Aging and bone loss: new insights for the clinician

Ther Adv Musculoskel Dis
(2012) 4(2) 61–76
DOI: 10.1177/1759720X11430858

Oddom Demontiero, Christopher Vidal and Gustavo Duque

Abstract: It is well known that the underlying mechanisms of osteoporosis in older adults are different than those associated with estrogen deprivation. Age-related bone loss involves a gradual and progressive decline, which is also seen in men. Markedly increased bone resorption leads to the initial fall in bone mineral density. With increasing age, there is also a significant reduction in bone formation. This is mostly due to a shift from osteoblastogenesis to predominant adipogenesis in the bone marrow, which also has a lipotoxic effect that affects matrix formation and mineralization. We review new evidence on the pathophysiology of age-related bone loss with emphasis upon the mechanism of action of current osteoporosis treatments. New potential treatments are also considered, including therapeutic approaches to osteoporosis in the elderly that focus on the pathophysiology and potential reversal of adipogenic shift in bone.

Keywords: osteoporosis, aging, elderly, osteoblast, osteoclast, treatment

Introduction

As a result of the aging process, the bone deteriorates in composition, structure and function, which predisposes to osteoporosis. Osteoporosis is defined as deterioration in bone mass and micro-architecture, with increasing risk to fragility fractures [Raisz and Rodan, 2003]. Owing to the close relationship between the aging process of bone and the pathogenesis of osteoporosis, research on the mechanisms of age-related bone loss has increased significantly in recent years involving a combination of basic, clinical, observational and translational studies.

Bone is a dynamic organ that serves mechanical and homeostatic functions. It undergoes a continual self-regeneration process called remodeling. Remodeling removes old bone and replaces it with new bone. This regenerative process occurs in distinct areas of bone known as bone metabolic units (BMUs) [Riggs *et al.* 2002]. Within each BMU bone formation by osteoblasts and bone resorption by osteoclasts is coupled tightly in a delicate balance to maintain bone mass and strength to resist deformity. With aging this balance shifts in a negative direction, favoring greater bone resorption and less bone formation. This combination of bone mass deficiency and reduction in strength ultimately results in osteoporosis and fractures.

Aging in combination with intrinsic and extrinsic factors accelerates the decline in bone mass that predisposes to fractures. Intrinsic factors include genetics, peak bone mass accrual in youth, alterations in cellular components, hormonal, biochemical and vasculature status. Extrinsic factors include nutrition, physical activity, comorbid medical conditions and drugs. In this article we review the mechanisms of age-related bone deterioration and their impact on the pathogenesis of osteoporosis. In addition, current and future therapeutic approaches focused on the correction of mechanisms associated with aging bone will also be outlined.

Bone remodeling in aging bone

Remodeling is continuous and coordinated cycle of removal of old bone by osteoclasts followed by the deposition of new bone by osteoblasts in response to micro damage and variable mechanical loadings. Bone remodeling is a continuous process throughout life. In the first three decades of life, bone turnover is coupled tightly to maintain a steady state between bone resorption and bone formation. Although there are variances in turnover rates, peak bone mass and size is achieved around the age of 15–20 years in women and later in men [Raisz and Seeman, 2001]. After this, long before sex steroids deficiency occurs, bone loss

Correspondence to:
Gustavo Duque, MD, PhD, FRACP
Nepean Clinical School, Level 5, South Block, Nepean Hospital, Penrith, NSW 2750, Australia
gustavo.duque@sydney.edu.au

Oddom Demontiero, MBBS, FRACP and Christopher Vidal, PhD, BSc (Hons)
Ageing Bone Research Program, Sydney Medical School Nepean, The University of Sydney, Penrith, NSW, Australia

becomes evident [Slemenda *et al*. 1996]. After reaching the peak of bone mass, bone turnover continues at a slower rate as suggested by a rapid decline in biochemical measures of bone remodeling with the predominance of bone resorption over bone formation [Raisz and Seeman, 2001]. Later in life, menopause in women significantly increases bone resorption over formation due to low levels of estrogens thus inducing accelerated bone loss. In contrast to the mechanisms of bone loss during menopause, which have been studied extensively, the triggers of an age-related transition from a steady state to one of negative net bone loss (both in women and men) remain poorly understood.

At the bone surface level, age-related bone loss is the consequence of two simultaneous but opposing processes: subperiosteal apposition, which takes place on the outside of the bone, and endosteal bone resorption, which takes place on the inside of the bone. With increasing age, bone remodeling is reduced leading to a negative bone balance at individual BMU sites. After the fourth decade of life, there is a reduction in the formation of periosteal bone and at the same time there is increasing number of remodeling units within endosteal bone resulting in a linear increase in endosteal bone resorption in both sexes. The overall consequences of these age-related changes are cortical thinning, increased cortical porosity, thinning of the trabeculae and loss of trabecular connectivity, all of which reduce bone quality and consequently bone strength [Rosen *et al*. 1994].

The above opposing processes are consistent with longitudinal and cross-sectional studies which showed a relatively slow rate of decline in areal bone mineral density (aBMD) in both sexes beginning at age 40 and continuing throughout the adult life [Khosla and Riggs, 2005]. Large decreases in lumbar spine volumetric BMD (vBMD) secondary to predominant vertebral trabecular bone loss beginning in the third decade and linear decrease in cortical vBMD in the wrist were also demonstrated in both sexes with advancing age [Riggs *et al*. 2004]. The changes were greater in women than men, owing to accelerated bone loss in the menopausal stage.

In terms of the effect of aging on periosteal bone formation, the increasing levels of endosteal bone loss are concomitant with steady levels of periosteal apposition somewhat compensating for the loss of bone mass. Therefore, cortical bone loss is less in men than in women because periosteal bone formation is greater and is independent of endosteal bone resorption. Bone loss reflects the net result of all of the periosteal bone formed during aging minus all of the bone irreversibly removed from the endosteal surface [Seeman, 2002], a process that seems to be independent of hormones and closely related to potential age-related mechanisms.

In terms of vBMD in the hip, a study by Center and colleagues in 852 women and 635 men (60 years and older) without fractures reported an age-related decline in vBMD in the hip [Center *et al*. 2004]. In addition, vBMD was more sensitive than areal BMD in older men and similar to that in women, in whom sensitivity was similar for both areal (73%) and estimated volumetric (78%) BMD cutoffs. The authors conclude that men and women have hip fractures at the same estimated femoral neck vBMD suggesting that vBMD can provide a useful single measure that could be used in both men and women.

Mechanisms of age-related bone loss

Secondary hyperparathyroidism

Both calcium and vitamin D deficiency can contribute to secondary hyperparathyroidism [Lips, 2001]. Vitamin D deficiency is prevalent in the older population irrespective of latitude [Lips, 2007]. A low serum 25(OH)D concentration leads to a small decrease in serum 1,25-$(OH)_2$D and calcium absorption which then stimulates an increase in parathyroid hormone (PTH) secretion. In addition vitamin D is required for osteoblastogenesis and bone formation [Duque and Troen, 2008]. The increased serum PTH subsequently increase osteoclastic activity and bone resorption, resulting in primarily cortical bone loss [Lips, 2001]. A chronic negative calcium balance state can also occur independently of vitamin D as a result of age-related reduced intestinal calcium absorption [Eastell *et al*. 1991] associated with reduced dietary intake. This deficiency, when not adequately compensated through dietary means or calcium supplements, contributes to physiological secondary hyperparathyroidism.

With age, a number of other factors can also cause an increasing PTH levels. Common factors include impaired renal function, the use of loop diuretics such as furosemide and estrogen deficiency. In women, there is some suppression of PTH secretion during the rapid phase of bone loss in early

postmenopausal period. In the later stage however there is gradually increasing PTH secretion which increases bone turnover [Ledger *et al.* 1995].

PTH secretion also increases in aging men, similar to what is seen in aging women [Khosla *et al.* 2008a; Khosla, 2010]. Normal circulating gonadal sex steroid levels in aging men may help to protect against bone resorption promoted by increased PTH levels. Thus it has been more difficult to demonstrate a direct role for PTH in causation of age-related bone loss in men [Kennel *et al.* 2003].

Gonadal sex steroid deficiency

It is well known that sex steroids have significant effects on skeletal health. The cessation of ovarian function associated with reduced estrogen levels at menopause is the start of rapid bone loss in women. During the menopause transition, serum 17β-estradiol levels decrease by 85–90% and serum estrone levels decrease by 65–75% from mean premenopausal levels [Khosla *et al.* 1997]. In fact, there may be a threshold level of serum bioavailable (non-sex hormone binding globulin [non-SHBG]-bound) estradiol below 11 pg/ml and below 11 pg/ml at which trabecular and cortical bone loss occurs, respectively [Khosla *et al.* 2005]. This phase of accelerated bone loss may persist for up to 10 years after menopause in most women.

The mechanisms of estrogen deficiency related bone loss are multiple and their relative importance in the pathogenesis of this process remains poorly understood [McCauley *et al.* 2003]. In general, effect of estrogen deficiency on bone is the result of loss of restraint and control estrogen has over mediators of bone resorption. Usually, estrogen may inhibit osteoclast formation and activity by increasing the production of osteoprotegerin (OPG), or transforming growth factor β (TGF-β) [Hofbauer *et al.* 1999; Hughes *et al.* 1996]. OPG is a soluble decoy receptor for receptor activator of nuclear factor kappa-B ligand (RANKL) and TGF-β induces osteoclast apoptosis [Lundberg *et al.* 2001]. *In vitro* and *in vivo* studies have also shown that estrogen suppresses RANKL production by osteoblastic cells and T and B lymphocytes [Eghbali-Fatourechi *et al.* 2003; Clowes *et al.* 2005]. Estrogen also directly stimulates apoptosis of osteoclast precursor cells, and decreases osteoclast precursor differentiation by blocking RANKL/macrophage colony-stimulating factor (M-CSF)-induced activator protein-1-dependent transcription by reducing c-jun activity [Lundberg *et al.* 1999; Mitnick *et al.* 2001]. Indirectly, estrogen may suppress the production of bone-resorbing cytokines such as interleukin (IL)-1, IL-6, TNF-α, M-CSF and prostaglandins [Charatcharoenwitthaya *et al.* 2007]. Finally, estrogen is also capable of inhibiting the activity of mature osteoclasts by direct, receptor-mediated mechanisms [Oursler *et al.* 1994]. In addition to changes to estrogen levels, a reduction in ovarian inhibin B across the menopause transition and perimenopausal elevated follicle-stimulating hormone (FSH) also increase bone turnover [Perrien *et al.* 2006].

In men, traditionally it was assumed that decreased serum testosterone was responsible for age-related bone loss. However estrogen has also been found to play a dominant role in age-related bone loss in men similar to women. A combination of cross-sectional and observational studies of aging men showed better correlations between serum estradiol and BMD than testosterone and BMD at various skeletal sites [Slemenda *et al.* 1997; Khosla *et al.* 2001, 2008b; Mellstrom *et al.* 2008; Szulc *et al.* 2001]. Further studies looking at differential effects between estrogen and testosterone confirmed that estrogen deficiency was more important than testosterone deficiency in causation of bone loss in aging men [Falahati-Nini *et al.* 2000; Leder *et al.* 2003] and that the effects of estrogen on bone were independent of FSH [Sanyal *et al.* 2008]. More recently, a large prospective study of older men again showed a low bio-available estradiol level to be associated with significant increased fracture risk and that testosterone in the presence of high SHBG is associated with significant increased fracture risk when adjusted for estradiol levels [LeBlanc *et al.* 2009]. Nevertheless, testosterone contributes to reduced fracture risk in men because of its influence on increasing bone size in men during growth and development [Clarke and Khosla, 2010].

Bone marrow fat

The predominant feature of age-related bone loss is the accumulation of bone marrow fat at the expense of osteoblastogenesis [Rosen and Bouxsein, 2006]. This accumulation of marrow fat appears to be an active process independent of estrogen since it is evident during the third and fourth decade of life [Perrien *et al.* 2007]. Biopsy studies with animal models [Duque *et al.* 2009] and humans [Verma *et al.* 2002; Meunier *et al.* 1971] have consistently demonstrated a

significant increase in marrow fat in aging bone. More recently MRI studies have also demonstrated an age-related increase in marrow fat [Griffith *et al*. 2005; Shen *et al*. 2007]. In addition, there is an inverse relationship between marrow fat volume and bone volume that was independent of sex and correlated with the changes seen in people with osteoporosis [Justesen *et al*. 2001].

Mechanistically, there appears to be a predominant differentiation of mesenchymal stem cells (MSCs) into adipocytes at the expense of osteoblasts [Rosen *et al*. 2009]. The differentiation of MSC into osteoblasts involves the recruitment of MSCs, release of appropriate amount of growth factors and activation of lineage-specific transcription factors [Duque, 2007; Chamberlain *et al*. 2007], recruitment of appropriate numbers of MSCs to achieve proper density and confluence [Zhou *et al*. 2008] and adequate oxygen tension and blood supply within the bone marrow [Wang *et al*. 2007]. Changes induced by aging can alter these conditions thus facilitating MSCs differentiation into adipocytes [Zhou *et al*. 2008]. Furthermore, the main lineage-specific transcription factors that direct the differentiation of MSCs are the runt-related transcription factor 2 (Runx2) for osteoblastogenesis and peroxisome proliferator-activator gamma 2 (PPARγ2) for adipogenesis [Rosen and Bouxsein, 2006; Gimble *et al*. 2006]. With aging, there is a predominant expression of PPARγ2 by MSCs with a concurrent decrease in Runx2 expression and therefore lower levels of osteoblast differentiation [Lecka-Czernik, 2006].

Recently, a protein of the nuclear envelope known as lamin A/C has been reported as an essential factor in the osteogenic differentiation of MSCs. Lamins are intermediate filament proteins present in the nuclear lamina and matrix and are important regulators of stem cells differentiation [Hutchinson and Worman, 2004; Pajerowski *et al*. 2007]. Most adult mammalian somatic cells contain three major lamins grouped into two classes: A type (A, AΔ10 and C) and B type (B1 and B2) [Li *et al*. 2011]. With aging there is a decrease in lamin A expression in normal osteoblasts [Duque and Rivas, 2006]. The importance of lamins in bone biology is evident from changes in bone mass seen in patients suffering from Hutchinson Gilford Progeria Syndrome (HGPS). Patients suffering from HGPS have mutations in the lamin A/C gene [Sandre-Giovannoli *et al*. 2003] and show major bone changes including severe osteoporosis, osteolysis, bone deformities and spontaneous fracture [Rodrigues *et al*. 2002]. A similar syndrome in mice caused by defects in type A lamins resulting in low levels of lamin A/C was associated with low BMD [Mounkes *et al*. 2003]. Mice lacking the enzyme responsible for lamin A/C processing ($Zmpste24^{-/-}$) also show accelerated bone loss and typical features of senile osteoporosis [Rivas *et al*. 2009].

Recently, an *in vivo* study of knockout lamin A/C mice demonstrated that the absence of lamin A/C increased the expression of MAN-1 protein which co localizes with Runx2 thus affecting its ability as an osteogenic transcription factor [Li *et al*. 2011]. This demonstrates that lamin A/C is required in osteoblastogenesis and bone formation *in vivo*.

In fact, aging *per se*, independently of hormonal changes, appears to contribute significantly to bone marrow adipogenesis raising the possibility that senile osteoporosis is a type of lipotoxic disease [Duque and Troen, 2008]. Indeed bone marrow adipocytes appear to exert a toxic effect on osteoblasts [Maurin *et al*. 2000]. Cocultures of adipocytes and osteoblasts reveal that adipocytes inhibit osteoblast activity and survival, possibly secondary to the release of adipokines and fatty acids by the increased number of adipocytes within the bone marrow [Musacchio *et al*. 2007]. Further evidence of the lipotoxicity of marrow adipocytes on bone comes from the observation of PPARγ induction by thiazolidenediones. The use of thiazolidenediones in diabetic patients was associated with bone loss and higher incidence of fractures [Grey, 2008]. The increasing levels of PPARγ induced by thiazolidenediones within the bone marrow not only affect bone formation, but also induce bone resorption [Lazarenko *et al*. 2007]. Furthermore, in a mice model, PPARγ was proven to functions as a direct regulator of osteoclastogenesis [Wan *et al*. 2007]. Given this observation, it was considered that blocking PPARγ could provide a new therapeutic approach for osteoporosis. Although PPARγ knockout mice showed high levels of bone formation [Akune *et al*. 2002], pharmacological attempt to increase bone mass by blocking PPARγ in diabetic mice was unsuccessful despite decreased marrow fat [Botolin and McCabe, 2006].

Other factors

A number of clinical studies demonstrated that body fat and bone mass were directly related [Felson *et al*. 1993; Lindsay *et al*. 1992; Glauber

et al. 1995; Khosla *et al.* 1996]. It was further observed that serum leptin levels were increased in obesity and correlated positively with fat mass [Considine *et al.* 1996]. Subsequently the hormone mediating the relationship between fat mass and bone mass was demonstrated to be leptin. An *in vitro* study showed that leptin acted on human marrow stromal cells to enhance osteoblast differentiation and inhibited adipocyte differentiation [Thomas *et al.* 1999]. Further animal studies also reported a central regulatory role of leptin [Ducy *et al.* 2000; Takeda *et al.* 2002]. More recently, in a loss of function of its receptor mice study, leptin was shown to regulate bone mass accrual *in vivo* by acting through neuronal means [Shi *et al.* 2008].

Serotonin was also shown to regulate bone mass in rodents [Yadav *et al.* 2009]. In humans the role for circulating serotonin in regulating bone mass was recently suggested by the findings from a study of premenopausal and postmenopausal women [Mödder *et al.* 2010]. Serotonin levels were inversely associated with body and spine aBMD, and with femoral neck total and trabecular vBMD. Serotonin levels remained significant negative predictors of femur neck total and trabecular vBMD, as well as trabecular thickness at the radius, after adjusting for age and BMI.

Attainment of peak bone mass is another factor contributing to later age-related bone loss. Those persons who achieve a higher peak bone mass are less likely to develop osteoporosis later in life as age-related bone loss ensues, whereas those with low levels are at greater risk [Seeman, 1997]. Numerous other factors such as corticosteroids usage, diseases such as malabsorption, anorexia nervosa and idiopathic hypercalciuria, and behavioral factors such as smoking, alcohol abuse and inactivity can also contribute to fracture risk in 40% of men and 20% of women in the older population [Riggs and Melton, 1986]. Finally, although controversial still, sarcopenia, probably through reduced muscle loading on bone, may also contribute to age-related bone loss [Mödder *et al.* 2010; Seeman, 1997].

The role of exercise

Aging is associated with a decline in physical activity and mechanical loading. Reduced mechanical loading exert diminished effects upon osteoblasts resulting in decreased osteoblast secretion of OPG and increased expression and secretion of RANKL, IL-1, IL-6, IL-11, and TNF-α. In turn, these compounds directly stimulate greater osteoclast formation and activity. The reduced OPG also permits greater binding of RANKL to RANK, which further facilitates increased osteoclastogenesis and resorption [Duque and Troen, 2008]. Animal studies of complete immobilization showed a striking remodeling imbalance with a rapid, although transient, increase in bone resorption followed by a sustained decrease in bone formation [Weinreb *et al.* 1989]. Recently, an anabolic response to exercise was shown to be related to the presence of lamin A/C [Duque *et al.* 2011b]. Lamin A/C haploinsufficient mice exposed to strenuous exercise demonstrated significant trabecular and cortical thinning and a reduction in osteoblasts and osteocyte numbers compared with their sedentary counterparts whilst the wild type exposed to exercise showed a significant increase in bone volume and number of bone cells. In humans, functional loading has been shown to improve bone mass [Leichter *et al.* 1989] and exercise training programs can prevent or reverse almost 1% of bone loss per year in both lumbar spine (LS) and femoral neck (FN) for both premenopausal and postmenopausal women [Wolff *et al.* 1999].

Bone loss due to decreased bone formation

Although sex steroids deficiency may contribute to age-related impairment of bone formation, reductions in key growth factors important for osteoblast differentiation/function may also contribute. Aging is associated with decreases in the amplitude and frequency of growth hormone secretion from the anterior pituitary [Marie *et al.* 1993] with growth hormone levels declining by up to 14% per decade in both elderly men and women [Rosen *et al.* 1994] leading to a decrease in hepatic production of insulin-like growth factor (IGF)-1 [Boonen *et al.* 1999; Pfeilschifter *et al.* 2000] and smaller decreases in IGF-2 [Boonen *et al.* 1999]. In addition to decreased systemic and local skeletal production of IGF-1 and IGF-2, growth factor binding proteins may also contribute to age-related bone loss. Higher serum IGF binding protein (IGFBP)-2 predicted lower BMD, and was associated with increased markers of bone resorption independent of age, body mass, and sex hormones [Amin *et al.* 2007].

Changes in key proteins such as sclerostin have also been implicated in bone formation reduction. Sclerostin (SOST), a glycoprotein primarily secreted by osteocytes is a potent inhibitor of

osteoblastogenesis. SOST binds to coreceptors LRP5 and LRP6 and prevents colocalization with frizzled protein and Wnt signaling, thereby reducing osteoblastogenesis and bone formation [Kneissel, 2009]. Loss-of-function mutations of the SOST gene are associated with an autosomal-recessive disorder, sclerosteosis, which causes progressive bone overgrowth [Balemans *et al*. 2001], a deletion downstream of this gene, which causes reduced SOST expression, is associated with a milder form of the disorder called van Buchem disease [Balemans *et al*. 2002] and finally SOST-null mice have a high bone mass phenotype [Li *et al*. 2008]. Consistent with these observations, pharmacologic inhibition of SOST has shown significant anabolic effects. In aged ovariectomized rat model of postmenopausal osteoporosis, treatment with SOST neutralizing monoclonal antibody resulted in marked increases in bone formation on trabecular, periosteal, endocortical, and intracortical surfaces [Li *et al*. 2009]. In addition, the increases in bone formation induced by antisclerostin antibody are not associated with increases in bone resorption [Lane and Silverman, 2010]. SOST also appears to have a role in mediating bone responses to mechanical unloading. SOST knockout mice were resistant to bone loss induced by mechanical unloading [Lin *et al*. 2009]. In humans, a recent phase I study of a single dose of a SOST monoclonal antibody (AMG 785) administered to healthy men and postmenopausal women was associated with dose-related increases in the bone-formation markers procollagen type 1 N-propeptide (P1NP), bone-specific alkaline phosphatase (BAP) and osteocalcin, and with a dose-related decrease in the bone-resorption marker serum C-telopeptide (sCTx) [Padhi *et al*. 2011].

Cathepsin K

Normal bone resorption and remodeling critically depend upon the synthesis and secretion of cathepsin K (CTSK) by osteoclasts [Troen, 2004; Yasuda *et al*. 2005; Motycykova and Fisher, 2002]. Bone resorption begins when osteoclasts bind firmly to bone surfaces forming resorption pits. An acid medium is produced within these pits resulting in the dissolution of the osseous mineral component exposing the organic matrix. The matrix is then degraded by the enzymes metalloproteinases and CTSK. In fact, RANKL, which plays a critical role in osteoclast differentiation and activation, has been shown to stimulate CTSK mRNA and protein expression in human osteoclasts [Shalhoub *et al*. 1999]. Indeed many of the agents that have been shown to induce osteoclast formation and activation or to inhibit osteoclast activity enhance and suppress, respectively, CTSK gene expression [Troen, 2006].

Agents that stimulate the osteoclast to produce increased amounts of CTSK include NFAT, TNF, IL-1, PPARΔ/β, stretching, and extracellular matrix proteins (ECM). Inhibitors of CTSK expression include estrogen, interferon-γ (IFN-γ), and OPG [Troen, 2004]. A phase I study of a CTSK inhibitor, odanacatib (ODN), showed that it was well tolerated, had a long half life, and exhibited significant and sustained suppression of bone resorption markers with weekly and daily regimen with no effects on markers of bone formation [Stoch *et al*. 2009]. A subsequent phase II study of postmenopausal women [Bone *et al*. 2010] demonstrated dose-dependent increases in BMD in all sites. The greatest increase was seen with the highest dose. Resorption markers fell in a dose-dependent manner for the first 6 months after which they increased and the difference with placebo disappeared. Bone formation markers increased with significant differences compared with placebo observed at 12 and 24 months. Recently, results of an extension of the phase II study for another year was reported [Eisman *et al*. 2011]. Continued treatment with 50 mg of ODN for 3 years produced significant increases from baseline and from year 2 in BMD at the spine (7.9% and 2.3%) and total hip (5.8% and 2.4%). Urine cross-linked *N*-telopeptide of type I collagen (NTx) remained suppressed at year 3 (−50.5%), but bone-specific alkaline phosphatase (BSAP) was relatively unchanged from baseline. Treatment discontinuation resulted in bone loss at all sites, but BMD remained at or above baseline. After ODN discontinuation at month 24, bone turnover markers increased transiently above baseline and resolved by month 36. There were similar overall adverse event rates in both treatment groups.

Finally, an important extrinsic factor causing reduced bone formation is glucocorticoids.

Glucocorticoids suppress bone formation by inhibiting Wnt/β-catenin signaling thereby impairing osteoblastogenesis, inhibit osteoblastic function directly and by inhibiting IGF-I synthesis [Canalis *et al*. 2007].

Table 1. Pharmacological effect of osteoporosis treatments on the typical features of age-related bone loss (adapted from Duque and Troen, 2008).

Compound	Osteoblast	Adipocyte	Osteoclast
Bisphosphonates	↑ differentiation ↑ activity ↓ apoptosis	↓ differentiation	↓ differentiation ↓ activity ↑ apoptosis
Calcitonin			↓ activity ↑ apoptosis
PTH	↑ activity ↑ survival ↑ differentiation	↓ differentiation	↑ activity
SERMs			↓ differentiation ↓ activity
Strontium ranelate	↑ activity ↑ differentiation		↓ activity ↓ survival
Vitamin D	↑ activity ↑ differentiation ↓ apoptosis	↓ differentiation ↑ trans-differentiation to osteoblasts	↑ activity
Denosumab			↓ differentiation ↓ activity ↑ apoptosis

PTH, parathyroid hormone; SERM, selective estrogen-receptor modulator

Osteoporosis therapy: present and future

Based on the mechanisms underlying age-related bone loss, the main goals of therapy should include the inhibition/restriction of osteoclastic activity, the enhancement of osteoblastic activity, and the regulation of bone marrow adipogenesis. In addition, contributing factors should be corrected or minimized. Currently the main classes of agents are antiresorptives, which suppress osteoclastic activity, and anabolic agents, which target osteoblasts (Table 1).

Antiresorptives

This class comprises bisphosphonates, hormone replacement therapy (HRT), selective estrogen-receptor modulators (SERMs), strontium ranelate, and RANKL antibody.

The nitrogen-containing bisphosphonates, such as alendronate, risedronate, ibadronate, and more recently zoledronate are first-line agents for the treatment and prevention of osteoporosis. These agents inhibit bone resorption by inducing osteoclasts apoptosis, thus reducing the number of osteoclasts in the BMU [Riggs and Melton, 1986], suppressing the capacity of osteoclasts to resorb bone by modifying their shape and nullifying their enzymatic capacity and also enhances secondary mineralization of preformed osteons [Russell, 2006]. There is also evidence suggesting that bisphosphonates (specifically alendronate) may promote osteoblast proliferation and maturation [Boonen *et al.* 1999], while inhibiting bone marrow adipogenesis [Duque and Rivas, 2007; Duque *et al.* 2009].

Most of the bisphosphonates have well-established antifracture efficacy. Alendronate has vertebral fracture reduction efficacy in postmenopausal women [Black *et al.* 1996], in men [Orwoll *et al.* 2000], in glucocorticoid-induced osteoporosis [Adachi *et al.* 2001] and nonvertebral fracture reduction including hip fractures [Wells *et al.* 2008a]. Similarly risedronate reduce vertebral fractures [Harris *et al.* 1999; Reginster *et al.* 2000], nonvertebral fractures [Wells *et al.* 2008b], hip fractures in osteoporotic older women [McClung *et al.* 2001], and glucocorticoid-induced osteoporosis [Reid *et al.* 2000].

Ibandronate has demonstrated efficacy against vertebral fractures in postmenopausal women and non vertebral fractures in higher-risk subgroup (femoral neck BMD T score < -3.0) [Chesnut *et al.* 2004]. The intravenous bisphosphonate zoledronate also has fracture reduction efficacy for vertebral, hip, and nonvertebral fractures, in both men and women [Black *et al.* 2007;

Table 2. Novel approaches to osteoporosis treatment.

Compound	Osteoblast	Adipocyte	Osteoclast
Cathepsin K inhibitor			↓ differentiation ↓ activity ↑ apoptosis
SOST antibody	↑ activity		
Interferon γ	↑ activity ↑ differentiation	↓ differentiation	↑ activity (uncoupling favoring formation)
BMP agonists	↑ activity ↑ differentiation		

BMP, bone morphogenetic protein; SOST, sclerostin.

Lyles *et al*. 2007] and glucocorticoid-induced osteoporosis [Reid *et al*. 2009].

Among these bisphosphonates, there are differences in side-effect profile, tolerability, and compliance rate. A review of these clinical considerations including treatment duration is discussed in an earlier issue [Langdahl and Harsløf, 2011] in this journal.

Denosumab

Since identified as a key molecule in mediating osteoclast development, activity, and survival [Lacey *et al*. 1998], the inhibition of RANKL activity has been tested as a therapeutic target for osteoporosis. Recently, a fully human monoclonal antibody to RANKL called denosumab was developed and tested. Denosumab blocks RANKL binding to RANK thus inhibiting the development and activity of osteoclasts. Denosumab administered every 3 or 6 months to postmenopausal women with low BMD over 12 months resulted in an increase in bone mineral density at the lumbar spine of 3.0–6.7%, at the total hip of 1.9–3.6%, and at the distal third of the radius of 0.4–1.3% [McClung *et al*. 2006]. Near-maximal reductions in mean levels of serum C-telopeptide from baseline were evident 3 days after the administration of denosumab.

Six-monthly subcutaneous injections of denosumab for 36 months was shown to reduce the risk of new radiographic vertebral fractures by 68%, reduce hip fractures by 40% and reduced the risk of nonvertebral fractures by 20% [Cummings *et al*. 2009]. There was no increase in the risk of cancer and infection compared with placebo; however, the major concern about the long-term use of denosumab relates to its possible effects on the immune system, since RANKL is expressed not just on bone cells but also on immune cells. Although not statistically significant there was a significant increase in rates of eczema and hospitalizations for cellulitis [Cummings *et al*. 2009] and more neoplasms and serious infections in the denosumab group compared with placebo [McClung *et al*. 2006]. These results suggest ongoing surveillance of patients receiving denosumab is prudent, particularly when the drug is used in the wider community in patients with comorbidities that might not have been included in clinical trials. Nevertheless it has several advantages over the bisphosphonates: (1) convenient biannual subcutaneous administration that could improve adherence; (2) lack of gastrointestinal side effects; (3) reversibility, because it targets RANKL and is not incorporated into the bone mineral; and (4) useful for impaired renal function because of nonelimination by the kidneys.

Other antiresorptives

The other antiresorptives such as HRT and SERMs have largely fallen out of recommendation in recent years. Although hormone therapy reduces vertebral, nonvertebral, and hip fractures, this is offset by increased risk of breast cancer and cardiovascular diseases [Mödder *et al*. 2010; Seeman, 1997]. Whilst raloxifene, the only SERM approved for the prevention and treatment of postmenopausal osteoporosis, only has vertebral fracture efficacy [Riggs and Melton, 1986] and is associated with increased risks of venous thromboembolic events and hot flushes. A newer SERM, lasofoxifene, showed a small advantage over raloxifene with a 24% reduction in nonvertebral fracture at 5 years (primarily peripheral fractures, however) [Becker, 2010].

Anabolics

PTH increases bone formation through several actions, including increasing commitment of MSCs to the osteoblast lineage, increasing osteoblast maturation and possibly life span, and reducing the osteocyte production of sclerostin to further stimulate bone formation. PTH stimulation of osteoblastogenesis also increases RANKL production, which then stimulates osteoclast maturation and activity, increasing bone remodeling overall; however, the overall effect is a positive formation balance [Lane and Silverman, 2010].

The anabolic effect of exogenous PTH was first reported in humans 20 years ago. Paired bone biopsies from a small group of patients receiving teriparatide by daily sc injections for 6–24 months demonstrated substantial increases in iliac trabecular bone volume, with evidence of new bone formation [Reeve *et al.* 1980]. Since then two anabolic agents have been approved for the treatment of osteoporosis, teriparatide, a 1–34 amino acid fragment of human recombinant PTH [PTH (1–34)] and in Europe, the full-length PTH (1–84) molecule.

Teriparatide has shown vertebral and nonvertebral fracture reduction in postmenopausal women with osteoporosis [Neer *et al.* 2001]. In men with osteoporosis, those who received teriparatide and who may have received follow-up antiresorptive therapy had a decreased risk of moderate and severe vertebral fractures [Kaufman *et al.* 2005]. Teriparatide also has efficacy in glucocorticoid-induced osteoporosis. Compared with alendronate, teriparatide induced earlier and greater gains in BMD at the lumbar spine and total hip and was more effective in preventing new vertebral fractures [Saag *et al.* 2007]. As for PTH(1–84) efficacy against vertebral fractures in postmenopausal women has been demonstrated [Greenspan *et al.* 2007].

Dual mode of action

Strontium ranelate appears to have a mixed mode of action by increasing bone formation and reducing bone resorption leading to rebalancing of bone remodeling in favor of bone formation. Mechanisms by which strontium acts include: increases osteoblast replication, differentiation, and activity [Canalis *et al.* 1996; Caverzasio, 2008], downregulates osteoclast differentiation and activity [Baron and Tsouderos, 2002; Takahashi *et al.* 2003], increases the OPG/RANKL ratio directly [Atkins *et al.* 2009] or via a calcium-sensing receptor [Brennan *et al.* 2009] and increases apoptosis of osteoclasts [Mentaverri *et al.* 2003].

Strontium ranelate reduced vertebral fractures [Meunier *et al.* 2004] and nonvertebral fractures including hip [Reginster *et al.* 2005] in postmenopausal osteoporosis. Trial evidence suggests that it is effective in a wide range of patient profiles, from early postmenopausal women with osteopenia to elderly women over the age of 80 years, and its antifracture efficacy is independent of baseline severity of osteoporosis, bone turnover level or the presence of clinical risk factors [Reginster *et al.* 2010]. Furthermore, the antifracture efficacy of strontium ranelate is sustained over 8 years [Reginster *et al.* 2009].

Novel approaches to osteoporosis treatment

Newer agents with novel modes of actions are under investigation in clinical trials or have shown promissory results in animal studies (Table 2).

SOST antibody

Positive bone formation results from a recent phase I study [Padhi *et al.* 2011] make the SOST antibody a promising therapeutic drug. At this time, however, the monoclonal antibody to SOST is in early phase II clinical trials in men and postmenopausal women with osteoporosis [ClinicalTrials.gov: NCT01101048]. The long-term safety of SOST is yet to be addressed.

Cathepsin K inhibitor (odanacatib)

At 36 months, ODN achieves increases in BMD similar to zoledronate and denosumab. However, compared with these agents, the reduction in resorption markers is less but there is also a smaller reduction in bone formation markers. What is not available yet is fracture data. The results of an ongoing randomized double-blind placebo-controlled clinical trial of 16,000 treatment-naïve postmenopausal women age 65 and above [ClinicalTrials.gov: NCT00529373] is eagerly anticipated.

New anabolic targets

Three regulatory proteins, which have also been intensely investigated as potential therapeutic targets, are bone morphogenetic proteins (BMPs), elements of the Wnt signaling pathways and IFNγ. BMPs [Canalis *et al.* 2003] and Wnt [Krishnan *et al.* 2006] induce the differentiation of mesenchymal cells toward mature osteoblasts. BMPs

may also induce osteoclastogenesis by enhancing the expression of RANKL [Kaneko *et al*. 2000]. Despite the use of locally administered BMPs for the treatment of nonunion fractures and to enhance the formation of spinal fusions, the systemic administration of BMPs would be limited by their nonskeletal effects, mitogenicity, and short half life. In terms of neutralizing Wnt antagonist such as with Dkk-1 antibodies, preclinical models have shown an increase in BMD, trabecular bone volume, osteoblast surface, and bone formation in rodents [Grisanti *et al*. 2006]. Currently, there is no information on their value for the treatment of osteoporosis.

Finally, recent evidence [Duque *et al*. 2011a] suggests that IFNγ, a protein that is produced by MSCs in the bone microenvironment, could be used as an anabolic treatment for osteoporosis at low doses. Oophorectomized and aged mice treated with IFNγ showed a significant gain in bone mass, which was mostly dependent on bone formation. Considering that IFNγ is currently used as a treatment for other diseases such as hepatitis C and osteopetrosis, this compound could become a promissory alternative as a bone anabolic in the near future.

Conclusion

Age-related bone loss is a complex and heterogeneous disease. A combination of genetic, hormonal, biochemical, and environmental factors underlie its pathophysiology. The result is a decline in bone quantity and quality that increases fracture risk in a progressive manner. Despite greater understanding of the mechanisms of these contributing factors through clinical and animal studies, more research is needed to determine the relative contributions of each of these factors in order to improve preventative and therapeutic options. In addition, despite the availability of an armamentarium of agents, the optimal agent remains a challenge.

Acknowledgements

Dr Demontiero has a postdoctoral scholarship in aging research from the Rebecca L. Cooper Medical Research Foundation.

Funding

This work was supported by project grants from the National Health and Medical Research Council (NHMRC) of Australia (grant numbers 632766 and 632767) and the Nepean Medical Research Foundation.

Conflict of interest statement

Dr Duque has served as a consultant for Sanofi-Aventis, Novartis, and Servier pharmaceuticals. He is regular speaker for Sanofi-Aventis, Amgen, and Servier Pharmaceuticals and has received research grants from Merck, Novartis, Sanofi-Aventis, and Key Pharmaceuticals.

References

Adachi, J., Saag, K., Delmas, P., Liberman, U., Emkey, R., Seeman, E. *et al.* (2001) Two-year effects of alendronate on bone mineral density and vertebral fracture in patients receiving glucocorticoids: a randomized, double-blind, placebo-controlled extension trial. *Arthritis Rheum* 44: 202–211.

Akune, T., Ogata, N., Hoshi, K., Kubota, N., Terauchi, Y., Tobe, K. *et al.* (2002) Insulin receptor substrate-2 maintains predominance of anabolic function over catabolic function of osteoblasts. *J Cell Biol* 159: 147–156.

Amin, S., Riggs, B., Melton III, L.J., Achenbach, S., Atkinson, E., Khosla, S. (2007) High serum IGFBP-2 is predictive of increased bone turnover in aging men and women. *J Bone Miner Res* 22: 799–807.

Atkins, G., Welldon, K., Halbout, P. and Findlay, D. (2009) Strontium ranelate treatment of human primary osteoblasts promotes an osteocyte-like phenotype while eliciting an osteoprotegerin response. *Osteoporos Int* 20: 653–664.

Balemans, W., Ebeling, M., Patel, N., Hul, E. V., Olson, P., Dioszegi, M. *et al.* (2001) Increased bone density in sclerostin is due to the deficiency of a novel secreted protein (SOST). *Hum Mol Genet* 10: 537–543.

Balemans, W., Patel, N., Ebeling, M., Hul, E. V., Wuyts, W. and Lacza, C. (2002) Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. *J Med Genet* 39: 91–97.

Baron, R. and Tsouderos, Y. (2002) In vitro effects of S12911-2 on osteoclast function and bone marrow macrophage differentiation. *Eur J Pharmacol* 450: 11–17.

Becker, C. (2010) Another selective estrogen-receptor modulator for osteoporosis (editorial). *N Engl J Med* 362: 752–754.

Black, D., Cummings, S., Karpf, D., Cauley, J., Thompson, D. and Nevitt, M. (1996) Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. *Lancet* 348: 1535–1541.

Black, D., Delmas, P., Eastell, R., Reid, I., Boonen, S. and Cauley, J. (2007) Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *N Engl J Med* 356: 1809–1822.

Bone, H., McClung, M., Roux, C., Recker, R., Eisman, J. and Verbruggen, N. (2010) Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density. *J Bone Miner Res* 25: 937–947.

Boonen, S., Mohan, S., Dequeker, J., Aerssens, J., Vanderschueren, D. and Verbeke, G. (1999) Down-regulation of the serum stimulatory components of the insulin-like growth factor (IGF) system (IGF-I, IGFII, IGF binding protein [BP]-3, and IGFBP-5) in age-related (type II) femoral neck osteoporosis. *J Bone Miner Res* 14: 2150–2158.

Botolin, S. and McCabe, L. (2006) Inhibition of PPAR-gamma prevents type I diabetic bone marrow adiposity but not bone loss. *J Cell Physiol* 209: 967–976.

Brennan, T., Rybchyn, M., Green, W., Atwa, S., Conigrave, A. and Mason, R. (2009) Osteoblasts play key roles in the mechanisms of action of strontium ranelate. *Br J Pharmacol* 157: 1291–1300.

Canalis, E., Economides, A. and Gazzerro, E. (2003) Bone morphogenetic proteins, their antagonists, and the skeleton. *Endocr Rev* 24: 218–235.

Canalis, E., Hott, M., Deloffre, P., Tsouderos, Y. and Marie, P. (1996) The divalent strontium salt S12911 enhances bone cell replication and bone formation in vitro. *Bone* 18: 517–523.

Canalis, E., Mazziotti, G., Giustina, A. and Bilezikian, J. (2007) Glucocorticoid induced osteoporosis: pathophysiology and therapy. *Osteoporos Int* 18: 1319–1328.

Caverzasio, J. (2008) Strontium ranelate promotes osteoblastic cell replication through at least two different mechanisms. *Bone* 42: 1131–1136.

Center, J.R., Nguyen, T.V., Pocock, N.A. and Eisman, J.A. (2004) Volumetric bone density at the femoral neck as a common measure of hip fracture risk for men and women. *J Clin Endocrinol Metab* 89: 2776-2782

Chamberlain, G., Fox, J., Ashton, B. and Middleton, J. (2007) Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells* 25: 2739–2749.

Charatcharoenwitthaya, N., Khosla, S., Atkinson, E., McCready, L. and Riggs, B. (2007) Effect of blockade of TNF-alpha and interleukin-1 action on bone resorption in early postmenopausal women. *J Bone Miner Res* 22: 724–729.

Chesnut, C., III, Skag, A., Christiansen, C., Recker, R., Stakkestad, J., Hoiseth, A. *et al.* (2004) Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. *J Bone Miner Res* 19: 1241–1249.

Clarke, B. and Khosla, S. (2010) Physiology of bone loss. *Radiol Clin North Am* 48: 483–495.

Clowes, J., Riggs, B. and Khosla, S. (2005) The role of the immune system in the pathophysiology of osteoporosis. *Immunol Rev* 208: 207–227.

Considine, R., Sinha, M., Heiman, M., Kriauciunas, A., Stephens, T. and Nyce, M. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292–295.

Cummings, S., Martin, J. S., McClung, M., Siris, E., Eastell, R., Reid, I. *et al.* (2009) Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 361: 756–765.

Ducy, P., Amling, M., Takeda, S., Priemel, M., Schilling, A., Beil, F. *et al.* (2000) Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* 100: 197–207.

Duque, G. (2007) As a matter of fat: new perspectives on the understanding of age related bone loss. *BoneKEy-Osteovision* 4: 129–140.

Duque, G., Huang, D.C., Dion, N., Macoritto, M., Rivas, D., Li, W., *et al.* (2011a) Interferon-γ plays a role in bone formation in vivo and rescues osteoporosis in ovariectomized mice. *J Bone Miner Res* 26: 1472–1483.

Duque, G., Li, W., Yeo, L., Vidal, C. and Fatkin, D. (2011b) Attenuated anabolic response to exercise in lamin A/C haploinsufficient mice. *Bone*, in press.

Duque, G. and Rivas, D. (2006) Age-related changes in lamin A/C expression in the osteoarticular system: laminopathies as a potential new aging mechanism. *Mech Ageing Dev* 127: 378–383.

Duque, G. and Rivas, D. (2007) Alendronate has an anabolic effect on bone through the differentiation of mesenchymal stem cells. *J Bone Miner Res* 22: 1603–1611.

Duque, G., Rivas, D., Li, W., Lic, A., Henderson, J., Ferlandd, G. *et al.* (2009) Age-related bone loss in the LOU/c rat model of healthy ageing. *Exp Gerontol* 44: 183–189.

Duque, G. and Troen, B. (2008) Understanding the mechanisms of senile osteoporosis: new facts for a major geriatric syndrome. *J Am Geriatr Soc* 56: 935–941.

Eastell, R., Yergey, A., Vieira, N., Cedel, S., Kumar, R. and Riggs, B. (1991) Interrelationship among vitamin D metabolism, true calcium

absorption, parathyroid function, and age in women: evidence of an age-related intestinal resistance to 1,25-dihydroxyvitamin D action. *J Bone Miner Res* 6: 125–132.

Eghbali-Fatourechi, G., Khosla, S., Sanyal, A., Boyle, W., Lacey, D. and Riggs, B.L. (2003) Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J Clin Invest* 111: 1221–1230.

Eisman, J., Bone, H., Hosking, D., McClung, M., Reid, I., Rizzoli, R. *et al.* (2011) Odanacatib in the treatment of postmenopausal women with low bone mineral density: Three-year continued therapy and resolution of effect. *J Bone Miner Res* 26: 239–241.

Falahati-Nini, A., Riggs, B., Atkinson, E., O'Fallon, W., Eastell, R. and Khosla, S. (2000) Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. *J Clin Invest* 106: 1553–1560.

Felson, D., Zhang, Y., Hannan, M. and Anderson, J. (1993) Effects of weight, and body mass index on bone mineral density in men and women. *J Bone Miner Res* 8: 567–573.

Gimble, J., Zvonic, S., Floyd, Z., Kassem, M. and Nuttall, M. (2006) Playing with bone and fat. *J Cell Biochem* 98: 251–266.

Glauber, H., Vollmer, W., Nevitt, M., Ensrud, K. and Orwoll, E. (1995) Body weight versus body fat distribution, adiposity, and frame size as predictors of bone density. *J Clin Endocrinol Metab* 80: 1118–1123.

Greenspan, S., Bone, H., Ettinger, M., Hanley, D., Lindsay, R., Zanchetta, J. *et al.* (2007) Effect of recombinant human parathyroid hormone (1–84) on vertebral fracture and bone mineral density in postmenopausal women with osteoporosis: a randomized trial. *Ann Intern Med* 146: 326–339.

Grey, A. (2008) Skeletal consequences of thiazolidinedione therapy. *Osteoporos Int* 19: 129–137.

Griffith, J., Yeung, D., Antonio, G., Lee, F., Hong, A., Wong, S. *et al.* (2005) Vertebral bone mineral density, marrow perfusion, and fat content in healthy men and men with osteoporosis: dynamic contrast-enhanced MR imaging and MR spectroscopy. *Radiol Clin North Am* 236: 945–951.

Grisanti, M., Niu, Q., Fan, W., Asuncion, F., Lee, J., Steavenson, S. *et al.* (2006) Dkk-1 inhibition increases bone mineral density in rodents. *J Bone Miner Res* 21(Suppl. 1): S25 (abstract).

Harris, S., Watts, N., Genant, H., McKeever, C., Hangartner, T., Keller, M. *et al.* (1999) Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. Vertebral Efficacy With Risedronate Therapy (VERT) Study Group. *JAMA* 282: 1344–1352.

Hofbauer, L., Khosla, S., Dunstan, C., Lacey, D., Spelsberg, T. and Riggs, B. (1999) Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic

cells. *Endocrinology* 140: 4367–4370.

Hughes, D., Dai, A., Tiffee, J., Li, H., Mundy, G. and Boyce, B. (1996) Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta. *Nat Med* 2: 1132–1136.

Hutchinson, C. and Worman, H. (2004) A-type lamins: guardians of the soma? *Nat Cell Biol* 6: 1062–1067.

Justesen, J., Stenderup, K., Ebbesen, E., Mosekilde, L., Steiniche, T. and Kassem, M. (2001) Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. *Biogerontology* 2: 165–171.

Kaneko, H., Arakawa, T., Mano, H., Kaneda, T., Ogasawara, A., Nakagawa, M. *et al.* (2000) Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. *Bone* 27: 479–486.

Kaufman, J., Orwoll, E., Goemaere, S., Martin, J. S., Hossain, A., Dalsky, G. *et al.* (2005) Teriparatide effects on vertebral fractures and bone mineral density in men with osteoporosis: treatment and discontinuation of therapy. *Osteoporos Int*, 16: 510–516.

Kennel, K., Riggs, B., Achenbach, S., Oberg, A. and Khosla, S. (2003) Role of parathyroid hormone in mediating age-related changes in bone resorption in men. *Osteoporos Int* 14: 631–636.

Khosla, S. (2010) Update in male osteoporosis. *J Clin Endocrinol Metab* 95: 3–10.

Khosla, S., Amin, S. and Orwoll, E. (2008a) Osteoporosis in men. *Endocr Rev* 29: 441–464.

Khosla, S., Amin, S., Singh, R., Atkinson, E., Melton L., III and Riggs, B. (2008b) Comparison of sex steroid measurements in men by immunoassay versus mass spectroscopy and relationships with cortical and trabecular volumetric bone mineral density. *Osteoporos Int* 19: 1465–1471.

Khosla, S., Atkinson, E., Melton L., III and Riggs, B. (1997) Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: a population-based study. *J Clin Endocrinol Metab* 82: 1522–1527.

Khosla, S., Atkinson, E., Riggs, B. and Melton, L., III (1996) Relationship between body composition and bone mass in women. *J Bone Miner Res* 11: 857–863.

Khosla, S., Melton, L., Atkinson, E. and O'Fallon, W. (2001) Relationship of serum sex steroid levels to

longitudinal changes in bone density in young versus elderly men. *J Clin Endocrinol Metab* 86: 3556–3561.

Khosla, S. and Riggs, B. (2005) Pathophysiology of age-related bone loss and osteoporosis. *Endocrinol Metab Clin N Am* 34: 1015–1030.

Khosla, S., Riggs, B., Robb, R., Camp, J., Achenbach, S., Oberg, A. *et al.* (2005) Relationship of volumetric bone density and structural parameters at different skeletal sites to sex steroid levels in women. *J Clin Endocrinol Metab* 90: 5096–5103.

Kneissel, M. (2009) The promise of sclerostin inhibition for the treatment of osteoporosis. *IBMS BoneKEy* 6: 259–264.

Krishnan, V., Bryant, H. and Macdougald, O. (2006) Regulation of bone mass by Wnt signaling. *J Clin Invest* 116: 1202–1209.

Lacey, D., Timms, E., Tan, H., Kelley, M., Dunstan, C., Burgess, T. *et al.* (1998) Osteoprotegerin 1. ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93: 165–1676.

Lane, N. and Silverman, S. (2010) Anabolic therapies. *Curr Osteoporos Rep* 8: 23–27.

Langdahl, B. L. and Harsløf, T. (2011) Medical treatment of osteoporotic vertebral fractures. *Ther Adv Musculoskel Dis* 3: 17–29.

Lazarenko, O., Rzonca, S., Hogue, W., Swain, F., Suva, L. and Lecka-Czernik, B. (2007) Rosiglitazone induces decreases in bone mass and strength that are reminiscent of aged bone. *Endocrinology* 148: 2669–1280.

LeBlanc, E., Nielson, C., Marshall, L., Lapidus, J., Barrett-Connor, E. and Ensrud, K. (2009) The effects of serum testosterone, estradiol, and sex hormone binding globulin levels on fracture risk in older men. *J Clin Endocrinol Metab* 94: 3337–3346.

Lecka-Czernik, B. (2006) PPARs and bone metabolism. *PPAR Res* 2006: 18089.

Leder, B., Leblanc, K., Schoenfeld, D., Eastell, R. and Finkelstein, J. (2003) Differential effects of androgens and estrogens on bone turnover in normal men. *J Clin Endocrinol Metab* 88: 204–210.

Ledger, G., Burritt, M., Kao, P., O'Fallon, W., Riggs, B. and Khosla, S. (1995) Role of parathyroid hormone in mediating nocturnal and age-related increases in bone resorption. *J Clin Endocrinol Metab* 80: 3304–3310.

Leichter, I., Simkin, A., Margulies, J., Bivas, A., Steinberg, R., Giladi, M. *et al.* (1989) Gain in mass density of bone following strenuous physical activity. *J Orthop Res* 7: 86–90.

Li, W., Yeo, L., Vidal, C., McCorquodale, T., Hermann, M., Fatkin, D. *et al.* (2011) Decreased bone formation and osteopenia in lamin a/c deficient mice. *PLoS One* 6: e19313.

Li, X., Ominsky, M., Niu, Q., Sun, N., Daugherty, B., D'Agostin, D. *et al.* (2008) Targeted deletion of sclerostin gene in mice results in increased bone formation and strength. *J Bone Miner Res* 23: 860–869.

Li, X., Ominsky, M., Warmingtom, K., Morony, S., Gong, J., Cao, J. *et al.* (2009) Sclerostin antibody treatment increases bone formation, bone mass and bone strength in a rat model of postmenopausal osteoporosis. *J Bone Miner Res* 24: 574–588.

Lin, C., Jiang, X., Dai, Z., Guo, X., Weng, T., Wang, J. *et al.* (2009) Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. *J Bone Miner Res* 24: 1651–1661.

Lindsay, R., Cosman, F., Herrington, B. and Himmelstein, S. (1992) Bone mass and body composition in normal women. *J Bone Miner Res* 7: 55–62.

Lips, P. (2001) Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* 22: 477–501.

Lips, P. (2007) Vitamin D status and nutrition in Europe and Asia. *J Steroid Biochem Mol Biol* 103: 620–625.

Lundberg, P., Bostrom, I., Mukohyama, H., Bjurholm, A., Smans, K. and Lerner, U. (1999) Neuro-hormonal control of bone metabolism: Vasoactive intestinal peptide stimulates alkaline phosphatase activity and mRNA expression in mouse calvarial osteoblasts as well as calcium accumulation mineralized bone nodules. *Regul Pept* 85: 47–58.

Lundberg, P., Lundgren, I., Mukohyama, H., Lehenkari, P., Horton, M. and Lerner, U. (2001) Vasoactive intestinal peptide (VIP) pituitary adenylate cyclase-activating peptide receptor subtypes in mouse calvarial osteoblasts: Presence of VIP-2 receptors and differentiation-induced expression of VIP-1 receptors. *Endocrinology* 142: 339–347.

Lyles, K., Colón-Emeric, C., Magaziner, J., Adachi, J., Pieper, C., Mautalen, C. *et al.* (2007) Zoledronic Acid in Reducing Clinical Fracture and Mortality after Hip Fracture. *N Engl J Med* 357: 1799–1809.

Marie, P., Hott, M., Launay, J., Graulet, A. and Gueris, J. (1993) In vitro production of cytokines by bone surface-derived osteoblastic cells in normal and osteoporotic postmenopausal women: relationship with cell proliferation. *J Clin Endocrinol Metab* 77: 824–830.

Maurin, A., Chavassieux, P., Frappart, L., Delmas, P., Serre, C. and Meunier, P. (2000) Influence of mature adipocytes on osteoblast proliferation in human primary cocultures. *Bone* 26: 485–489.

McCauley, L., Tozum, T., Kozloff, K., Koh-Paige, A., Chen, C., Demashkieh, M. *et al.* (2003) Transgenic models of metabolic bone disease: Impact of estrogen receptor deficiency on skeletal metabolism. *Connect Tissue Res* 44: S250–S263.

McClung, M., Geusens, P., Miller, P., Zippel, H., Bensen, W., Roux, C. *et al.* (2001) Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. *N Engl J Med* 344: 333–340.

McClung, M., Lewiecki, E., Cohen, S., Bolognese, M., Woodson, G., Moffett, A. *et al.* (2006) Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med* 354: 821–831.

Mellstrom, D., Vandenput, L., Mallmin, H., Holmberg, A., Lorentzon, M., Oden, A. *et al.* (2008) Older men with low serum estradiol and high serum SHBG have an increased risk of fractures. *J Bone Miner Res* 23: 1552–1560.

Mentaverri, R., Hurtel, A., Kamel, S., Robin, B. and Brazier, M. (2003) Extracellular concentrations of strontium directly stimulates osteoclast apoptosis. *J Bone Min Res* 18: M237.

Meunier, P., Aaron, J., Edouard, C. and Vignon, G. (1971) Osteoporosis and the replacement of cell populations in the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. *Clin Orthop Relat Res* 80: 147–154.

Meunier, P., Roux, C., Seeman, E., Ortolani, S., Badurski, J., Spector, T. *et al.* (2004) The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N Engl J Med* 350: 459–468.

Mitnick, M., Grey, A., Masiukiewsicz, U., Bartkiewicz, M., Rios-Velez, L., Friedman, S. *et al.* (2001) Parathyroid hormone induces hepatic production of bioactive interleukin-6 and its soluble receptor. *Am J Physiol Endocrinol Metab* 280: E405–E412.

Mödder, U., Achenbach, S., Amin, S., Riggs, B., Melton, L. and Khosla, S. (2010) Relation of serum serotonin levels to bone density and structural parameters in women. *J Bone Miner Res* 25: 415–422.

Motycykova, G. and Fisher, D. E. (2002) Pycnodysostosis: role and regulation of cathepsin K in osteoclast function and human disease. *Curr Mol Med* 2: 407–421.

Mounkes, L., Kozlov, S., Hernandez, L., Sullivan, T. and Stewart, C. (2003) A progeroid syndrome in mice is caused by defects in A-type lamins. *Nature* 423: 298–230.

Musacchio, E., Priante, G., Budakovic, A. and Baggio, B. (2007) Effects of unsaturated free fatty acids on adhesion and on gene expression of extracellular matrix macromolecules in human osteoblast-like cell cultures. *Connect Tissue Res* 48: 34–38.

Neer, R., Arnaud, C., Zanchetta, J., Prince, R., Gaich, G., Reginster, J. *et al.* (2001) Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* 344: 1434–1441.

Orwoll, E., Ettinger, M., Weiss, S., Miller, P., Kendler, D., Graham, J. *et al.* (2000) Alendronate for the treatment of osteoporosis in men. *N Engl J Med* 343: 604–610.

Oursler, M., Pederson, L., Fitzpatrick, L., Riggs, B.L. and Spelsberg, T. (1994) Human giant cell tumors of the bone (osteoclastomas) are estrogen target cells. *Proc Natl Acad Sci U S A* 91: 5227–5231.

Padhi, D., Jang, G., Stouch, B., Fang, L. and Posvar, E. (2011) Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *J Bone Miner Res* 26: 19–26.

Pajerowski, J., Dahl, K., Zhong, F., Sammak, P. and Discher, D. (2007) Physical plasticity of the nucleus in stem cell differentiation. *Proc Natl Acad Sci U S A* 104: 15619–15624.

Perrien, D., Achenbach, S., Bledsoe, S., Walser, B., Suva, L., Khosla, S. *et al.* (2006) Bone turnover across the menopause transition: correlations with inhibins and follicle-stimulating hormone. *J Clin Endocrinol Metab* 91: 1848–1854.

Perrien, D., Akel, N., Dupont-Versteegden, E., Skinner, R., Seigel, E., Suva, L. *et al.* (2007) Aging alters the skeletal response to disuse in the rat. *Am J Physiol Regul Integr Comp Physiol* 292: R988–R996.

Pfeilschifter, J., Diel, I., Kloppinger, T., Bismar, H., Schuster, E., Balbach, S. *et al.* (2000) Concentrations of insulin-like growth factor (IGF)-I, -II, and IGF binding protein-4 and -5 in human bone cell conditioned medium did not change with age. *Mech Ageing Dev* 117: 109–114.

Raisz, L. and Rodan, G. (2003) Pathogenesis of osteoporosis. *Endocrinol Metab Clin North Am* 32: 15–24.

Raisz, L. and Seeman, E. (2001) Causes of age related bone loss and bone fragility: An alternative view. *J Bone Miner Res* 16: 1948–1952.

Reeve, J., Meunier, P., Parsons, J., Bernat, M., Bijvoet, O., Courpron, P. *et al.* (1980) Anabolic effect of human parathyroid hormone fragment on trabecular bone in involutional osteoporosis: a multicentre trial. *Br Med J* 280: 1340–1344.

Reginster, J., Bruyère, O., Sawicki, A., Roces-Varela, A., Fardellone, P., Roberts, A. *et al.* (2009) Long-term treatment of postmenopausal osteoporosis

with strontium ranelate: results at 8 years. *Bone* 45: 1059–1064.

Reginster, J., Hiligsmann, M. and Bruyere, O. (2010) Strontium ranelate: long-term efficacy against vertebral, nonvertebral and hip fractures in patients with postmenopausal osteoporosis. *Ther Adv Musculoskel Dis* 2: 133–143.

Reginster, J., Minne, H., Sorensen, O., Hooper, M., Roux, C., Brandi, M. *et al.* (2000) Randomized trial of the effects of risedronate on vertebral fractures in women with established postmenopausal osteoporosis. Vertebral Efficacy with Risedronate Therapy (VERT) Study Group. *Osteoporos Int* 11: 83–91.

Reginster, J., Seeman, E., Vernejoul, M. D., Adami, S., Compston, J., Phenekos, C. *et al.* (2005) Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. *J Clin Endocrinol Metab* 90: 2816–2822.

Reid, D., Devogelaer, J., Saag, K., Roux, C., Lau, C., Reginster, J. *et al.* (2009) Zoledronic acid and risedronate in the prevention and treatment of glucocorticoid-induced osteoporosis (HORIZON): a multicentre, double-blind, double-dummy, randomised controlled trial. *Lancet* 373: 1253–1263.

Reid, D., Hughes, R., Laan, R., Sacco-Gibson, N., Wenderoth, D., Adami, S. *et al.* (2000) Efficacy and safety of daily risedronate in the treatment of corticosteroid-induced osteoporosis in men and women: a randomized trial. European Corticosteroid-Induced Osteoporosis Treatment Study. *J Bone Miner Res* 15: 1006–1013.

Riggs, B., Khosla, S., Melton, L.J. III (2002) Sex steroids and the construction and conservation of the adult skeleton. *Endocr Rev* 23: 279–302.

Riggs, B. and Melton, L. (1986) Medical progress series: Involutional osteoporosis. *N Engl J Med* 314: 1676–1686.

Riggs, B., Melton, L.J., III., Robb, R., Camp, J. J., Atkinson, E. J., Peterson, J. *et al.* (2004) A population-based study of age and sex differences in bone volumetric density, size, geometry and structure at different skeletal sites. *J Bone Miner Res* 19: 1945–1954.

Rivas, D., Li, W., Akter, R., Henderson, J. and Duque, G. (2009) Accelerated features of age-related bone loss in zmpste24 metalloproteinase-deficient mice. *J Gerontol A Biol Sci Med Sci* 64: 1015–1024.

Rodrigues, G.D.P., Tâmega, I.D.E., Duque, G. and Neto, V.S.D. (2002) Severe bone changes in a case of Hutchinson-Gilford syndrome. *Ann Genet* 45: 151–155.

Rosen, C., Ackert-Bicknell, C., Rodriguez, J. and Pino, A. (2009) Marrow fat and the bone microenvironment: developmental, functional, and pathological implications. *Crit Rev Eukaryot Gene Expr* 19: 109–124.

Rosen, C. and Bouxsein, M. (2006) Mechanisms of disease: Is osteoporosis the obesity of bone? *Nat Clin Pract Rheumatol* 2: 35–43.

Rosen, C., Donahue, L. and Hunter, S. (1994) Insulin-like growth factors and bone: the osteoporosis connection. *Proc Soc Exp Biol Med* 206: 83–102.

Russell, R. (2006) Bisphosphonates: From bench to bedside. *Ann N Y Acad Sci* 1068: 367–401.

Saag, K., Shane, E., Boonen, S., Marín, F., Donley, D., Taylor, K. *et al.* (2007) Teriparatide or alendronate in glucocorticoid-induced osteoporosis. *N Engl J Med* 357: 2028–2039.

Sandre-Giovannoli, A. D., Bernard, R., Cau, P., Navarro, C., Amiel, J., Boccaccio, I. *et al.* (2003) Lamin a truncation in Hutchinson–Gilford progeria. *Science* 300: 2055.

Sanyal, A., Hoey, K., Mödder, U., Lamsam, J., McCready, L., Peterson, J. *et al.* (2008) Regulation of bone turnover by sex steroids in men. *J Bone Miner Res* 23: 705–714.

Seeman, E. (1997) From density to structure: growing up and growing old on the surfaces of bone. *J Bone Miner Res* 12: 509–521.

Seeman, E. (2002) Pathogenesis of bone fragility in women and men. *Lancet* 359:1841–1850.

Shalhoub, V., Faust, J., Boyle, W. J., Dunstan, C. R., Kelley, M., Kauffman, S. *et al.* (1999) Osteoprotegerin and osteoprotegerin ligand effects on osteoclast formation from human peripheral blood mononuclear cell precursors. *J Cell Biochem* 72: 251–261.

Shen, W., Chen, J., Punyanitya, M., Shapses, S., Heshka, S. and Heymsfield, S. (2007) MRI measured bone marrow adipose tissue is inversely related to DXA-measured bone mineral in Caucasian women. *Osteoporos Int* 18: 641–647.

Shi, Y., Yadav, V., Suda, N., Lui, X., Guo, X., Myers, M. J. *et al.* (2008) Dissociation of the neuronal regulation of bone mass and energy metabolism by leptin in vivo. *Proc Natl Acad Sci U S A* 105: 20529–20533.

Slemenda, C., Longscope, C., Peacock, M., Hui, S. and Johnston, C. (1996) Sex steroids, bone mass and bone loss. A prospective study of pre-, peri-, and postmenopausal women. *J Clin Invest* 1: 14–21.

Slemenda, C., Longcope, C., Zhou, L., Hui, S., Peacock, M. and Johnston, C. (1997) Sex steroids and bone mass in older men: positive associations with serum estrogens and negative associations with androgens. *J Clin Invest* 100: 1755–1759.

Stoch, S. A., Zajic, S., Stone, J., Miller, D. L., Dyck, K. V., Gutierrez, M. J. *et al.* (2009) Effect of the cathepsin K inhibitor odanacatib on bone resorption biomarkers in healthy postmenopausal women: two double blind, randomised placebo controlled phase I studies. *Clin Pharmacol Ther* 86: 175–182.

Szulc, P., Munoz, F., Claustrat, B., Garnero, P., Marchand, F., Duboeuf, F. *et al.* (2001) Bioavailable estradiol may be an important determinant of osteoporosis in men: the MINOS study. *J Clin Endocrinol Metab* 86: 192–199.

Takahashi, N., Sasaki, T., Tsouderos, Y. and Suda, T. (2003) S 12911-2 inhibits osteoclastic bone resorption in vitro. *J Bone Miner Res* 18: 1082–1087.

Takeda, S., Elefteriou, F., Levasseur, R., Liu, X., Zhao, L., Parker, K. *et al.* (2002) Leptin regulates bone formation via the sympathetic nervous system. *Cell* 111: 305–317.

Thomas, T., Gori, F., Khosla, S., Jensen, M., Burguera, B. and Riggs, B. (1999) Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology* 140: 1630–1638.

Troen, B.R. (2004) The role of cathepsin K in normal bone resorption. *Drug News Perspect* 17: 19–28.

Troen, B.R. (2006) The regulation of cathepsin K gene expression. *Ann N Y Acad Sci* 1068: 165–172.

Verma, S., Rajaratnam, J., Denton, J., Hoyland, J. and Byers, R. (2002) Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis. *J Clin Pathol* 55: 693–698.

Wan, Y., Chong, L. and Evans, R. (2007) PPAR-gamma regulates osteoclastogenesis in mice. *Nat Med* 13: 1496–1503.

Wang, Y., Wan, C., Gilbert, S. and Clemens, T. (2007) Oxygen sensing and osteogenesis. *Ann N Y Acad Sci* 1117: 1–11.

Weinreb, M., Rodan, G. and Thompson, D. (1989) Osteopenia in the immobilized rat hind limb is associated with increased bone resorption and decreased bone formation. *Bone* 10: 187–194.

Wells, G., Cranney, A., Peterson, J., Boucher, M., Shea, B., Robinson, V. *et al.* (2008a) Alendronate for the primary and secondary prevention of osteoporotic fractures in postmenopausal women. *Cochrane Database Syst Rev*, 1.

Wells, G., Cranney, A., Peterson, J., Boucher, M., Shea, B., Robinson, V. *et al.* (2008b) Risedronate for the primary and secondary prevention of osteoporotic fractures in postmenopausal women. *Cochrane Database Syst Rev*, CD004523.

Wolff, I., Croonenborg, J. V., Kemper, H., Kostense, P. and Twisk, J. (1999) The effect of exercise training programs on bone mass: a meta-analysis of published controlled trials in pre and post-menopausal women. *Osteoporos Int* 9: 1–12.

Yadav, V., Oury, F., Suda, N., Liu, Z., Gao, X., Confavreux, C. *et al.* (2009) A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. *Cell* 138: 976–989.

Yasuda, Y., Kaleta, J. and Bromme, D. (2005) The role of cathepsins in osteoporosis and arthritis: rationale for the design of new therapeutics. *Adv Drug Deliv Rev* 57: 973–993.

Zhou, S., Greenberger, J., Epperly, M., Goff, J., Adler, C. and Leboff, M. (2008) Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. *Aging Cell* 7: 335–343.