cancers (lung, bladder, oesophagus, kidney, pancreas) (11-12, 5). These diseases are significantly related to mortality and most of them to disability. Statistics show that chronic tobacco smoking reduces the life expectancy by an average of 7 years (11). Unfortunately, most of the damage done by tobacco to health does not become evident until years or even decades after the onset of use. Therefore, whereas tobacco use is rising globally, the epidemic of tobacco-related disease and death has yet to reach its peak.

Since limited and heterogeneous scientific information has been made available on the possible marked effects of these harmful lifestyles on bone over the last decade, this review should be seen as a synthetic update of data in support of the hypothesis that obesity, alcoholism and tobacco smoking, may be important risk factors for osteoporosis and fractures. Even though a great deal on the effect of physical activity on bone status is available in the literature, the range of risk factors considered does not include these because attention has been focused on three harmful lifestyles belonging to addictive behaviors. To the current authors knowledge there are no specific articles where the former lifestyles and physical activity were considered together as risk factors for bone health status, but physical activity was found to be related at least to body weight and tobacco smoking,(13-14) or to tobacco smoking and alcohol intake (13,15).

3. SEARCH STRATEGIES

On January 2011, a literature search of the entire MEDLINE database (PubMed research engine) using the following MeSH database terms was carried out:

("Obesity"[MeSH]) AND ("Bone and Bones"[Mesh] OR "Bone Density"[Mesh] OR "Fractures, Bone"[Mesh] OR "Osteoporosis"[Mesh]).

("Smoke"[MeSH] OR "Smoking"[MeSH]) AND ("Bone and Bones"[Mesh] OR "Bone Density"[Mesh] OR "Fractures, Bone"[Mesh] OR "Osteoporosis"[Mesh]).

("Alcoholism"[Mesh] OR "Alcohol Drinking"[Mesh] OR "Alcohol-Related Disorders"[Mesh]) AND ("Bone and Bones"[Mesh] OR "Bone Density"[Mesh] OR "Fractures, Bone"[Mesh] OR "Osteoporosis"[Mesh]).

All papers on the effects of obesity, alcohol and smoking on bone implant osseointegration rate were excluded (NOT "Osseointegration"[MesH]) as well as those on dentistry (NOT "Dentistry"[MeSH]). The search was limited to the last 10 years (from 2001/01/01 to 2010/12/31), English language, and abstract availability. Then, the references of these studies and pertinent reviews were manually assessed by three reviewers using predefined criteria. In particular, papers related to cannabis smoking and metabolic and degenerative pathologies or syndromes (i.e. diabetes, Prader-Willi, etc...), infectious diseases and cancer were excluded. Citations regarding human subjects aged less than 19 years were excluded, considering that primary osteoporosis and related fractures are more common in the elderly. Physical activity was not included in the search, but yielded citations, considering this lifestyle is an alternative to improving bone health status.

The number of unique papers from the electronic search was 1470, which after abstract review was reduced by 1264. Two hundred six (14.0%) papers were included in the final paper, which belonged to different types of articles as defined in PubMed. These papers included 70% human, 22% animal and 8% *in vitro* studies (Figure 1). Most of the human studies were observational (63%, 90 out of 144) and controlled studies (15%, 21 out of 144) (Figure 2). Few meta-analyses were yielded by the electronic search (n = 9) and only 5 matched the selection criteria (n = 2 for alcohol and n = 3 for smoking).

4. OBESITY AND BONE

4.1 Clinical studies

It is generally accepted that bone mineral density (BMD) is closely and positively related to body weight (BW) according to different mechanisms: a) an accommodation mechanism to a greater mechanical loading on bone, that also stimulates osteoblast activity; b) adipocytes produce estrogen that inhibits osteoclast activity and thus bone resorption; and c) obesity has been associated with insulin resistance, characterized by high plasma levels of insulin that may contribute to increased sex hormone levels, leading to an increased bone mass due to the reduction and augmentation of osteoclast and osteoblast activity, respectively (16-19). Another positive effect of increased BW is the reduced fracture rate and a cushioning effect produced by a greater thickness of adipose tissue during a fall (20). Finally, a recent review

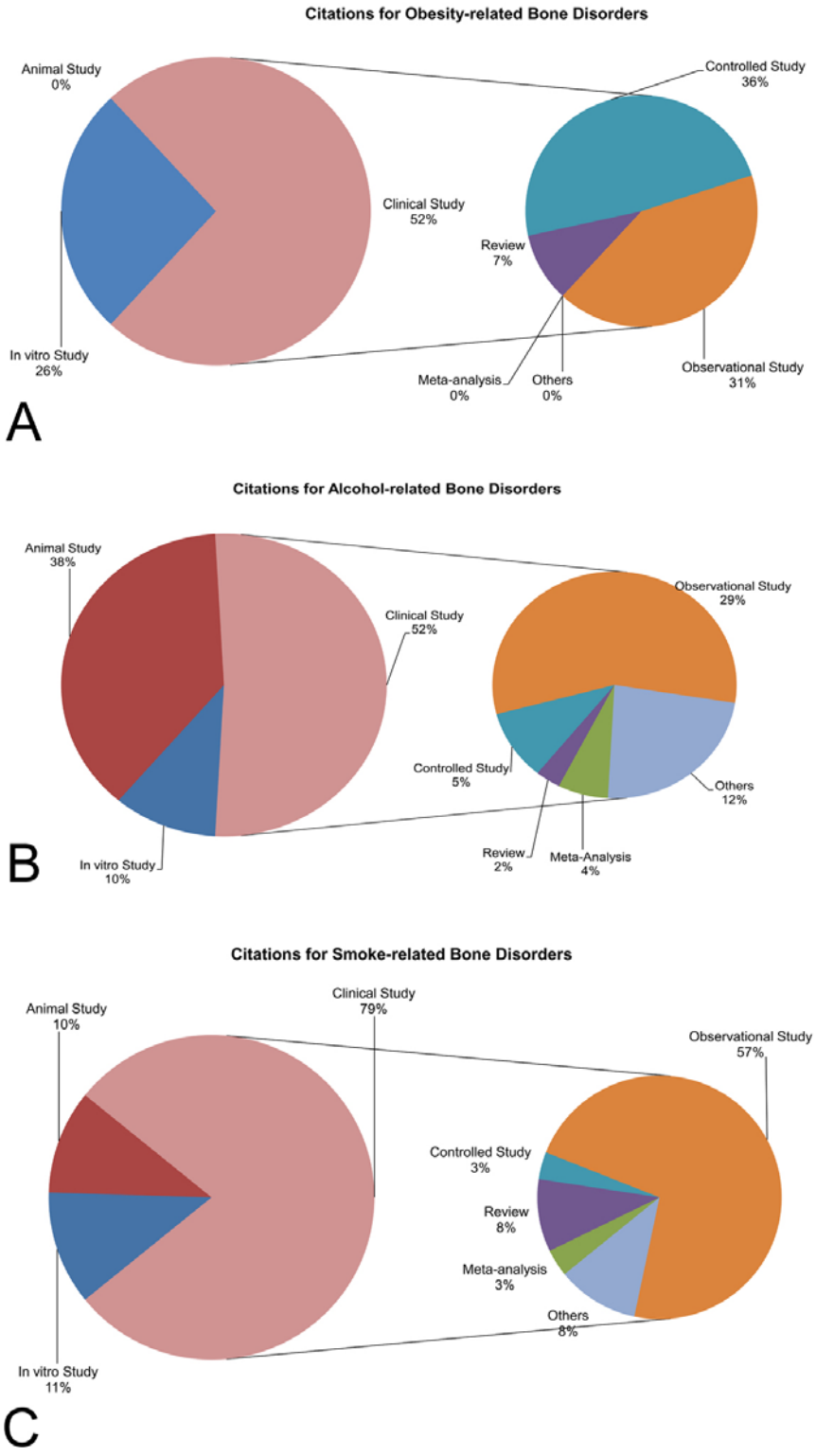

Figure 1. Number and type of publications found in accordance to chosen research strategies for A) obesity, B) alcohol and C) smoke-related bone disorders. *The research for this type of article was performer using only the following MeSH terms: (("Smoke"[MeSH] OR "Smoking"[MeSH] OR "Nicotine"[MeSH]) AND ("Osteoblasts" [MeSH])). ** The research for this type of article was performer using only "Ethanol"[MeSH] AND "Osteoblasts" [MeSH]).

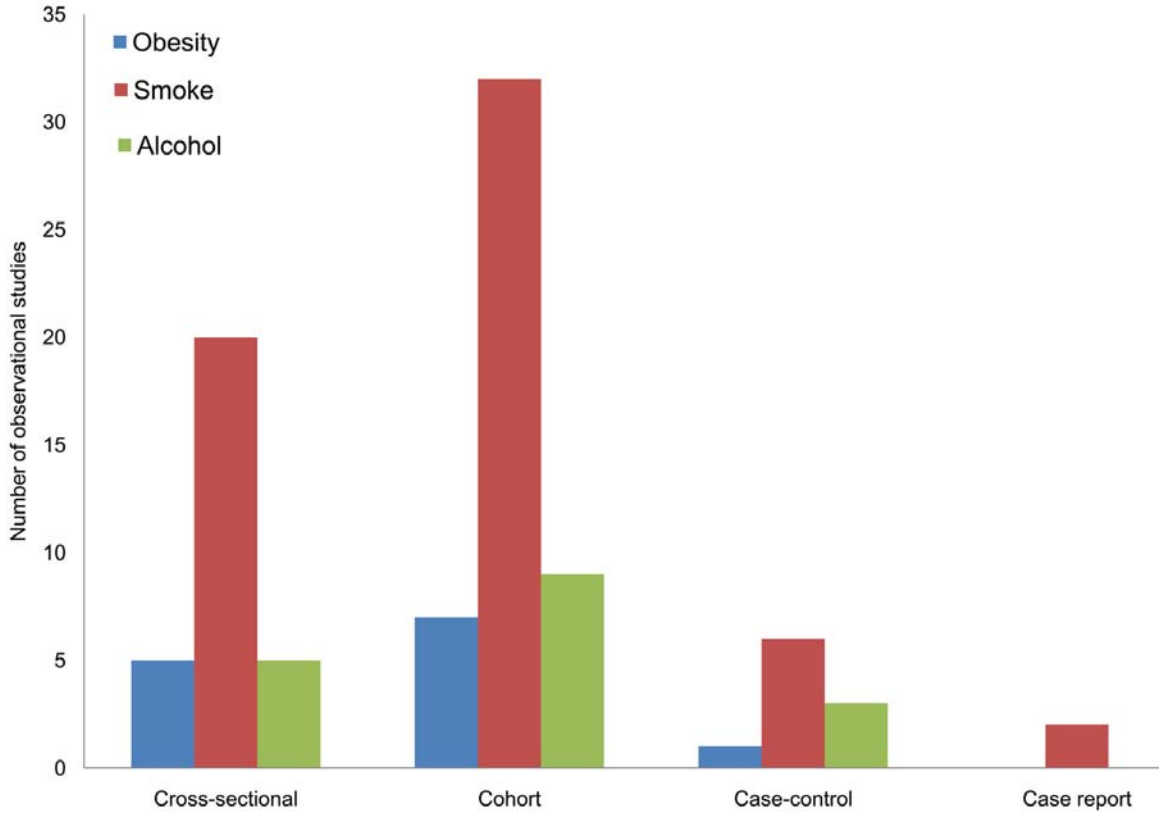

Figure 2. Histograms of the observational studies examined for obesity, alcohol and smoke-related bone disorders.

showed an indirect relationship between body weight/BMI and fracture risk. Therefore, overweight and obesity have a protective effect on bone thus reducing the fracture risk and the development of osteoporosis in older adults, however this protection is overtaken by age and long standing deficiency of estradiol (21- 22).

However, even if body weight is one of the strongest predictors of BMD, the relative contribution of fat mass (FM), the most important index of obesity, and lean mass (LM) to the weight-bone relationship has not yet been well defined (23). LM measures muscle mass and its changes during physical activity may have a greater effect on bone mass than changes in FM (24). The relationship between FM, LM and bone is reported to be different according to gender (FM is more important for BMD in women), age (LM has a greater effect on BMD in young women, whereas FM has a greater effect after pubertal growth and in postmenopausal women), skeletal site (LM is found to be strongly related to BMD of proximal femur), menopausal state (LM is a positive predictor of BMD in pre-menopausal women, whereas FM in post-menopausal women), and activity (LM influences BMD positively in exercising subjects, whereas FM has a positive influence in sedentary ones); however discordant results have been reported in the literature (23-25).

The positive relationship between BMD and BW has not been confirmed in extreme obesity (BMI > 40 kg/m²) where the relationship between body weight and bone mass is inverted (21). In particular, human and animal studies showed that extremely high levels of obesity are associated with a greater loss of BMD in estrogen-deficient osteopenia (21). These results suggest a complex interaction between fat and bone metabolism at cellular and molecular levels. On one hand, FM has a beneficial effect on increasing bone mass and "rapid" bone losers have significantly lower FM than the "slow" bone losers. On the other hand, excessive FM may not protect against decreases in bone mass, thus suggesting that when the mechanical loading effect of total body weight is removed, FM is negatively correlated with bone mass.

Moreover, obesity is reported to be responsible for peripheral or axial joint osteoarthritis. The risk of developing knee osteoarthritis in patients with BMI ≥ 30 is 6.8 times higher than that of healthy patients. The pathogenesis of osteoarthritis in obese patients is due not only to the increased mechanical load but also metabolic changes induced by an interaction between insulin-resistance and systemic inflammation (26). Acute joint inflammation determines increased levels of Thelper-1 and tumor necrosis factor (TNF), cytokines that produce an insulin-resistance condition by suppressing the sensitivity of insulin receptors on the membrane of muscle cells and adipocytes. This decreases glycogen production and, consequently, a muscle weakness leads to further traumatic events of peripheral or axial joints, thus resulting in

Table 1. Main clinical studies on obesity effects on bone

Ref. Study	Design	Analysis	Sample Characteristics	Magnitude of Association
19	Population-based cohort study	Radius BMD associated with physical activity and nutritional and dietary factors	1222 women aged 70-73 y	
			BMI ≤ 25.1	RR=0.7 95% CI (0.3-1.4) for low daily physical exertion RR=1.0 95% CI (0.7-1.4) for average daily current dietary calcium intake ≤ 654.0 mg
			25.1<BMI<28.5	RR=0.4 95% CI (0.1-2.5) for low daily physical exertion RR=1.8 95% CI (1.0-3.1) for average daily current dietary calcium intake ≤ 654.0 mg
			BMI ≥ 28.5	RR=1.2 95% CI (0.3-4.1) for low daily physical exertion RR=1.0 95% CI (0.3-3.1) for average daily current dietary calcium intake ≤ 654.0 mg
23	Cross-sectional population-based study	Effects of sex and age on the impact of FM on femoral neck BMD.	Men 47-50 y	FM <1 5 Kg OR=2.22 95% CI (1.15-4.28) FM 16-10 Kg OR=2.41 95% CI (1.25-4.64) FM 21-24 Kg OR=1.90 95% CI (0.96-3.73) FM 25-30 Kg OR=1.38 95% CI (0.65-2.95) FM ≥30 Kg OR=1
			Women 47-50 y	FM <1 5 Kg OR=2.95 95% CI (1.79-4.86) FM 16-10 Kg OR=2.60 95% CI (1.63-4.16) FM 21-24 Kg OR=2.25 95% CI (1.42-3.57) FM 25-30 Kg OR=1.48 95% CI (0.88-2.48) FM ≥30 Kg OR=1
			Men 71-75 y	FM <1 5 Kg OR=1.44 95% CI (0.74-2.80) FM 16-10 Kg OR=1.37 95% CI (0.71-2.65) FM 21-24 Kg OR=1.02 95% CI (0.52-1.99) FM 25-30 Kg OR=0.99 95% CI (0.47-2.08) FM ≥30 Kg OR=1
			Men 71-75 y	FM <1 5 Kg OR=8.43 95% CI (4.46-15.93) FM 16-10 Kg OR=2.46 95% CI (1.38-4.38) FM 21-24 Kg OR=2.13 95% CI (1.23-3.69) FM 25-30 Kg OR=3.20 95% CI (1.89-5.42) FM ≥30 Kg OR=1
195	Population-based study	A study of persistent high BMD in a multivariate logistic regression analysis	Women 53.5 y old (48.0-59.6)	BMI 25-30 kg/m^2 OR=2.84 95% CI (1.83-4.42) BMI > 30 kg/m^2 OR=5.94 95% CI (3.47-10.16) Menopause OR=0.57 95% CI (0.38-0.85) Age OR=0.94 95% CI (0.87-1.01) Daily Ca intake >1.500 mg OR=1.44 95% CI (0.52-3.97) Physical activity OR=0.86 95% CI (0.59-1.33)
16	Population-based study	Evaluate the relationship between obesity and osteoporosis (Pearson correlations FM-weight-adjusted bone area, p< 0.01)	Chinese premenopausal women and men (27.2±4.5 y old),	r=-0.25 lumbar spine r=-0.16 femoral neck r=-0.22 total body bone area
			Caucasian females and males (47.8±16.2 y old)	r=-0.17 lumbar spine, r=-0.12 femoral neck r=-0.15 total body bone area
24	Cross-sectional study	Relationship between BMD and age, BMI, FM and LM	Postmenopausal women (50-85 y old)	BMD-age and BMI: R^2= 0.30, 0.37, 0.35 for lumbar spine, femoral neck and whole body respectively. BMD- age, LM and FM: R^2=0.33, 0.38, 0.39 for lumbar spine, femoral neck and whole body respectively.

stretching and breaking of the tenoperiosteal junction with abrasive damage to cartilage. As far as cartilage is concerned, increased BMI, BW and adiposity in healthy adults with no clinical knee osteoarthritis have been found to be associated with an increased annual rate of patella cartilage volume loss due to increased loading and dysregulation of lipid homeostasis (27).(Table 1)

4.2. Experimental studies

Few experimental studies have been performed on the effect of increased BMI on bone status. Increased BMI has been reported to have a positive effect on bone mass in wild type mice and in ob/ob mice, independently of leptin signaling that exerts its positive effects on cancellous bone volume. (28) In a model of Growth Hormone (GH)-deficient rats, increasing adiposity and circulating leptin by maintenance on a high-fat diet (HFD) had no effect on femoral strength, thus indicating that obesity and hyperleptinemia do not influence the strength of cortical bone in the absence of GH (29). Some studies focused on the negative correlation between BW and bone mass, thus indicating that biomechanical loading, due to BW in growing mice, decreased BMD because of a reduction in the number of trabecolae without thickening them (30). A significant reduction in material and structural trabecular bone properties in growing rats fed on an HFD was observed and FM was morphologically and mechanically inversely correlated with bone mass. Moreover, obese mice on HFD had increased osteoclast formation and decreased osteocalcin and femoral bone mass, thus suggesting that bone resorption augments more than formation. In fact, a decreased femoral trabecular bone mass by increasing trabecular separation and reducing trabecular number and connectivity density was observed in obese mice (31).

4.3. Pathophysiology and the role of cytokines

To explain the pathophysiological linkage between obesity and osteoporosis, it has been thought that: a) both diseases are affected by genetic and environmental factors; b) bone remodeling and adiposity are both regulated through the hypothalamus and sympathetic nervous system; and c) adipocytes and osteoblasts derive from a common progenitor – the pluripotent mesenchymal stem cells found in bone marrow and adipose tissue (32). In fact, there is a large degree of plasticity between adipogenesis and osteoblastogenesis and this relationship is reciprocal in bone marrow stromal cells, leading to the concept that inhibition of adipogenesis might enhance bone formation (33). Numerous transcription factors and multiple extracellular and intracellular signals regulating adipogenesis and osteoblastogenesis have been identified. Briefly, the Wnt/beta-catenin pathway induces osteoblastogenesis and inhibits adipogenesis. TNF-alpha, interleukin-1 (IL-1) and trasforming growth factor-beta, (TGF-beta) induce a signaling cascade that attenuates peroxisome proliferator activated receptor-gamma (PPAR-gamma) functions, which is an adipogenesis inducer and osteoblastogenesis inhibitor. As a result of the previous signaling cascade, PPAR-gamma induces osteoblastogenesis (34).

Adipose tissue allows vertebrates to store excess calories such as lipids and acts as an endocrine tissue by secreting hormones known as adipokines. Numerous proteins are included under this heading, from proteins related to the immune system (TNF-alpha, IL-6) to growth factors (TGF-beta) and proteins of the alternative complement pathway (adipsin). Furthermore, other adipokines are involved in the regulation of pressure (angiotensinogen), blood coagulation (plasminogen activator inhibitor-1), glycemic homeostasis (adiponectin, resistin, visfatin, leptin) and angiogenesis (vascular endothelial growth factor) (35). Adipokines affect human energy homeostasis and may be involved in bone metabolism regulation even if their effect on bone is actually complex (35).

In all processes observed in obese and overweight patients and investigated with *in vitro* and *in vivo* experimental studies, leptin has an important pathophysiological role in the protective effect of increased FM on BMD and consequently on reducing the fracture risk and development of osteoporosis. Leptin is known to affect the immune system, reproduction, development, haemopoiesis and angiogenesis. Besides all these functions, leptin promotes the growth of osteoblasts and chondrocytes, and increases synthesis of bone matrix proteins such as type I collagen and OC (32). However, it is difficult to define the real effects of leptin on bone metabolism, because contrasting experimental and clinical results have been reported on the positive relationship between serum leptin levels and BMD (22, 32, 36). On one hand leptin can be a mediator of the protective effects of FM on bone tissue, as confirmed by the inverse relationship found between serum leptin and bone resorption markers; on the other hand, by adjusting BMD results for BMI or FM, the correlation between leptin and BMD is lost, thus suggesting that circulating leptin does not exert a protective effect on bone mass (36).

Briefly, it has been highlighted in *in vitro* models that leptin (a) induces the phosphorylation of PPAR-gamma, which prevents adipogenesis and enhances osteoblastic activity by inhibiting apoptosis (22); (b) induces MAPK-dependent cell proliferation by enhancing osteoblastic differentiation (22); and (c) increases osteoprotegerin (OPG) mRNA expression and decreases RANKL (Receptor Activator for Nuclear Factor kappa B Ligand) mRNA expression, thus limiting osteoclastogenesis (37). Conversely, it has been shown in some clinical and experimental models that *in vivo* leptin does not act directly on osteoblasts, but through a central pathway (hypothalamic-pituitary axis), which mediates its antiosteogenic function (32). Finally, leptin effects on bone may result from a balance between negative central effects and positive peripheral effects (32). The latter seem to be predominant only when serum leptin levels rise above a certain threshold as a signal of elevated energic storage.

Finally, another adipose-specific protein that has a pathophysiological role in the protective effect of visceral FM on BMD is adiponectin, which regulates energy homeostasis and has anti-inflammatory and anti-atherogenic effects.(38, 39). Unlike leptin, adiponectin levels decrease in obesity and type 2 diabetes. It is present within normal bone marrow and inhibits fat cell formation by marrow-derived stromal cells through a COX-2–dependent mechanism (38).

5. ALCOHOL-RELATED BONE DISORDERS

5.1. Clinical studies

Conflicting results on the relationship between alcohol consumption and fracture risk are reported in the literature (Table 2). Two recent metanalyses confirmed that a reported history of high intake of alcohol carries a substantial risk for future fractures and that this risk is largely independent of BMD (40-41). A threshold effect was found above which fracture risk increases; individuals who took 2 units (8 g of pure alcohol; 1 unit) or less per day of alcohol presented no increased risk of osteoporotic fracture. In particular, J-shaped and U-shaped relationships between alcohol consumption and hip fracture risk or BMD was found; moderate drinkers had lower hip fracture risk, whereas heavier drinkers had a higher hip fracture risk both compared with abstainers (40-42). Recently, Pye *et al.* highlighted a U-shaped association between quantitative heel ultrasound parameters and frequency of alcohol consumption in middle-aged and elderly men (43) Except for the prospective studies that showed higher BMD levels at different sites in drinkers,(13, 44-45) most clinical trials performed in the general population found neither a decrease in BMD nor an increase in fracture incidence associated with alcohol consumption (43, 46-51).

Alcohol-related bone disorders seem to depend mainly on consumed volume, type of drinking and drinker gender. Various studies on alcohol-induced bone loss have highlighted that bone remodeling is disrupted, new bone

Table 2. Main clinical studies on alcohol effects on bone

Study	Design	Units of Analysis of Alcohol	Sample Characteristics	Magnitude of Association
40	metanalysis drinkers compared with abstainers (1 drink= 14g/day)	≤ 0.5 drinks/day	both	RR = 0.84 95% CI (0.70-1.01) hip fracture
		0.5 – 1.0 drinks/day		RR = 0.80 95% CI (0.71-0.91) hip fracture
		> 2 drinks/day (28g/day)		RR = 1.39 95% CI (1.08-1.79) hip fracture
41	metanalysis	> 2 units/day (16g/day) (1 unit = 8g)	16971 men and women	RR = 1.23 95% CI (1.06-1.43) any fracture
				RR = 1.38 95% CI (1.16-1.65) osteoporotic fracture
				RR = 1.68 95% CI (1.19-2.36) hip fracture
48	population-based case-control study aged > 50y	≥ 2 units/day	725 women	RR = 2.9 95% CI (1.0-8.6) hip fracture
			451 men	RR = 1.9 95% CI (1.1-3.2) hip fracture
44	cross-sectional and prospective study aged > 65y	< 12 drinks/y	5974 men	
		≥ 12 drinks/y - < 2 drinks/day		RR = 0.77 95% CI (0.65-0.92) incident falls
		≥ 2 drinks/day		RR = 0.83 95% CI (0.63-1.10) incident falls
59	population-based case-control study 50-81 y	<3 g/day	4589 women	OR_{adj} = 0.78 95% CI (0.64-0.95) hip fracture
		3-6 g/day	BMI = 18.9-29.4 kg/cm²	OR_{adj} = 0.80 95% CI (0.65-0.99) hip fracture
		> 6 g/day		OR_{adj} = 0.84 95% CI (0.66-1.08) hip fracture
42	prospective cohort study aged > 65y	≤ 2 drinks/day	5888 men and women	HR_{adj} = 0.78 95% CI (0.61-1.00) hip fracture
		> 2 drinks/day	BMI 21.8-32.4 kg/cm²	HR_{adj} = 1.18 95% CI (0.77-1.81) hip fracture
120	cross-sectional study 68.6 ± 7.1 y	~0.5 – 1.0 drinks/day	136 women	R^2_{adj} = 53.3 total BMD
			BMI = 26.0 ± 3.8	R^2_{adj} = 56.1 lumbar spine BMD
63	cross-sectional study 48.44 ± 12.59 y	non-drinkers	1697 women	β = 1.06 Ad-SoS* vs beer intake
		light drinkers < 15.7g/day	BMI = 19-32 kg/cm²	β = -3.86 Ad-SoS* vs BMI
		moderate drinkers 15.7-40g/day		
65	prospective cohort study 36.87 ± 7.95 y	< 3.4 g /day	400 men	β = 0.19 femoral BMD
		3.4 – 7.3 g/day		
		7.4 – 15 g/day	BMI = 23.8 ± 2.4 kg/cm²	
13	population-based cohort study 45-92 y	< 0.43 drinks/days (referent)	507 men	OR = 0.68 95% CI (0.46 – 0.99) femoral BMD
		≥ 0.43 drinks/wk	BMI = 24-28 kg/cm²	

formation is suppressed (decreased osteoblast activity, reduced serum osteocalcin, malnourished resulting in vitamin D deficiency) and only relatively small changes occur in bone resorption (52-54).

Excessive alcohol consumption is usually associated with liver toxicity, poor nutrition status, cigarette smoking and reduced physical activity, which have all been reported to interfere with human bone homeostasis and repair in several ways (52-54, 47). It has been found that high alcohol intake drinkers had higher concentrations of pro-inflammatory markers (IL-1, Il-6, TNF-alpha) responsible for severe damage to the vascular bed above all that of the liver (55). Increased alcohol consumption has addictive effects on bone formation, thus confirming that comorbidity factors may contribute to the high incidence of osteoporosis in alcoholics (56). Furthermore, heavy drinkers, above all binge drinkers, are at a higher risk of fracture, because excessive alcohol consumption also impairs balance and fractures occur when falling while intoxicated (10,47, 49, 57-58).

Conversely, moderate alcohol consumption has a modest protective effect against hip fracture (45, 48, 59-60). Alcohol at moderate levels seems to be a powerful stimulant of calcitonin secretion, which inhibits resorption and stimulates the formation of bone, and is positively correlated with the bone mass and BMD (10, 57, 61-62). In addition, moderate alcohol consumption might induce anti-inflammatory cytokines (TGF-beta and IL-10) and endogenous estrogen synthesis, thus promoting an increase in BMD, also in men, by the aromatization of androgens to estrogens; however the level of alcohol intake to produce this effect is not evidence-defined (55, 57, 61). Furthermore, some authors suggest that the beneficial effect of moderate doses of alcohol on bone health might be due to the presence of nonalcoholic constituents such as polyphenol compounds, including flavonols and proanthocyanidins, present in alcoholic beverages (45, 62-63). Polyphenols are typically considered as antioxidants, but most of them have also estrogen-mimetic properties which might plausibly influence bone metabolism (45, 62-63). Finally, two recent studies showed the positive effect of consciously-defined lifestyle conditions, regular physical activity and balanced diet, on moderate alcohol consumption that does not lead to unhealthy bone status (64-65). (Table 2)

5.2. Experimental studies

The mechanisms of action of alcohol on bone turnover have not been fully understood and may involve both direct and indirect actions. A few *in vitro* studies have shown that alcohol modifies human bone-forming cells (undifferentiated and differentiated) directly by reducing proliferation, extracellular matrix and mineralization index synthesis (54, 66-67). Alcohol diffuses into osteoblasts, where alcoholdehydrogenase class 1 is expressed, and is metabolized to acetaldehyde and produces reactive oxygen species (ROS) that can have biological effects: (1) it may activate the p53/p21 complex, a tumor suppressor gene regulating cell growth, apoptosis and genome integrity (p53) and a downstream effector of p53 (p21); (2) it may activate alcohol responsive elements; and/or (3) it may also block the estrogen receptor nuclear translocation (68-69). This action of alcohol and negative cross-talk between alcohol signaling and estrogen receptor-mediated pathways contributes to osteoporotic bone loss and protective effects of estrogens on alcohol bone loss (69).

Gong *et al.* observed a dose-dependent inhibition of stem cell proliferation, the down-regulation of type I collagen gene expression and consequently the decrease of type I collagen C-terminal propeptide synthesis (54). They also found that alcohol reduced alkaline phosphatases (ALP) levels without influencing ALP mRNA levels, which did not affect OC gene expression (Osf2/Cbfa1), and protein synthesis by induced stem cells (54). More recently, Torricelli *et al.* found that osteoblast proliferation rate, ALP and OC synthesis and TGF-beta1, IL-6 and TNF-alpha releases were all negatively affected after *in vivo* alcohol exposure. Rosa *et al.* showed that chronic alcohol intake *in vivo* and subsequent alcohol exposure *in vitro* inhibited the differentiation of osteoblasts by increasing their proliferation rate and reducing bone-like nodule formation (70).

In vivo studies have mainly focused on the effect of alcohol on BMD and fracture healing (71-73). Direct detrimental effects of chronic or binge alcohol abuse on bone health have been highlighted by various *in vivo* biomechanical and densitometric studies using alcohol-fed rat models. First of all, it was found that chronic alcohol intake produces progressive bone loss and an increased risk of secondary osteoporosis in adult rats, whereas it suppresses skeletal growth and reduces peak bone mass in young rats (52, 74). The inhibitory effect of alcohol consumption on bone formation occurred within 2 weeks of initiating treatment, and it was transient with the same time on steady-state mRNA levels for bone matrix proteins (75). In chronic alcohol-exposed models mechanical deficiencies of femoral and tibial cortical bone, disruption of femoral osteoblasts and reduction of bone mass in trabecular bone were found; the diaphysis expanded radially to create a higher cross-sectional moment of inertia to maintain the same flexural rigidity in response to the lower elastic modulus (52, 76-80). Secondly, Maddalozzo *et al.* highlighted that alcohol has effects on bone that are both dependent and independent of caloric intake (81): the self-imposed caloric restriction that occurs with high alcohol intake clearly plays an important role in the skeletal manifestations. Alcohol consumption decreased whole body fat and serum leptin levels, but increased bone marrow fat, producing a decrease in osteoblastogenesis and an increase in osteoclastogenesis (81). Site-specific differences in bone mass were found in a binge alcohol-exposed rat model (82). It was observed that bone loss did not recover during prolonged alcohol abstinence and suggested that young adults engaging in binge alcohol consumption may experience persistent changes in the skeleton leading to a greater susceptibility to fracture and osteoporosis in later decades of life (82). Furthermore, physical activity had no effect on the bone and did not attenuate any of the negative effects of alcohol (83). Finally, Chakkalakal *et al.* observed that alcohol exposure inhibited osteoid synthesis with the formation of a mixture of osteoid and fibrous tissue in fracture repair. Since fibrosis was predominant, they concluded that the inferior bone repair was due mostly to the inhibition of osteoblast number rather than to the inhibition of mineralization (77, 73).

5.3. Pathophysiology and the role of cytokines

Alcohol decreases blood insulin growth factor levels by decreasing insulin growth factor-I mRNA levels and increases insulin growth factor-I-binding proteins by reducing insulin growth factor-I bioavailability; bone loss and muscle wasting follow (79, 84-85). Alcohol interferes with liver enzymes that are necessary for converting the inactive into the active form of vitamin D. Finally, alcohol increases the release of catecholamines (norepinephrine and epinephrine) and glucorticoids (cortisol, corticosterone), which negatively influence bone metabolism (86).

Chronic alcohol intake (a) increases the local expression of mRNA transcripts of both TNF-alpha and IL-

beta with a consequent enhancement of RANK-ligand induced bone resorption; (b) inhibits the proliferation of osteoblasts and their precursors; (c) increases RANKL mRNA expression in bone marrow cells and osteoblasts; (d) increases protective effects of OPG on alcohol-induced bone loss by blocking increased bone resorption; and (e) increases proinflammatory cytokines (76, 87-89).

By using a binge alcohol rat model, some authors highlighted the relationship between bone damage and modulation of bone transcriptome (82, 72). Acute binging affects the integrin-signaling pathway with a significant increase in mRNA levels of beta3 integrin (Itgb3), which consequently increases alphavbeta3 integrin, the main integrin expressed by osteoclasts (72). The alphavbeta3 integrin enhances both alphavbeta3-mediated matrix attachments and intracellular signaling, leading to the organization of the osteoclast cytoskeleton and formation of the protected resorptive microenvironment required for bone resorption (72). Chronic alcohol binging affects the Wnt signaling pathway at different levels. This deactivates Wnt/beta-catenin signaling, which is important for the trasformation of mesenchymal progenitor cells into the osteoblast lineage (72). Callaci *et al.* reported that chronic alcohol binging influences the expression of several "bone-forming" genes and the modulation of important regulators of bone resorption: (a) a general decrease in gene expression of bone morphogenetic proteins; (b) a decrease in parathyroid hormone receptor expression levels on osteoblasts by more than 40%; (c) an increase in sclerostin mRNA, a Wnt inhibitor, with a consequent decrease in the maturation of osteoblast precursors and thus in bone formation; (d) a differential expression of RANKL-OPG system by osteoblasts with an increase in RANKL-mediated signaling that decreases the attenuating effect of OPG leading to bone resorption; (e) a decrease in RANK expression as a possible negative feedback loop modulating alcohol-induced osteoclast over-activity; and (f) an increase in IL-6 and oncostatin M expression levels that stimulate bone resorption by increasing expression of RANKL and decreasing OPG expression (82).

6. CIGARETTE SMOKING AND NICOTINE EFFECTS ON BONE

6.1. Clinical studies

The literature is full of reports highlighting smoking as a risk factor for primary and secondary osteoporosis, bone fracture incidence and increased nonunion rates (90-105). The relative effects of tobacco use on the attainment of peak bone mass and on age-related bone loss are uncertain, as are the contributions to bone loss and fracture of a number of lifestyle variables that are commonly linked to smoking (106-113). However, it was clearly shown that smoking cessation decreases fracture risk, thus indicating that the detrimental effects of smoking are reversible (114-116). It was also highlighted that the effect of smoking on bone is mainly time and not dose dependent. (116-119) The clinical implication of all these studies was that even moderate smoking has a harmful effect on bone, and thus it should be stopped.(104, 115, 117)

Clinical studies performed on post-menopausal women have shown decreased BMD and increased fracture risk in smokers (15, 13, 107, 120-125). However, the biological mechanisms by which smoking influences bone loss have not been clearly understood (106, 117). Tobacco smoking also contributes to bone loss by interfering with calcium and vitamin D homeostasis, which are vital for bone metabolism (127-128). A negative effect of smoking on BMD has been found in different skeletal sites such as the spine, femoral neck and total body (47, 103, 109, 128-135), but some authors did not find any negative effects on BMD (108, 136-138). In particular, it has been reported that former smokers had similar BMD to that of current smokers and lower compared with that of never smokers at most skeletal sites and lifetime smoking seems to be an independent predictor of BMD.(108-139)

Both current smokers and lifetime tobacco smoking were associated with a deterioration in bone structure and elasticity that were found differently influenced by sex and menopausal status (60, 140-144). Recently, Wust *et al.* found that smoking compromises bone strength by diaphyseal marrow cavity expansion and epiphyseal trabecular bone content reduction, and that these effect seem to wane with age (145). Smoking-induced changes in gonadal hormones appears to be an unlikely explanation for the gender differences found in many studies. (116-117, 146-147). Nevertheless, the antiestrogenic effects in women are responsible for an increased incidence of early menopause and osteoporosis (113, 135, 146, 148-156).

In recent years there has been increasing interest in the possible adverse effects of cigarette smoke on soft tissue and bone healing following injury (41, 91, 157-163). It has been found that smoking can influence the fracture healing process by reducing blood supply to the injury site, increasing reactive oxygen species in the circulation, and reducing endothelial nitric oxide synthase (94,157, 164-165). A higher incidence of delayed union in fracture healing (43, 60, 91-92, 94, 166-173) and nonunion following spinal fusion (94) have been highlighted. Conversely, a prospective case-control series reported that smoking appears not to affect the preoperative or postoperative course of hip fracture patients(174). Moghaddam *et al.* showed that tobacco smoking reduces the osteoblast release of TGF-beta1 during fracture healing, and advocated the use of this growth factor as a marker of fracture healing in smokers soon after injury (93). (Table 3).

6.2. Experimental studies

Many experimental studies were performed on the effect of smoking on bone status but most of them were focused mainly on the nicotine effect. Recent studies analyzing the effect of global tobacco smoke suggest that smoking significantly decreases bone mineral density and alters bone structure (175-176). A recent study examined how the duration and cessation of cigarette smoke exposure affects lymphocyte distribution and function in normal mice and in mice predisposed to low or high bone mass due to disruption or mutation of gene Lrp5, a gene involved in

Table 3. Main clinical studies on cigarette smoking effects on bone

Study	Design	Analysis	Sample Characteristics	Magnitude of Association
116	meta-analysis	current, previous and never smokers	40753 men and women	
104	meta-analysis	current, previous and never smokers	512399 men and women	RR (current smokers)=1.26 all fracture types
41	meta-analysis	current or past smoking	59232 men and women	RR (smoker vs. non smokers)=1.25 any fracture
149	systematic review	factors associated with osteoporosis	Women>65y	
148	systematic review	effects of smoking on bone health	Pre and postmenopausal women and man	
102	systematic review	Risk factors for low BMD	Men age≥50 y	
129	cohort study	risk of low bone status at the lumbar spine of ever-smokers	789 premenopausal women aged 20-40 years	OR=2.03; OR=1.55 in those with < 3 pack-years of tobacco use; OR= 2.55 in those with ≥ 3 pack-years of tobacco use
13	prospective cohort study	predictors of bone loss	507 ambulatory community-dwelling men aged 45 to 92 years	Total hip: OR Never smokers=1.0 OR Former smokers=0.93 OR Current smokers=1.42 Femoral Neck: OR Never smokers=1.0 OR Former smokers=1.02 OR Current smokers=2.09 Lumbar spine: OR Never smokers=1.0 OR Former smokers=1.11 OR Current smokers=1.39
105	case-control study	risk factors for hip fracture in men	7495 men aged 46-56 years	HR in never smokers=1.00 HR in ex-smokers=1.06 HR in smokers=1.58
98	case-control study	risk factors for hip fracture	40,279 long-stay (>60 days) home care clients aged 65	RR in smokers=1.41 RR in smokers with osteoporosis=1.59

canonical Wnt signaling that regulates bone metabolism.(177) This study provided the first evidence that smoke exposure reduces bone marrow B cells, providing a plausible mechanism for how smoking contributes to osteoporosis.(177) Regarding cigarette smoking effects on fracture healing, it has been found that acute cigarette smoke inhalation delays but does not prevent spinal fusion, (178-180) and delays chondrogenesis, and thus, fracture healing.(181) Akhter *et al* showed the negative effects of high nicotine dose exposure on bone biomechanical properties (decrease in most of the structural strength and apparent material properties of the femoral mid-shaft), bone mass and structure in growing female mice (182-184). Conversely, lower nicotine doses in growing adult female rats, producing serum concentrations similar to those in smokers, had no effect on the biomechanical properties of bone (182).

A link between environmental toxicants found in the tar fraction of tobacco smoke and bone loss was reported by Lee *et al.* using an ovariectomized rat model (185). They focused on the polycyclic aromatic hydrocarbons interacting with the aryl hydrocarbon receptor as the potential mediators of the toxic effects of tobacco smoking. Benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene are two polycyclic aromatic hydrocarbons found in high concentrations in the tar fraction of tobacco smoke (94). The study suggested that Benzo(a)pyrene / 7,12-dimethylbenz(a)anthracene reduced bone mechanical properties and lowered the BMD and bone connectivity, while stepping up bone formation; these changes are the result of an antiestrogenic effect, leading to an increase in bone turnover.

6.3.Pathophysiology and the role of cytokines

The mechanism of detrimental effects of smoking on bone is complex and probably includes a combination of factors. Nicotine is a potent vasoconstrictor that reduces blood flow and delivery of essential nutriments to injured tissue. It has been shown to increase platelet aggregation, decrease microvascular prostacyclin levels and inhibit the biological function of fibroblasts, red blood cells and macrophages (186). Nicotine inhibits the cell growth of various cell types, including skin fibroblasts, gingival fibroblasts, lung cancer cells and osteosarcoma cells (186). Nicotine modulates osteoblast cell proliferation and differentiation by specific receptors, and reduces collagen synthesis and ALP activity (90, 186-189). Conversely, a study by Gullihorn *et al.* suggests that nicotine acts as a direct stimulant of ALP activity, total protein and collagen syntheses (190). Recently, Rothem *et al.* demonstrated that the addiction of nicotine concentrations analogous to those acquired by a light-to-moderate smoker yields increased osteoblast proliferation and bone metabolism (191). Nicotine has a direct effect on human bone cells up-regulating c-fos oncoprotein and osteopontin synthesis, a bone matrix protein indicator of bone turnover and

degradation, IL-6 and TNF-alpha (90, 186-189). The high expression of osteopontin might be associated with nicotine effects on bone turnover and, after long-term administration, determining a decrease in BMD or reducing fracture healing rate (90). Recently, Katono *et al.* observed that nicotine stimulated bone matrix turnover thereby tipping the balance between bone matrix formation and resorption toward the latter process (193). Additionally, smoking was also associated with lower osteocalcin levels indicating a slow and reduced osteoblasts formation and regeneration leading to a decrease in BMD (194).

Furthermore, in *in vitro* models Nakayama *et al.* identified three different elements in the rat bone sialoprotein proximal gene promoter that mediate the negative effects of nicotine on bone sialoprotein transcription, a sulphated and phosphorylated glycoprotein involved in osteoblast differentiation and bone matrix mineralization, with a consequent decrease in bone turnover (186).

In relation to the effect of other toxic smoke components, moieties contained in tobacco smoke extract can influence bone cell recruitment and contraction of extracellular matrices through the inhibition of bone cell fibronectin production (188).

7. PERSPECTIVE

While for years we have been witnessing the progressive education on smoking and the risk of lung cancer and cardiovascular disease, obesity, diabetes and hypertension, and alcohol or severe liver disease, there is a lack of information about the serious side effects on bone metabolism such as osteoporosis and fractures. Numerous pre-clinical and clinical studies have begun to explore these issues, taking into account many factors, which may negatively influence the effect of these harmful lifestyles on bone (20-21, 32, 43, 60, 120-122,169-171). In this article the authors collected evidence that lifestyles that includes non-smoking, no alcohol abuse and avoidance of weight loss is advantageous for maintaining bone heath through life. Documentation of not only the association, but also the size of the effect between lifestyles and bone health can be helpful in promoting public health advice.

The risk factors analyzed by most of the clinical studies selected are related to hip fracture or less frequently to decrease in BMD. Concerning the risk factors associated with BMD variations, it has been reported to be OR=2.84 with a BMI=25-30 kg/m^2 and OR=5.94 with a BMI>30 kg/m^2 for postmenopausal women (195). Korpelainen *et al.* reported in postmenopausal women significant RR for low daily physical exertion and daily dietary calcium intake in relation to different ranges of BMI (Table 1) (19). It appears that daily physical activity and modification of dietary intake contribute positively to BMD results. Weight loss is important for the reduction of chronic conditions, such as heart diseases and osteoarthrosis (196), but weight loss or repeated cycles of weight loss and regaining weight increase fracture risk (197) and reduce BMD, mainly by malnutrition (198-201). Therefore, a balanced diet with correct intakes of protein and calcium is mandatory. In fact, inadequate or excessive protein intakes adversely affect BMD (202-203) through calcium mobilization from bone to balance endogenous acidity produced during protein intake, increasing urinary calcium excretion and stimulating PTH hormone circulation (203). Conversely, a high-protein diet may protect against bone loss during weight reduction (204), because overweight patients who consume energy-restricted diets with protein do not experience adverse changes in markers of bone turnover, preventing some of the inevitable loss of lean body mass (205). Calcium supplementation attenuates these changes in bone metabolism during energy restriction (198, 206) by the activation of calcium-parathyroid hormone axis (207) and it is recommended during weight loss (200). Furthermore, physical activity may help to prevent bone loss through both direct effects on skeleton and indirect effects on muscle mass (19, 32); however the literature shows discordant results (32, 208).

Lifestyle changes to address modifiable risk factors are beneficial and defining an intake limit of alcohol beverages is mandatory. The risk associated with alcohol consumption has been reported to be J-shaped or U-shaped for hip fracture (RR = 0.84 for ≤ 0.5 drinks/day, RR=0.80 for 0.5 – 1.0 drinks/day, and RR=1.39 for > 2 drinks/day) in that abstainers from alcohol have a higher risk that that of individuals consuming 1 or 2 units daily (40,41). Even though the beneficial association between moderate drinking of alcohol and bone health is consistent across many studies, this association deserves further exploration. Evidence is insufficient to determine relative associations between alcohol consumption and bone density in moderate compared with heavy drinkers. In addition, clinical human studies that are aimed at delineating more fully the etiology and mechanisms of the effects of alcohol are often complicated by difficulties in carefully controlling for lifestyle factors such as nutrition and activity level (209).

Finally, as far as the risk associated with smoking is concerned, it has been reported to be linear for osteoporosis and fracture. In particular, the hip fracture risk was RR = 1.41 in smokers, RR = 1.59 in smokers with osteoporosis (98) and up to RR = 2.20 in ever-smokers (163). Trimpou *et al.* reported a linear association for HR in hip fracture: HR = 1.00 in never smokers, HR = 1.06 in ex-smokers e di HR = 1.58 in smokers (105). Osteoporosis also is associated with higher odds of femoral neck bone loss: OR=1.0 in never smokers; OR = 1.02 ex smokers; and OR=2.09 in smokers. However, many of these surveys suffer from an incorrect, usually underestimated number of cigarettes smoked. Furthermore, the number of cigarettes considered as a risk varies among studies.

Smoking is again generating controversy in the medical press even though there is now adequate evidence from this and other studies to advise patients to stop smoking to minimize these complications. There are many factors that contribute to the smoking habit, unrealistic perception of personal susceptibility to the health hazards of smoking may be an important determinant of cigarette smoking and a barrier to smoking cessation. Perception of

the risk is important in several well-established models of preventive behavior (1-2, 12). The available evidence largely shows that smokers in general acknowledge the health risk associated with smoking but underestimate the magnitude and personal relevance. Despite large-scale public health campaigns to inform the public of the heath risk of smoking, the majority of smokers in the population did not perceive that they were at increased risk produced by the effects of cigarette smoking. Although communication of the risk is one aspect of the campaign to decrease smoking, the extent of the role of communicating the risk to stop smoking cannot be known until researchers are able to show an accurate perception of the risk of smoking (1-2, 12).

As for future preclinical studies, the pathophysiological mechanisms of harmful lifestyles underlying the metabolic alterations of bone tissue occurring in some osteoporotic patients should be clarified. In particular, preclinical models resolving some problems such as experimental models and setup, routes of administration, dosages and methods of assessment should be further developed. However, health information campaigns on these harmful lifestyles should be strengthened by using available scientific information to increase the awareness of their consequences on the musculoskeletal system.

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