

Sex steroids and bone: current perspectives

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Although the process of bone remodelling or its control has not yet been fully elucidated there is, at present, sufficient information available to conclude that ovarian steroids (estrogens, androgens, progesterone) play an essential role in skeletal homeostasis. The mechanism of action of sex steroids on the skeleton is still not entirely clear, but it has traditionally included indirect effects on systemic hormones that regulate calcium balance and a direct receptor-mediated action. More recently, changes in cytokine production within the bone marrow, as well as pro-apoptotic and anti-apoptotic effects in the osteoblastic cells, have been proposed as new perspectives on the cellular and molecular mechanisms by which sex steroids influence adult bone homeostasis. Mechanical loading, when combined with estrogens or androgens, results in a greater osteogenic response than either condition separately. Women are especially at risk for osteoporosis if they have had a premature or surgical menopause and have not received hormone replacement therapy (HRT). Other reproductive factors that can help to identify women with osteopenia and emphasize the role of sex steroids in preserving bone mass in premenopausal women include: age at menarche, menstrual history and irregularities (including those associated with excessive exercise), age at menopause, previous hysterectomy, hyperprolactinaemia, anorexia nervosa, scoliosis, ovarian dysgenesis, pregnancy and lactation, and pharmacological ovarian suppression. The prevention of osteoporosis starts with the onset of the menarche. A combination of exercise, appropriate nutrition and a healthy lifestyle all maximize bone mineral accrual and result in optimal peak bone mass; normal ovarian function is essential to this process. Unfortunately, many women actually become aware of the need for osteoporosis prevention much later in life, usually after they have already become menopausal. HRT, however, has important limitations for prevention of fractures in post-menopausal women. Future perspectives for treatment of osteoporosis include androgen therapy and anabolic agents. Specifically, synthetic ligands of the estrogen receptor that can evoke the non-genotrophic but not the genotrophic signal of the receptor may be bone anabolic agents, as opposed to natural estrogens or selective estrogen receptor modulators that are anti-resorptive agents. The same ligands may circumvent the side effects associated with conventional HRT.

Key words: bone homeostasis/bone remodelling/cytokines/hormone replacement therapy/osteoporosis/ovarian steroids

Introduction

It was Fuller Albright who, in the 1940s in a series of elegant clinical descriptions, first highlighted the adverse effects of sex steroid deficiency on bone (Albright *et al.*, 1940; Albright and Reifenstein, 1947). In his original observations, Albright pointed out that the prevalence of ovariectomy among osteoporotic women was higher than expected, and almost invariably in these women the surgery had been performed at an age younger than the average age of the natural menopause. He also showed that the negative calcium balance characteristic of osteoporotic post-menopausal women was reversed by estrogen administration. Thus, Albright postulated that estrogen in some unknown fashion stimulated

osteoblast function, and this is still considered today to be a potential mechanism of estrogen action on bone (Lindsay, 1995). In addition, Albright reported that loss of androgens in males as a result of either chemical or surgical castration or an age-associated decline of androgen levels—albeit not as universal or abrupt as menopause—had the same adverse effect on the skeleton as estrogen (Albright, 1947).

Post-menopausal and ovariectomized women have lower circulatory levels of a variety of sex steroids, in addition to estrogens (Lindsay, 1995). Androgens are the most abundant circulating sex steroids in both men and women. In women, androgens circulate in the concentration range of nanomolar to micromolar; this contrasts with the estrogens, which have

circulating concentrations in the picomolar range. Androgens are obligatory precursors in the biosynthesis of estrogens, and in the female are secreted by the adrenal gland and the ovary. Androgens are formed peripherally, particularly from dehydroepiandrosterone sulphate (Burger, 2002a). The concept of an androgen deficiency syndrome in women is a relatively old one, although it has attracted a substantial increase in attention during recent years (Burger, 2002b).

The premenopausal ovary produces significant amounts of progesterone during the luteal phase of each cycle. In a review of the literature (Prior, 1990) it was indicated that progesterone appears to act directly on bone remodelling and may play a role in the coupling of bone resorption with bone formation. This observation, and the fact that the addition of a progestogen to estrogen therapy is essential to endometrial protection for post-menopausal women who have not had a hysterectomy, raises the question about the role of progestins on bone mass preservation.

Although the process of bone remodelling or its control are not yet completely understood, there is sufficient information available to conclude that sex steroids play an important role in skeletal homeostasis (Compston, 1990; Lindsay, 1995). The loss of sex steroids from the ovary, mainly before the age of menopause, causes a net loss of bone tissue. When provided to estrogen-deprived women, estrogen reverses many of the effects of lost ovarian function. Thus, it has been suggested that menopausal women should use hormone supplements (hormone replacement therapy; HRT) for the rest of their lives in order to prevent a variety of ills, including osteoporosis and fractures (Rekers, 1991; Bilezikian and Silverberg, 1992; Bush, 1992; Burkman *et al.*, 2001). However, the following facts should be considered in this respect:

Ovarian function is an important, but not the only, determinant of skeletal status among ageing women (Parfitt, 1987; Khosla *et al.*, 1995). In fact, early menopause is associated with a self-limiting loss of bone which does not progress further until ageing exerts its effect. Thus, a 70-year-old woman has lost 11% of her bone due to menopause and 18% as a function of age; thereafter, the age-related function is dominant. The main conclusion is that estrogen deficiency represents one of the multiple causes of involuntional osteoporosis, and its effect is largely limited to bone loss in women during the first 5 years after menopause. Other aspects of age-related bone loss (rather than sex steroid deficiency) dominate the older years and thus, the significance of early menopause as a risk factor for osteoporosis has been overstated (Nordin *et al.*, 1990; Orwoll and Nelson, 1999; Riggs *et al.*, 2002).

Although all post-menopausal women are estrogen-deficient, osteoporosis develops in only 10 to 20% of them (Khosla *et al.*, 1995).

Menopausal women are indeed deficient in endogenous estrogens, relative to premenopausal women, if the focus is solely on the role of endogenous estrogens as preventives of fractures. If the focus is shifted to the role of endogenous estrogens in the aetiology of breast cancer, ovarian cancer, endometrial cancer and uterine fibroids, the premenopausal women are 'hyperestrogenic' and post-menopausal women have a more desirable level (Rosenberg, 1993; Hulka and Stark, 1995; Rodriguez *et al.*, 2001; Noller, 2002; Wang and Arnold, 2002).

For protection against fractures, estrogen should be initiated soon after menopause and continued indefinitely, but this long-

term HRT is associated with increased risk of breast cancer and the risk increases with duration of treatment (Cauley *et al.*, 1995; Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Stampfer and Hankinson, 1997; Dixon, 2001; Marcus, 2002). Even a small increase in relative risk has a large impact on the number of women developing breast cancer because of the high baseline rates (Hulka, 1994).

There are other treatment options to prevent osteoporosis. These options extend far beyond HRT and include lifestyle and dietary changes such as increasing weight-bearing activity, enhancing calcium and vitamin D intake, as well as incorporating pharmacological agents such as the bisphosphonates and selective estrogen receptor modulators (SERMs) (Notelovitz, 1988; 1993; Bassey, 1995; Marcus, 2002; Pinkerton and Santen, 2002). Regrettably, the different available options are recommended by very few physicians, which suggests that many women are not aware of all treatment options (Randall, 1993).

Therefore, the aims of the present report were to: (i) analyse the effects of sex steroids on bone mass; (ii) illustrate those reproductive factors potentially associated with osteopenia; and (iii) analyse potential therapeutic implications of the relationship between sex steroids and bone mass, stressing limitations of universal HRT for prevention of post-menopausal osteoporosis and analysing future therapeutic perspectives. The MEDLINE database was used to identify relevant English-language articles on these topics published between 1980 and August 2002. The medical subject heading terms used for searching included those for the main topics in this review: sex steroids, estrogens, androgens, progestins, bone, bone mass; risk factors, reproductive factors, osteopenia; hormone replacement therapy, osteoporosis, fractures. Additional relevant citations were identified by reviewing references from retrieved articles and by consulting relevant books on osteoporosis published during the past 10 years.

Effects of sex steroids on bone mass

The capability of measuring bone mass, using non-invasive techniques, and the development of biochemical markers to assess bone turnover allowed a more detailed evaluation to be made of the relationship between sex steroids and the skeleton (Delmas, 1995; Johnston and Melton, 1995).

Estrogens and bone

A large number of studies have been conducted to evaluate the effect of estrogen administration on bone mass in post-menopausal women. In general, all studies have drawn similar conclusions. Estrogen intervention reduces the bone remodelling that is seen across menopause, and thus reduces the rate of loss of skeletal tissue (Lindsay, 1995).

All estrogens (both natural and synthetic, and whether administered by mouth, percutaneously, subcutaneously or transdermally as appropriate) appear capable of inhibiting bone loss, provided that adequate doses are administered and adequate serum levels are obtained (Christiansen, 1994; Lindsay, 1995; Riggs and Khosla, 1995). Effective doses of conjugated equine estrogens are about 0.625 mg/day (no additional effect is seen at double this daily dose; Lindsay, 1987; 1995), with comparable doses required for other

estrogens (1–2 mg/day for micronized estradiol). For oral synthetic estrogens the effective doses are as low as 0.02 mg/day (ethinyl estradiol). For transdermal estrogen, a 0.05 mg patch applied every 3.5 days appears to reduce bone loss.

The effects persist for as long as therapy is provided. Moreover, the earlier the treatment is begun, the more beneficial is the effect, as bone which has already been lost cannot be replaced to any significant extent (Lindsay, 1987; 1995). Therefore, estrogen—like other anti-resorptive agents (e.g. calcium and bisphosphonates)—primarily prevents further loss of bone mass and thus might be expected to be more effective when used for prevention (i.e. in early post-menopausal women). However, when estrogen therapy is stopped, bone loss recurs at a rate similar to that after oophorectomy (Notelovitz, 1993; Lindsay, 1995; Marcus, 2002). For the long-term preservation of bone mineral density, continuous estrogen therapy is needed; even 10 years of treatment after menopause will have little residual effect on bone density among women aged 75 years or more, who have the highest risk of fracture (Felson *et al.*, 1993; Cauley *et al.*, 1995; Marcus, 2002). This is of paramount importance considering that exogenous hormones are considered a risk factor for breast cancer (Hulka, 1994; Dixon, 2001). Of greater impression is the fact that older women (aged >70 years) continue to lose bone density in spite of estrogen therapy, which suggests that the causes of bone loss at these ages are more complex than during the early post-menopausal period, and that estrogen alone is incapable of ensuring skeletal integrity (Orwoll and Nelson, 1999).

Despite all the above evidence, the mechanism of action of estrogen on the skeleton is still not entirely clear. The consensus is that the principal action of estrogen is inhibition of bone resorption, although several studies have pointed to a positive effect of estrogen on bone formation. Specific receptors for estrogens (in low concentrations) have been identified in cells of osteoblast lineage (Eriksen *et al.*, 1988; Komm *et al.*, 1988); hence, the effects of estrogen might result from either direct, receptor-mediated actions or from indirect actions on systemic hormones (Compston, 1990; Schot and Schuurs, 1990).

Estrogen may affect the hormones that regulate calcium balance, such as calcitonin and parathyroid hormone (Lindsay, 1987; Schot and Schuurs, 1990; DeCherney, 1993). However, the identification of estrogen receptors in normal osteoblast-like cells invites speculation that estrogen may have a direct effect on bone rather than affect bone indirectly via secondary effects on other systemic hormones such as calcitonin and parathyroid hormone. Estrogen receptors, however, are present only in low concentrations (much lower than in reproductive tissues), and their significance has yet to be demonstrated (DeCherney, 1993; Riggs *et al.*, 2002).

An attractive hypothesis is that estrogen effects are cell-mediated, the release of cytokines and growth factors being altered in response to estrogenic stimulation (Compston, 1994; Lindsay, 1995; Riggs *et al.*, 2002). A number of cytokines (mainly interleukins 1 and 6, macrophage-colony-stimulating

factor, tumour necrosis factor and nitric oxide), prostaglandins (mainly prostaglandin E₂) and growth factors (mainly insulin-like growth factor 1) have been shown to affect bone cells *in vitro* (Compston, 1990; Lindsay, 1995; Riggs *et al.*, 2002). Many—though not all—of these cytokines and growth factors are produced by bone cells, and their secretion into the bone matrix provides a potential mechanism for the regulation of bone remodelling (resorption/formation). The results of different studies have raised the possibility that estrogen may act directly on cells in the bone microenvironment rather than, or in addition to, bone cells themselves (Schot and Schuurs, 1990; Compston, 1994; Lindsay, 1995; Riggs *et al.*, 2002). Alterations in cytokine production within the bone marrow would have significant paracrine or autocrine effects on the cells responsible for remodelling the skeleton. However, the precise role for any of the reported changes within bone is not clear; neither has any cause-and-effect relationship been demonstrated between the alterations observed in cell or tissue culture and estrogen deficiency bone loss (Lindsay, 1995). Despite that, the autocrine and paracrine basis for sex steroid action on bone cells has during the past few years come into sharper focus, and how to weigh the importance of the various cytokines and quantify their complex interactions in mediating the sex steroid effects are subjects for further research (Riggs *et al.*, 2002).

Recently, an interesting new perspective on the cellular and molecular mechanisms by which sex steroids (estrogens and androgens) influence adult bone homeostasis and, by extension, the pathogenetic mechanisms responsible for the development of osteoporosis following sex steroid deficiency, have been developed by Manolagas' group (Manolagas *et al.*, 2002). Until the 1990s, investigations into the pathophysiology of osteoporosis had focused on the functions of differentiated cells: the rates and/or total amounts of bone resorbed or formed by individual osteoclasts or osteoblasts. However, these cells have relatively short active lifespans that are within the range of lifespans of other cells originating in the bone marrow. Such cells must be continually replaced: the number present depends on the birth rate, which reflects the frequency of cell division of the appropriate precursor cell; and also on the lifespan, which most likely reflects the timing of death by apoptosis. The importance of the distinction between cell number and individual cell function has often been obscured by using the vague term 'activity' (Manolagas, 1999; Manolagas *et al.*, 2002). The essential hypothesis tested by Manolagas' group is that the balance between bone resorption and bone formation depends more on the number of cells carrying out these processes than on their individual capacities.

Results obtained from studies conducted by Manolagas' group during the past 10 years have confirmed that estrogens and androgens decrease the number of bone remodelling cycles by attenuating the birth rate of osteoclasts and osteoblasts from their respective progenitors (for a review, see Manolagas *et al.*, 2002). These effects result, in part, from the transcriptional regulation of genes responsible for osteoclastogenesis and mesenchymal cell replication and/or differentiation, and are

exerted through interactions of the ligand-activated receptors with other transcription factors. However, increased remodeling alone cannot explain why a loss of sex steroids tilts the balance of resorption and formation in favour of the former. Estrogens and androgens also exert effects on the lifespan of mature bone cells: pro-apoptotic effects on osteoclasts, but anti-apoptotic effects on osteoblasts and osteocytes. These latter effects stem from a heretofore unexpected function of the classical 'nuclear' sex steroid receptors outside the nucleus, and result from the activation of a Src/Shc/extracellular signal-regulated kinase signal transduction pathway, probably within pre-assembled scaffolds called caveolae (Kousteni *et al.*, 2001). Remarkably, either estrogen receptor alpha or beta or the androgen receptor can transmit anti-apoptotic signals with similar efficiency, irrespective of whether the ligand is an estrogen or an androgen. Even more importantly, these non-genotrophic, sex non-specific actions are mediated by the ligand-binding domain of the receptor and can be functionally dissociated from transcriptional activity with synthetic ligands (Manolagas *et al.*, 2002).

Overall, the above lines of evidence strongly suggest that, in sex steroid deficiency, loss of transcriptional effects may be responsible for the increased osteoclastogenesis and osteoblastogenesis, and thereby the increased rate of bone remodeling. Loss of non-genotrophic anti-apoptotic effects on mature osteoblasts and osteocytes, in combination with an opposite effect on the lifespan of mature osteoclasts, may be responsible for the imbalance between formation and resorption and the progressive loss of bone mass (Manolagas *et al.*, 2002).

Less well recognized in the bone-remodelling cycle is the role of mechanical strain and its effect on the modulating activity of osteocytes. However, the importance of this interaction of sex steroid action and biomechanical forces in determining the level of bone mass has recently been emphasized (Notelovitz, 2002a; Riggs *et al.*, 2002). There are clear associations among muscle mass, muscle strength and bone density. Mechanical loading—combined exercise and gravity—rather than weight-dependent gravity exerts a higher stimulus on bone formation. Biomechanical strain, and especially that induced by muscle contraction, encourages the activation of bone-forming sites on periosteal bone surfaces. These changes in bone remodelling will adjust bone mass and distribution to a level that is appropriate for the ambient biomechanical forces. This new bone formation is mediated through the synthesis and activity of both prostaglandin E₂ and nitric oxide produced by bone surface osteocytes. Prostaglandin E₂ and nitric oxide are responsible for osteogenesis by modifying the activity of osteoblasts (stimulated by prostaglandin E₂) and osteoclasts (inhibited by nitric oxide) (Notelovitz, 2002a; Riggs *et al.*, 2002).

Mechanical loading, when combined with estrogen, results in a greater osteogenic response than does either condition separately. It is the interaction between the two that requires understanding and which holds the key to success (Notelovitz, 2002a). This is probably the result of estrogen's anti-resorptive effect and of the stimulation of bone formation with exercise

(Notelovitz, 2002a). This hypothesis is supported by the results of a study conducted in young men which showed an increase in the biochemical markers of bone formation after resistance exercise training, with a transient suppression of bone resorption markers (Fujimura *et al.*, 1997).

Approximately 4% of muscle mass is lost during the first 3 years after menopause, and this is associated with a significant decline in muscle strength. In men, muscle strength is preserved until the age of 60 years, and reaches levels found in menopausal women at about 75 years of age—a factor that might explain the greater tendency for falls in older women (Notelovitz, 2002a).

Androgens and bone

Androgens have a profound effect on the physiology of bone and muscle in women, as androgens modulate the bone-remodelling cycle by direct androgenic activity and transformation into estrogens (estrone is formed by the aromatization of androstenedione and estradiol by the aromatization of testosterone).

Androgen deficiency in males—like estrogen deficiency in females—can cause osteoporosis, and androgen-related anabolic steroids are sometimes used in the treatment of this condition. However, their use is limited by adverse side effects related to blood lipids and virilization (Rodan and Rodan, 1995). Clinical evidence of androgen effects on bone mass comes from women with hirsutism, polycystic ovarian disease and secreting ovarian tumours who often have increased bone mineral density (Gregoriou *et al.*, 2000; Zborowski *et al.*, 2000; Castelo-Branco *et al.*, 2003). Also, the combination of androgens and estrogen replacement therapy in post-menopausal women increases bone mass to a greater extent than estrogen alone; its effects on lipids, however, are clearly adverse (Castelo-Branco *et al.*, 2000). In contrast, the use of a gonadotrophin-releasing hormone (GnRH) agonist associated with low-dose add-back HRT as a prototype contraceptive to prevent breast cancer was associated with osteopenia because of the inhibition of ovarian androgen production (Spicer *et al.*, 1993; Pike and Spicer, 2000).

Androgen receptors are found in all three bone cells: osteoblasts, osteoclasts and osteocytes, but are mainly expressed in osteoblasts. Specific binding receptors for the following androgens have been identified: testosterone, dihydrotestosterone and dehydroepiandrosterone. Androgens stimulate osteoblast proliferation, enhance osteoblast differentiation and the synthesis of extracellular matrix proteins, and stimulate mineralization (Notelovitz, 2002a;b). Likewise, androgens influence bone cell function through their effect on local factors that control the bone cells' microenvironment; moreover, androgens also have pro-apoptotic effects on osteoclasts, but anti-apoptotic effects on osteoblasts and osteocytes, and increase muscle mass and strength (Manolagas *et al.*, 2002; Notelovitz, 2002a; Riggs *et al.*, 2002). The latter results in an improvement in physical activity, and leads to the activation of bone-forming sites and the stimulation of bone formation-modulating cells, the osteocytes.

These effects of androgens contribute to their action on enhancing bone formation. However, androgen may also act at the tissue level by reducing bone resorption (Riggs *et al.*, 2002).

Progestins and bone

The role of progestins in preventing bone loss is less well understood than that of estrogen. There is general agreement, however, that the synthetic nortestosterone-derived C₁₉ progestins with androgenic properties (e.g. norethindrone and norethindrone acetate), at doses higher than those needed for HRT, do have beneficial effects on bone density. Thus, they can increase bone density in women with post-menopausal osteoporosis (Horowitz *et al.*, 1993) and ameliorate the effects of estrogen deficiency in younger women receiving GnRH agonists (Abdalla *et al.*, 1985; Riis *et al.*, 1990; Eldred *et al.*, 1992). However, data regarding the effects on bone of the progesterone-related C₂₁ progestins such as medroxyprogesterone acetate (MPA) are contradictory. Thus, it has been reported that in premenopausal women with luteal phase deficiency, MPA (10 mg/day; 10 days/cycle) could significantly improve spinal bone density (Prior *et al.*, 1994). In contrast, 20 mg/day of that progestin were unable to arrest spinal bone loss in post-menopausal women (Gallagher *et al.*, 1991). Also, premenopausal women using parenteral depot MPA (DMPA) for contraception or prescribed high-dose (50 mg/day) MPA for gynaecological disorders had significantly reduced bone density (Cundy *et al.*, 1991; 1996). The variable findings in these studies clearly suggest that the effects of MPA on bone density may vary according to the dose used and the estrogen status of the user. When given in doses sufficient to induce hypogonadism, MPA use is associated with a significant and rapid loss of bone density from the lumbar spine. Traditionally, it has been accepted that this bone loss is the consequence of estrogen deficiency and occurs despite a gain in body weight, although it appears to be at least partially reversible (Cundy *et al.*, 1996). Recently, however, it has been argued that bone loss associated with MPA administration is caused by decreased osteoblast differentiation as a result of medroxyprogesterone occupying the glucocorticoid receptor, since increasing glucocorticoid receptor occupancy beyond that reached at optimal glucocorticoid concentrations attenuates osteoblast differentiation (Ishida and Heersche, 2002). If this hypothesis is confirmed, then progestins with no glucocorticoid activity would be a better choice for progestagen therapy in order to achieve more beneficial effects on bone metabolism.

While the majority of the data accumulated on estrogens and skeletal status have dealt with estrogen alone, the requirement to consider combined HRT for all women with a uterus demands that the effects of progestins be examined in this context. In addition, because of their other metabolic effects there is agreement that progestins should not be used themselves for osteoporosis prevention or treatment (Lobo and Speroff, 1994). Clinical trials have shown that post-menopausal women receiving norethindrone acetate in association with estrogen displayed a significant increase in their bone

mineral density when compared with estrogen-only-treated patients (Speroff *et al.*, 1996). In contrast, micronized progesterone and the less androgenic MPA did not contribute significantly to the bone-protective effects of estrogen-only therapy (The Writing Group for the PEPI Trial, 1996).

In contrast to estrogens, progestins are thought mainly to influence bone formation in the remodelling process. Progesterone receptors have been identified in primary cultures of human osteoblasts and osteoclasts (Slootweg *et al.*, 1992; Wei *et al.*, 1993). However, it is not clear whether the activities of a progestogen on bone can be ascribed to its progestational or other intrinsic hormonal actions. Besides their progestational activities, progestogens exert androgenic, corticosteroidal and even estrogenic activities. Furthermore, from a practical point of view, of more interest and importance is the question of whether progestogens can interact with the estrogen effects on the skeleton. In this regard it is interesting to note the results of a study (Slootweg *et al.*, 1992) where the authors investigated the action of a 'pure progestogen' (i.e. binding only to the progesterone receptor), Org 2058, in SaOS-2 human osteogenic osteosarcoma cells. The pure progestogen had no effect on cell proliferation when added alone. However, in combination with 17- β -estradiol, it had a highly synergistic action on SaOS-2 cell proliferation. The same effect was observed in primary rat osteoblasts, showing that non-transformed cells react similarly and thus, that this synergism is a general phenomenon in osteoblastic cells. The effects of estrogen and progestogen on bone cells suggested the presence of receptors for these compounds in SaOS-2 cells. The authors therefore performed classical biochemical receptor studies to demonstrate the presence of sex steroid receptors in these cells. Scatchard analysis revealed a significant number of high-affinity estrogen and progestagen receptors in the SaOS-2 cells, thus indicating that the effects of these steroids are mediated via the normal route of steroid receptors (Slootweg *et al.*, 1992). In this regard, it has also been shown that some synthetic progestagens exert estrogenic effects through the activation of estrogen (but not progesterone) receptors, and so can have a dual effect on estrogen target tissues, either to stimulate or differentiate cells (Jeng *et al.*, 1992; Jordan *et al.*, 1993). These investigations lead to the conclusion that some estrogen-like effects could be produced by synthetic progestins. Interestingly, 19-nortestosterone derivatives—but not MPA—were found to have marked estrogenic properties. On the other hand, experimental studies in rats have shown that estrogens may play an important role in maintaining bone mass by up-regulating progesterone receptor levels in a class of osteoprogenitor cells responsive to progesterone (Ishida and Heersche, 1999). Overall, these data provide a cellular basis for the clinically recognized positive effect of estrogen and progestogen combinations on bone mass.

Risk factors of osteopenia related to sex steroids

The prevention of osteoporosis is of utmost importance in reducing the costs related to this disease. In women, bone loss prior to menopause may contribute to later risk of fracture due to

osteoporosis. Therefore, an important prevention strategy is to optimize and maintain premenopausal bone mineral status (Tudor-Locke and McColl, 2000).

During the early years of life, adolescence and early adulthood, bone mass increases until, at a point in time which remains to be clearly defined, the peak bone mass is attained. Most of the bone mass will be accumulated by late adolescence after cessation of longitudinal growth (Tudor-Locke and McColl, 2000), but peak bone mass attainment probably occurs during the third decade of life. This may be followed by a period of relative stability until the point at which age-related bone loss begins, prior to menopause, in the fourth decade or early in the fifth decade (Compston, 1990). Peak adult bone mass (the actual amount of bone in the skeleton) is a major factor determining the risk of fracture. Thus, if a woman with a good peak adult bone mass at menopause loses the average 20% of bone mass, the loss is not sufficient to increase the risk of fracture to dangerous levels. By contrast, if a woman with a low peak adult bone mass at menopause loses only half as much bone—about 10%—the risk of fracture increases rapidly (Stevenson, 1990). Therefore, there exists the potential to provide premenopausal women with the information necessary to enter menopause with an individually optimized bone mineral status.

In the past, health education strategies have focused on the needs of post-menopausal women, and the potential of prevention strategies targeted at premenopausal women has largely been ignored. However, sufficient evidence-based knowledge is now available regarding risk factors for reduced mineral status in this population, to provide a guide for behavioural changes that are conducive to good bone health (Tudor-Locke and McColl, 2000). The risk factors for osteopenia in relation to ovarian sex steroids are discussed in the following sections.

Age at menarche

Early and continued exposure to endogenous estrogen during a time of accelerated bone growth may potentiate peak bone mass development in premenopausal women. The onset of the menarche is thus considered as an important biological indicator of future bone mass. Several studies of age at menarche and premenopausal bone have suggested the presence of an inverse relationship: the earlier the onset of menstruation, the greater the individual's subsequent bone mass (Notelovitz, 1993; Tudor-Locke and McColl, 2000). A later age of menarche may indicate underlying hormonal irregularities, and could therefore be a proxy measure for low bone density in premenopausal women. Thus, it has been reported that a later age of menarche was no longer a significant factor for low bone mineral content when controlled for nulliparity—itsself a potential proxy indicator for underlying hormonal abnormalities (Sowers *et al.*, 1992).

Menstrual history and irregularities

Cyclic disturbance of the menstrual cycle is an important marker of potential osteopenia. However, asymptomatic menstrual disturbances and no associated amenorrhoea have also been reported as a risk factor for low bone density in premenopausal women (the so-called 'marginal hormonal status') (Sowers and Galuska, 1993). Thus, lower levels of estradiol in eumenorrhoeic premenopausal women have been

associated with lower bone mineral content (Sowers *et al.*, 1998). Defective luteal phases have been linked with spinal bone density losses in some (Prior, 1990), but not all (Waller *et al.*, 1996; DeSouza *et al.*, 1997), studies. Bone loss in women with anovulatory menses is estimated at 4.2% per year (Notelovitz, 1993). Bone loss has also been associated with low levels of androgens in premenopausal women (Slemenda *et al.*, 1996).

A history of menstrual irregularities has been consistently associated with lower bone mineral density in premenopausal women. A reduced bone mineral content has been shown to be related to duration of amenorrhoea and severity of estrogen deficiency, regardless of the underlying cause of the irregularity (Tudor-Locke and McColl, 2000). It has been estimated that women who missed <50% of their expected menses had a vertebral bone mass that was 88% of their eumenorrhoeic peers, and those who missed >50% of their menses had values that were 69% of those of normally menstruating women. Serum estradiol concentrations were lower and subnormal in both groups of oligomenorrhoeic women (Notelovitz, 1993). Women who experience periods of regularity, interspersed with times of oligomenorrhoea and/or amenorrhoea, are intermediate to these other two groups with respect to bone mineral density, suggesting some protection with infrequent regularity (Tudor-Locke and McColl, 2000).

The deleterious effects of amenorrhoea on bone are immediate, and therefore early recognition and intervention are necessary. Increases in bone mineral density are associated with resumption of menses, which is the treatment goal—preferably by treating the underlying cause. Regardless, it is important to re-establish a regular estrogen–progesterone cycle with appropriate replacement therapy if necessary (Crosignani and Vegetti, 1996; Tudor-Locke and McColl, 2000).

Age at menopause

Delayed menopause, or alternatively an extended reproductive period characterized by a longer protective hormonal environment, is associated with higher bone mineral density and reduced risk of hip fracture in older women (Tudor-Locke and McColl, 2000). In contrast, women are specially at risk for osteoporosis and fracture if they have had a premature or surgical menopause and have not received HRT (Notelovitz, 1993; Lindsay, 1995).

Hysterectomy with ovarian conservation

Premenopausal women who underwent a hysterectomy with ovarian conservation were found to have bone densities significantly lower than those of normally menstruating controls (Hreshchyshyn *et al.*, 1988). It is not known whether this effect is due to removal of the uterus *per se*, to aberrant functioning of the retained ovaries (Notelovitz, 1993), or to premature loss of ovarian function (Souza *et al.*, 1986; Siddle *et al.*, 1987; Cooper and Thorp, 1999). Interestingly, both hysterectomy and tubal ligation have been reported to protect against ovarian cancer, even in

carriers of BRCA1 and BRCA2 mutations (Hankinson *et al.*, 1993; Rosenblatt *et al.*, 1996; Narod *et al.*, 2001).

Exercise

Exercise is generally considered to be beneficial to the skeleton and, in fact, high bone density is consistently observed in eumenorrhoeic athletes while intervention studies in non-athletic premenopausal women indicate that high-load activities (weight training, running, jumping and stepping) improve bone mineral status (Tudor-Locke and McColl, 2000). Excessive exercise, however (mainly running) may be less beneficial for at least a subpopulation of women (Marcus *et al.*, 1985). Ballet dancers are also subject to amenorrhoea and lower bone mass than average (Warren *et al.*, 1991). Amenorrhoeic athletes have a lower body weight than is found in those who remain eumenorrhoeic, and they also have lower circulating estradiol and progesterone levels (Lindsay, 1995). It seems likely that it is the reduction in sex steroids which is associated with reduced bone mass and thus, the return of menses (usually following a reduction in exercise) results in a degree of recovery (Drinkwater *et al.*, 1986). The lower bone mass of athletes who have lost steroids appears to be associated with a greater incidence of stress fractures, often affecting the small bones of the feet (Warren *et al.*, 1991). These athletes do not appear to be at increased risk of classical osteoporotic fractures (Lindsay, 1995).

Hyperprolactinaemia

Failure of the gonadal axis due to a prolactin-secreting pituitary tumour results in a significant loss of bone tissue (Schlechte *et al.*, 1983). That it is, the loss of sex steroids that is responsible for the low bone mass is suggested by the normal bone mass of hyperprolactinaemic women with intact ovarian function, but similar prolactin serum levels. Treatment of the underlying problem with return of ovarian function is also associated with significant gain in bone mass (Lindsay, 1995).

Anorexia nervosa

Patients with anorexia nervosa have significantly reduced bone mass compared with normal control women, mainly when anorexia begins before puberty. The reduction in bone mass may be more than 25% of that in age-matched individuals and is related to the duration of amenorrhoea. In addition to the calorie deprivation and low body weight, mechanisms for bone loss in these patients include also high endogenous cortisol production and suppression of the hypothalamic pituitary axis (Lindsay, 1995; Tudor-Locke and McColl, 2000). There are concerns that incurred bone deficits may incompletely recover from anorexia nervosa. Bone loss continues with ongoing amenorrhoea. HRT or oral contraceptives increase bone density, but the best remission is associated with natural resumption of menses. Not infrequently, however, recovery is slow and incomplete (Tudor-Locke and McColl, 2000).

Scoliosis

Although the cause of scoliosis is not known and is associated with a strong genetic tendency, it has been related to hypoestrogenism. Adult scoliotic women have a higher incidence of osteoporosis, and younger women with scoliosis should be regarded as being at greater potential risk for osteoporosis (Notelovitz, 1993).

Ovarian dysgenesis (Turner's syndrome)

The severe estrogen deficiency characteristic of Turner's syndrome is associated with a significant bone mass decrease ascribable to increased bone turnover, as indicated by histological studies and assays of bone turnover markers. Estrogen therapy is followed by a significant bone mass gain and a return to normal of bone turnover markers, suggesting that it is the estrogen deficiency rather than the chromosomal abnormality that causes the bone mass deficiency, although abnormalities in the renal metabolism of vitamin D have been reported (Breuil and Euler-Ziegler, 2001). If not treated with estrogen, patients with ovarian dysgenesis probably begin to lose bone at early age—a feature accompanied by postmenopausal serum levels of bone markers such as alkaline phosphatase and urinary hydroxyproline (Lindsay, 1995).

Pregnancy and lactation

There is no net effect of pregnancy on bone mineral density (Rosen and Rosenblatt, 2002). There is, at present, insufficient evidence to state that parity or age at first pregnancy are risks factors for reduced bone mineral status in premenopausal women (Tudor-Locke and McColl, 2000).

Secretion of calcium into the milk of lactating women approximately doubles the daily loss of calcium. Thus, lactation is associated with loss of bone of 1.5 to 4% in women who breast-feed for 6 months or more, even in the presence of a high calcium intake (Rosen and Rosenblatt, 2002). The bone loss is due to increased bone resorption, probably secondary to the relatively low estrogen levels associated with lactation. However, bone density returns to baseline levels in the 6 months after weaning. Recovery of bone density appears to be linked with resumption of menses (Speroff *et al.*, 1999; Tudor-Locke and McColl, 2000). It is possible that recovery is impaired in women with inadequate calcium intake. Total calcium intake during lactation should be at least 1500 mg daily. However, calcium supplementation has no effect on the calcium content of the breast milk or on bone loss in lactating women who have normal diets (Speroff *et al.*, 1999). Lactation followed immediately by a second pregnancy does not adversely affect recovery of bone density, and there is no increased risk of osteoporosis and fractures in postmenopausal women who lactated earlier in life (Tudor-Locke and McColl, 2000; Rosen and Rosenblatt, 2002).

Pharmacological ovarian suppression

Suppression of ovarian function is used mainly for contraception and treatment of endometriosis, and produces varying degrees of estrogen deficit. Indeed, prolonged suppression in

premenopausal women may have a significant impact in accelerating bone loss. Data in the literature do not support any differences in bone mass in the spine, hip or forearm of normal women from those with endometriosis; thus, women with endometriosis are more likely to be at risk from their medical therapy rather than any inherent initial osteopenia (Dawood, 1994; Tudor-Locke and McColl, 2000).

Oral contraceptives

Oral contraceptives are commonly recommended for the treatment of hypoestrogenic amenorrhoea, and it seems clear that a bone-sparing benefit of oral contraceptive use is observed in this group, though not to the extent seen with a natural resumption of menses (Tudor-Locke and McColl, 2000). However, the effects of oral contraceptives on bone in healthy premenopausal women with regular menses have been controversial (Speroff *et al.*, 1999; Tudor-Locke and McColl, 2000; Dayal and Barnhart, 2001). Traditionally, it has been suggested that oral contraceptives may preserve bone mineral density. In general, however, epidemiological study of the effects of oral contraceptives on bone mineral density has been limited by evaluation of post-menopausal, former high-dose oral contraceptive users. In fact, some retrospective studies have found a beneficial effect of oral contraceptives on premenopausal bone, whilst others have not. Discrepancies are due to dose, duration of use, characteristics of subject populations, including wide age ranges and variable menstrual status, and potential confounders, including calcium intake and smoking habits. Moreover, not all oral contraceptives are similar, making it difficult to generalize their effects (Tudor-Locke and McColl, 2000). Thus, studies reflecting modern use of low-dose products indicate little impact of oral contraceptive use on bone mass (Mais *et al.*, 1993; Hartard *et al.*, 1997; Speroff *et al.*, 1999).

However, measurements of bone density are not as important as the clinical outcome, namely fractures (Speroff *et al.*, 1999). In a population-based, case-control study of post-menopausal women who had formerly taken oral contraceptives during their premenopausal years, those aged over 40 years showed significant protection against hip fractures during their menopause, while those aged less than 40 years had smaller and non-significant reductions in hip fracture (Michaëlsson *et al.*, 1999). However, the available evidence suggests that any favourable effects on bone density in premenopausal women are not clinically important (Speroff *et al.*, 1999); this suggestion is supported by two large cohort studies (Cooper *et al.*, 1993; Vessey *et al.*, 1998). In the Royal College of General Practitioners study, the overall risk of fractures in ever-users of oral contraception was actually slightly increased (Cooper *et al.*, 1993). Similar results were observed in the Oxford Family Planning Association Study (Vessey *et al.*, 1998). It is likely that the increased risk reflects lifestyle effects among oral contraception users, but thus far there is no evidence of any protective effect against fractures (Speroff *et al.*, 1999). Therefore, prospective studies of longer duration are needed to determine the effects of oral contra-

ceptive use on bone in healthy premenopausal women with regular menses.

MPA and progestin implants

DMPA is a widely used injectable form of hormonal contraception (Kaunitz, 2002). In contraceptive doses, DMPA suppresses ovarian production of estradiol, which suggests a potential for an increased risk of osteopenia and subsequent post-menopausal osteoporosis. As discussed above, the impact of premenopausal DMPA use on post-menopausal osteoporosis remains controversial. Thus, although it is considered a safe and reliable form of contraception and its impact on bone mineral density similar to that of lactation (i.e. both lower ovarian production of estradiol, leading to reversible decreases in bone mineral density), the United States Food and Drug Administration recommended post-marketing studies to determine, prospectively, changes in bone density related to DMPA use; the results of a long-term study initiated in 1994 are expected in 2003 (Kaunitz, 1998; Tudor-Locke and McColl, 2000).

Fluctuating estradiol levels in progestin implant or progestin-releasing intra-uterine system users imply that loss of bone density would be unlikely. The potential different effects on bone mass of progestin implants or progestin-releasing intra-uterine systems and DMPA is probably attributable to less suppression of the pituitary gland by implants, permitting more follicular activity in the ovary and more estrogen production (Cromer, 1999; Luukkainen *et al.*, 2001; Meckstroth and Darney, 2001).

Danazol

Because of its androgenicity, danazol use is associated with either significant bone gain or no change in bone mineral density (Hornstein and Barbieri, 1988; Dawood, 1994).

GnRH agonists

The long-term consequences of the hypoestrogenic state induced by GnRH agonists on calcium metabolism and bone are of concern (Dawood, 1994; Speroff *et al.*, 1999). For this reason, therapy is usually limited to 6 months to avoid bone loss, though even during this time period there can be a 6–8% decrease in bone mineral density. This loss is reversed after cessation of therapy, but many patients (mainly those of advanced reproductive age or with lower calcium intakes) have not regained the bone that was lost, as much as one year later (Orwoll *et al.*, 1994; Speroff *et al.*, 1999). In this respect, it should be noted there is not only individual variability of bone density but also variability in the amount of bone lost by different individuals receiving the same dose of GnRH agonist over the same duration, as is the case with bone loss occurring after the menopause (Dawood, 1994).

In recent years, so-called ‘add-back’ therapy has been used to alleviate a lasting impact on the risk of osteopenia associated with long-term therapy with GnRH agonists (Deary *et al.*, 2002). The addition of a 19-nortestosterone progestin, a post-menopausal estrogen-progestin programme, tibolone or bi-

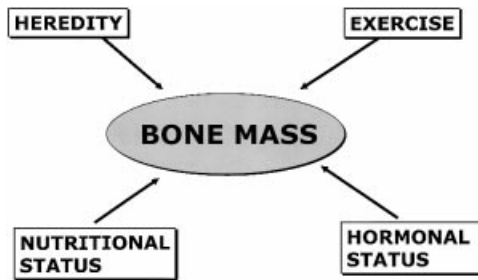


Figure 1. Schematic representation of the four major interactive factors determining bone mass in the adult female.

sphosphonate treatment may be effective in reducing bone loss associated with GnRH agonist therapy while maintaining treatment efficacy (Edmonds, 1996; Moghissi, 1996; Speroff *et al.*, 1999). Interestingly, however, it has been reported that a daily dose 0.625 mg conjugated estrogen associated with MPA is not sufficient to prevent the hypoestrogenic effects of GnRH agonists on skeletal bone, and 1.25 mg of conjugated estrogen or the equivalent dosage of other estrogens should be prescribed when planning long-term GnRH agonist ovarian suppression (Sugimoto *et al.*, 1993).

In addition to endometriosis, this add-back therapy is considered to be a breakthrough for the management of a number of gynaecological conditions including uterine leiomyoma, dysfunctional uterine bleeding and premenstrual syndrome (Studd and Leather, 1996). In contrast with treatment of uterine fibroids, add-back therapy for endometriosis does not need to be deferred for optimal results (Kiesel *et al.*, 1996). A matter of concern is that these gynaecological conditions are frequently recurrent diseases and thus further treatments may become necessary. Any medical treatment associated with a significant loss of bone mass, and without a complete return to baseline several months after treatment is stopped, may produce an additional cumulative deficit in trabecular bone mass with each succeeding treatment. With a sufficient treatment-free interval of perhaps more than a year, and with adequate calcium intake and elimination of risk factors for osteoporosis, it is likely that bone loss will be adequately recovered (Dawood, 1994), but in some women bone density is restored only by 2 years after a 6-month period of GnRH agonist therapy (Paoletti *et al.*, 1996). Therefore, it should be considered that re-treatment with GnRH agonist or any other medication producing bone loss after short or inadequate post-therapy intervals is likely to cause recurring additional residual bone losses, giving rise to an increasing deficit in total bone mass (Dawood, 1994).

Sex steroids and bone: therapeutic implications

Bone mass in the adult female is determined by four major interactive factors: (i) heredity (genes); (ii) exercise (mechanical loading); (iii) nutritional status (intake of calcium and other nutrients); and (iv) hormonal status of the person (sex steroids) (Heaney, 1987; Notelovitz, 1993; Dawood, 1994) (Figure 1). Superimposed on the complex interaction of these factors are

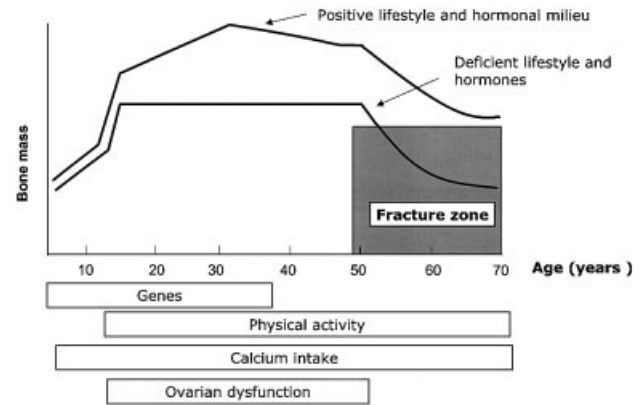


Figure 2. Diagrammatic representation of bone mass accrual in women and factors influencing the bone mass at different ages. The different factors are shown in the boxes on the horizontal axis. Modified from Heaney (1987).

lifestyle or social habits such as smoking and excessive caffeine, alcohol, sodium and animal protein intake (Heaney, 1987; Dawood, 1994; Tudor-Locke and McColl, 2000).

As discussed above, peak bone mass is usually attained by the third decade of life, and this maximum accrual of bone mass serves to put a woman into a more advantageous skeletal balance in preparation for the impending accelerated bone loss accompanying the early post-menopausal years. This emphasizes the paramount importance of maintaining or increasing bone mass before the decline observed after menopause. Despite genetic control of peak bone density, nutrition, exercise and hormonal milieu can impact on bone during growth, thereby either reducing or increasing the capacity to reach the maximum genetic potential. To influence bone density, therefore, intervention is required at a very early stage. Given the dynamic activity of the bone-remodelling cycle during puberty, this could be the optimal time to influence bone mass accrual (Stevenson, 1990; Notelovitz, 1993).

Therefore, the prevention of osteoporosis starts with the onset of the menarche. A combination of exercise (to stimulate new bone formation), appropriate nutrition (to mineralize the newly formed osteoids) and a healthy lifestyle (to avoid detrimental effects on bone formation) all maximize bone mineral accrual and result in optimal peak bone mass. Normal ovarian function (estrogen-replete state to modulate the rate of bone loss) is essential to this process (Figure 2) (Heaney, 1987; Notelovitz, 1993; Dawood, 1994). Unfortunately, many doctors rely on HRT only, and most women actually become aware of the need for osteoporosis prevention much later in life—usually after they have already become menopausal (Notelovitz, 1993). HRT, however, has important limitations for prevention of fractures in post-menopausal women, as discussed next.

Limitations of HRT for prevention of fractures

The first report from the Women's Health Initiative (WHI) study has recently been published (Writing Group for the Women's Health Initiative Investigators, 2002). The WHI is the first randomized clinical trial to demonstrate a reduction in hip and vertebral fracture risk with HRT use, and the findings are discussed below. Historically, and

before such a study, support for estrogen use to prevent bone loss and fractures in post-menopausal women had come from three lines of evidence. First, randomized trials have consistently shown that estrogen prevents post-menopausal bone loss. Many short-term studies and some longer-term studies of HRT and bone mineral density as the primary outcome have shown significant efficacy (Speroff *et al.*, 1996; The Writing Group for the PEPI Trial, 1996; NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). Second, observational studies consistently suggested that post-menopausal HRT reduces the risk of hip and other types of fracture (Grady *et al.*, 1992; Lindsay, 1995). Third, evidence was obtained from risk-versus-benefit studies of HRT for post-menopausal women who are considering long-term hormone therapy to prevent disease or to prolong life. According to these studies, HRT would increase life expectancy for most menopausal women, since protection against hip fractures and coronary heart disease would outweigh the increase in the risk of breast cancer (Grady *et al.*, 1992; Burkman *et al.*, 2001).

Reduced bone density has been considered to be a risk factor for fracture. However, change in bone density is an imperfect predictor of reduction in the risk of non-spine fractures (Grady and Cummings, 2001). Thus, for example, calcium with vitamin D decreases the risk of fracture, despite having little or no effect on bone density. In contrast, alendronate (which reduces fracture risk in women with osteoporosis) substantially increases bone density but does not reduce the risk of non-spine fractures in women without osteoporosis (Grady and Cummings, 2001).

Although the skeletal benefits of HRT for preventing bone loss can hardly be challenged, one might be wary of published evidence that prolonged HRT use unequivocally reduces the risk of fracture (Reginster *et al.*, 2000). Remarkably, unlike trials of the effects of estrogen replacement on bone mass in the early menopause (often placebo-controlled), the ability of estrogen to reduce fracture risk has been reported (until very recently, as discussed below) only in uncontrolled, frequently retrospective, observational or case-controlled studies (Orwoll and Nelson, 1999; Reeve, 2000; Reginster *et al.*, 2000; Grady and Cummings, 2001). Observational studies, however, are susceptible to selection bias and confounding. This may be especially true of studies of HRT because users tend to be healthier, wealthier and more active than non-users (Orwoll and Nelson, 1999; Reeve, 2000; Grady and Cummings, 2001; Laine, 2002; Vastag, 2002). Therefore, an important part of the protective effect associated with taking estrogen is mediated via other factors (exercise, diet and others). For this reason, concern has been raised as to the preferential treatment given to HRT products compared with other currently developed medications, where evidence of an anti-fracture efficacy at the level of the spine or hip is requested (Reginster *et al.*, 2000). As recently stressed (McNagny and Wenger, 2002),...“clinicians should alert women that, in 1999, the Food and Drug Administration removed the treatment of

osteoporosis as an indication for estrogen therapy because of the lack of evidence from randomized trials of the effect of estrogen on the risk of fracture”.

Ideally, clinical practice should be based on evidence from large randomized trials using fracture as the outcome measure. Such trials have found that bisphosphonates reduce non-vertebral fracture risk as well as vertebral fracture risk whilst raloxifene and calcitonin reduce vertebral fracture risk, and these drugs have been approved by the Food and Drug Administration for prevention and treatment of osteoporosis (Grady and Cummings, 2001). Surprisingly, no such large trial has been performed to determine the effect of estrogen on fracture risk in women with osteoporosis (Grady and Cummings, 2001). The Heart and Estrogen/progestin Replacement Study (HERS and HERS II) (Cauley *et al.*, 2001; Hulley *et al.*, 2002) and the WHI randomized controlled trial (Writing Group for the Women’s Health Initiative Investigators, 2002), however, have recently examined the effect of long-term post-menopausal hormone therapy on fractures. The HERS study was a randomized, blinded, placebo-controlled trial of estrogen plus progestin therapy after menopause of 4.1 years duration (HERS) and subsequent open-label observational follow-up for 2.7 years (HERS II). The study included 2763 post-menopausal women with coronary heart disease; 2321 of these women (93% of those surviving) consented to follow-up in HERS II. The WHI was the first randomized trial to directly address whether estrogen plus progestin has a favourable or unfavourable effect on coronary heart disease incidence and on overall risks and benefits in predominantly healthy women. A total of 16 608 women entered the randomized double-blind trial (planned duration, 8.5 years), and after a mean of 5.2 years of follow-up the data and safety monitoring board recommended stopping the trial because the test statistic for invasive breast cancer exceeded the stopping boundary for this adverse effect, while the global index statistic supported risks exceeding benefits.

The HERS main trial revealed little difference between the hormone and placebo groups in risk of any type of fracture (Cauley *et al.*, 2001). Surprisingly, the additional follow-up experience from HERS II suggests a risk of hip fracture among women in the hormone therapy group that was higher than that in the placebo group (relative hazard, 1.61; 95% CI, 0.98–2.66; $P = 0.06$). The WHI (Writing Group for the Women’s Health Initiative Investigators, 2002) was the first trial with data supporting the ability of post-menopausal hormones to prevent fractures at the hip, vertebrae and other sites (hazard ratio for all types of fractures, 0.76; 95% CI, 0.69–0.85). Discrepancies between studies may be explained by the fact that women included were not selected for osteoporosis and may thus not be well suited to revealing the effects of fracture-prevention treatments. Clinical trials of bisphosphonates have found an effect on the risk of fracture in women with osteoporosis, but not in women with normal bone density (Cummings *et al.*, 1998; McClung *et al.*, 2001). Overall, however, those studies cast doubt on the usefulness of HRT as a universal approach to prevent fractures in post-menopausal women. An evaluation of

incident fracture rates in the Study of Osteoporotic Fractures population further illustrates the limitations of HRT. In women who reported taking estrogen continuously since the menopause (an average of 25 years), the rate of bone loss was noted to accelerate with increasing age, and those on estrogen therapy continued to lose bone density, as did those without estrogen (Ensrud *et al.*, 1995). Furthermore, as reported very recently, almost 20% experienced a non-traumatic, non-vertebral fracture during a 10-year follow-up period (Nelson *et al.*, 2002a). This was fully two-thirds the number observed in women who had never taken estrogen. The extent to which estrogen was associated with lower rates of vertebral and hip fracture was similar. Clearly, many women who take estrogen will ultimately suffer osteoporotic fractures (Orwoll and Nelson, 1999). Therefore, low bone mass and fractures remain serious threats in older post-menopausal women, even in the presence of hormone replacement. Thus, as recently stressed (Grady and Cummings, 2001), perhaps the important clinical question now is not whether estrogen reduces risk of fracture, but whether it reduces risk as much as other proven useful treatments such as bisphosphonates.

Finally, since women in their 50s who do not have osteoporosis have a relatively low risk of fracture, the benefit of long-term treatment with estrogen to prevent bone loss and fractures may not exceed the risks. Previous guidelines for counselling post-menopausal women about preventive hormone therapy (American College of Physicians, 1992) were based on estimates of the potential risks and benefits coming from results of observational studies (Grady *et al.*, 1992). According to those estimates a 50-year-old healthy white woman has a 15% lifetime probability of suffering a hip fracture (median age at first hip fracture, 79 years) and a 1.5% probability of dying of a hip fracture. If treated with long-term HRT, the lifetime probability of hip fractures is reduced to 12%. However, the estimated lifetime probability of breast cancer is increased substantially and, even considering a beneficial effect on the cardiovascular system, the overall benefit is negligible: the estimated increase in life expectancy is only 0.1 years. For a woman who is at increased risk for hip fracture due to such factors as low bone mineral density, the overall benefit is again small (estimated increase in life expectancy of 0.2 years). In women at increased risk for breast cancer, treatment with combination hormones could result in a substantial increase in lifetime probability of breast cancer and a slight reduction in life expectancy (American College of Physicians, 1992; Grady *et al.*, 1992).

In patients at increased risk for hip fracture, the lifetime probability of hip fracture does not decrease as much as expected because the additional year of life gained occurs, on the average, at an advanced age when the incidence of hip fracture is very high. On the other hand, breast cancer is a more important cause of illness and death at younger ages than either cardiovascular disease or osteoporotic fractures (Grady *et al.*, 1992; Rosenberg, 1993). In this context, it should be noted that a review on potential selection bias in post-menopausal hormone therapy concluded that “surveys on the health impact

of estrogen therapy may have underestimated the risk of breast cancer and overestimated the prevention of fractures” (Hemminki and Sihvo, 1993). The results of that study supported the tenet that, until the 1990s, physicians in the USA tended to select healthier women for long-term hormone therapy, and women predisposed to osteoporosis or with an increased risk of breast cancer were excluded from therapy. This further limits the potential value attributed to HRT in early studies.

The studies that formed the foundation of the 1992 guidelines (American College of Physicians, 1992; Grady *et al.*, 1992) were biased because most of them did not adequately account for socioeconomic status, and we now know that the women who take estrogen are different from those women who do not: they are healthier; they are wealthier; and they have a better health profile. Thus, rather than HRT keeping women healthy, healthy women were taking HRT (Laine, 2002; Vastag, 2002).

Recent controlled trials (such as HERS, WHI, Estrogen Replacement and Atherosclerosis Trial, Women’s Estrogen for Stroke Trial, Papworth Hormone Replacement Therapy Atherosclerosis Study) have brought risks and benefits of HRT under scrutiny, and investigators renounced their earlier recommendations to prescribe such therapy for prevention in post-menopausal women (Friedrich, 2001; Vastag, 2002). Falling in line with the evidence-based medicine trend (Herrington, 1999; Cauley *et al.*, 2001; Hulley *et al.*, 2002; Humphrey *et al.*, 2002; Nelson *et al.*, 2002b; Writing Group for the Women’s Health Initiative Investigators, 2002), an international team of women’s health experts is discouraging the use of HRT for many post-menopausal conditions. Coronary heart disease, fractures, depression and urinary incontinence—all of which have in the past been cited as prime reasons to initiate HRT—are losing favour as valid indications for its use, as evidence from high-quality clinical trials accumulates (Vastag, 2002). Although short-term use (<5 years) of HRT with combined estrogen-progestin may help women cope with symptoms of the menopause, when used for longer periods this therapy may cause net harm. Whilst event rates are low, possible reductions in the risk of fracture and colorectal cancer are outweighed by increases in the risk of coronary heart disease, stroke, pulmonary embolism, breast cancer and biliary tract surgery (Simon *et al.*, 2001; Nelson *et al.*, 2002b; Writing Group for the Women’s Health Initiative Investigators, 2002). Increased risk of ovarian cancer is also of major concern (Lacey *et al.*, 2002; Riman *et al.*, 2002a;b; Rodriguez *et al.*, 2002). This implies a dramatic shift on best clinical practices for treating patients during menopause. This new perspective is summarized in the evidence-based recommendations from the *International Position Paper on Women’s Health and Menopause: A Comprehensive Approach*, published by the National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH). The International Position Paper is based on extensive international review and evaluation of the scientific evidence for current clinical practices as presented in the published literature. Further information is available at the

Office of Women's Health Research Web site: (http://www.nhlbi.nih.gov/health/prof/heart/other/wm_menop.htm).

Future therapeutic perspectives

The recognition during the mid-1980s of selective estrogen receptor modulation provided a unique opportunity to develop multifunctional drugs. Tamoxifen, the first SERM, is the first antiestrogen to be tested successfully for the prevention of breast cancer in high-risk women. However, the recognition that SERMs maintain bone density and lower circulating cholesterol suggested that the prevention of osteoporosis and coronary heart disease would be beneficial side effects of tamoxifen treatment. This hypothesis was not pursued in any clinical trial, but an alternate hypothesis—that SERMs could be developed to prevent osteoporosis and potentially reduce the risk of breast cancer—has been pursued with raloxifene (Jordan, 2001; Jordan *et al.*, 2001). Recent evidence from randomized trials suggests that raloxifene prevents bone loss and reduces the risk of vertebral fractures (Cranney *et al.*, 2002). The increase in bone density with raloxifene is smaller than that seen with other anti-remodelling therapies, such as HRT and bisphosphonates (Cranney *et al.*, 2002). The relatively large effect of the drug on vertebral fractures may suggest that raloxifene has a positive effect on other aspects of bone, such as bone quality. On the other hand, the very small effect on non-vertebral fractures does not support such an effect.

There is intense interest in understanding the molecular mechanisms of action of SERMs at target sites in a woman's body. Current molecule modelling of the SERM–estrogen receptor complex has identified the reason for the promiscuous estrogen-like actions of tamoxifen compared with raloxifene. Future studies of the signal transduction pathways of the estrogen receptor alpha and beta-SERM complexes hold the promise of new drug discoveries (Jordan 2001; Jordan *et al.*, 2001). An understanding of the targeted actions of this novel drug group will potentially result in the introduction of new multifunctional medicines with applications as preventive agents or treatments of breast cancer, osteoporosis, coronary heart disease and endometrial cancer (Jordan *et al.*, 2001).

Androgens are known to be essential for men's sexual performance and well-being, but their contribution to female health and sexuality is less well understood. The concept of androgen insufficiency in women has been controversial but is now viewed as potentially having a major impact on the quality and well-being of women's lives. There is, at present, a growing interest in the subject, and a wide spectrum of symptoms and signs—including bone loss and muscle wasting that may be associated with androgen insufficiency in post-menopausal women—has been strongly identified at a recent consensus conference (Bachmann, 2002; Bachmann *et al.*, 2002). Due to the potential adverse effects of diminished androgen, the issue of pharmacological intervention with androgen replacement assumes significance in the female population with androgen insufficiency, especially in the post-menopausal age group where it has been emphasized that we are only at the beginning of recognizing the importance of androgens (Bachmann, 2002; Burger, 2002b; Sarrel, 2002). Although no precipitous drop in androgen production is apparent at the time of the menopause, androgens decrease slowly

and progressively from early adulthood until old age. The impact of ageing and of estrogen replacement therapy—especially oral estrogen—significantly reduces androgen bioavailability after menopause. The combined effects of reduced production with ageing and the pharmacological effects of oral estrogen dramatically reduce the endogenous and bioavailable androgen milieu (Simon, 2002).

The matter of androgen therapy and bone mineral density has been analysed recently (Notelovitz, 2002a). A number of clinical trials, including appropriately designed randomized blinded studies, have shown that androgen therapy (testosterone by subcutaneous pellet implantation or oral methyltestosterone) when combined with estrogen therapy, has an additive effect on bone mineral density compared with estrogen-only therapy. These studies were preceded by the earlier empirical use of combination estrogen and androgen therapy in clinical practice, and by the observation in individual women of an anabolic effect on bone of combined estrogen and androgen treatment. Interestingly, using clinically available bone marker tests, the rationale for knowing when to use estrogen and androgen therapy in preference to estrogen-only when treating women with osteoporosis has been proposed (Raisz *et al.*, 1996). Non-response of bone mineral density to adequate estrogen therapy may be indicative of low-turnover osteoporosis, mainly when appropriate serum levels of estrogen have been achieved and the urinary excretion of the collagen cross-link peptides are appropriately suppressed. Under these circumstances the anabolic bone-building potential of androgens should be considered (Notelovitz, 2002a). Long-term clinical trials are needed to confirm the clinical efficacy of estrogen/androgen therapy in osteoporosis prevention and treatment. Safety concerns include potential virilizing effects, significant hepatic events, the generation of an adverse lipoprotein profile, and an increased risk of breast cancer (Slayden, 1998; Dorgan *et al.*, 2001; Liao and Dickson, 2002). This notwithstanding, while oral androgens lead to unfavourable changes in circulating lipid concentrations, there is no evidence that parenteral testosterone administration is likely to have any adverse cardiovascular consequences (Simon, 2001; Burger, 2002b). On the other hand, results from clinical and non-human primate studies have shown that androgen administration may induce down-regulation of mammary epithelial proliferation and estrogen receptor expression, suggesting that estrogen/androgen therapy might reduce the risk of breast cancer associated with estrogen replacement therapy (Simon, 2001; Dimitrakakis *et al.*, 2002).

Another new perspective in treatment of osteoporosis has been proposed by Manolagas' group (Manolagas *et al.*, 2002). Besides the critical role of sex steroid-controlled osteoblast and osteocyte apoptosis for the pathogenesis of osteoporosis caused by loss of sex steroids as discussed above, several additional studies from that group (Manolagas *et al.*, 2002) have demonstrated that osteoblast and osteocyte apoptosis are key pathogenetic mechanisms in other forms of osteoporosis. Moreover, control of osteoblast and osteocyte apoptosis would explain the anti-osteoporotic efficacy of the most commonly used drugs. As mentioned above, the effect of estrogens (and androgens for this matter) is mediated by a novel paradigm of sex steroid action that requires only the ligand-binding domain of the receptor, extranuclear localization of the protein, and sex non-specificity (Kousteni *et al.*, 2001). This fact, together with the demonstration

of the principle that increased work output of a cell population by suppressing apoptosis can augment tissue mass, points to an entirely new approach for the treatment of osteoporosis: one that could lead to cure rather than prevention or slowing of the disease process. Studies with the use of different anabolic agents such as fluoride, growth hormone, insulin-like growth factor I, the statins, and mainly the intermittent administration of parathyroid hormone (Neer *et al.*, 2001; Rosen and Bilezikian, 2001) strongly support this contention by showing that the anabolic property of parathyroid hormone restores bone mineral density in the normal range and prevents bone fractures to levels much greater than those seen with anti-resorptive agents (Neer *et al.*, 2001).

The optimal therapeutic modality for osteoporosis, mainly in patients who have already suffered significant bone loss, is clearly an anabolic agent that can restore bone mass by rebuilding bone within a short period of time. Since daily parathyroid hormone administration increases bone mass by preventing osteoblast apoptosis without slowing remodelling, Manolagas' group reasoned that estrogen receptor ligands with anti-apoptotic but not anti-resorptive/anti-remodelling properties will expand the pool of mature osteoblasts at sites of new bone formation and allow these cells more time to make bone, to a much greater degree than the anti-resorptive agents that also slow remodelling (Manolagas, 2000; Manolagas *et al.*, 2002). Based on their findings that the non-genotrophic estrogen-like activity of the estrogen receptor can be dissociated from its transcriptional activity with synthetic ligands (Kousteni *et al.*, 2001), that group of researchers coined the acronym 'ANGELS' for estrogen receptor ligands which function as 'Activators of Non-Genotrophic Estrogen-Like Signaling' (Manolagas *et al.*, 2002). These ligands lack, either completely or partially, the ability to induce the transcriptional activity of the estrogen receptor. Evoking the non-genotrophic but not the genotrophic signal, these synthetic ligands may be bone anabolic agents, as opposed to natural estrogens or SERMs that are anti-resorptive agents. The same ligands may also circumvent the side effects associated with conventional HRT (Manolagas *et al.*, 2002).

In supporting these postulates, the same authors (Manolagas *et al.*, 2002) have obtained evidence that synthetic ligands with potent anti-apoptotic but not genotrophic activity increase bone mineral density and bone strength significantly more than estrogens, in estrogen-deficient mice, without affecting the uterus or the breast. Future studies will be needed to establish the validity of the ANGELS concept on non-reproductive tissues other than bone. However, it is suggested that if non-genotrophic effects of estrogen are as important in other non-reproductive tissues as the evidence suggests, ANGELS may also retain at least some of the beneficial effects of estrogens on the vasomotor, cardiovascular and central nervous systems (Manolagas *et al.*, 2002).

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