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How do women develop fragile bones?

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1. Introduction

During childhood, while oestrogen levels are low compared to those in adult women, the skeleton expands rapidly. But no one knows how our bones regulate their growth so as to precisely match their eventual architecture to the mechanical loading environment experienced by the young adult. Giant strides are currently being made towards defining the molecular mechanisms regulating the growth of embryonic limb buds and their subsequent development, as well as of other parts of the skeleton. It is clear that specialised mechanisms exist which permits the skeleton to expand in an orderly fashion, so as to eventually reach adult size with sufficient (but not excessive) strength and the provision of mechanically efficient leverage for the muscles. There are also, in rodents and probably in man, genetic determinants of the density of bones; as yet, however, there are no fully defined molecular pathways by which bone density might be influenced in response to these genes. Clearly, therefore, conceptually there are a number of ways by which different women might have varying levels of skeletal susceptibility to oestrogen exposure or its withdrawal.

Internally, bones are remarkably well adapted to resist the forces that are regularly placed upon them by muscles or impact reactions. Thus the cancellous bone found at the ends of all long bones is specifically oriented to resist the forces arising from the neighbouring joints. At the proximal end of the femur, which is basically a cantilever structure, the trabeculae follow the major lines of force arising from the joint and the attachments of the major groups of muscles around the joint. The virtue of cancellous over compact bone as a material from which to make the ends of long bones is that it is more deformable. Thus it can accept more strain (deformation) before it begins to suffer structural damage, being in its properties intermediary between cartilage and compact bone in this respect. Without their largely cancellous ends, the hard parts of the skeleton might well be too unyielding to permit the long-term survival of healthy joint cartilage. In the mid-shafts of long bones, the increased strength of compact bone gives it a structural advantage.

It is crucial for the survival of nearly all vertebrates that their bones can accept sudden, occasional, very large forces, which are outside the range generally experienced. In any case, like nearly all hard materials, bone that is not renewed gradually suffers structural damage over many cycles of repeated loading, so the skeleton has to be somewhat mechanically over-specified (at least apparently) at the growth stage and after. However microdamage of bone, as with other hard materials, does not lead directly to fracture. In a single cycle of bending, if the load applied produces strain that exceeds the material's elastic limit, the bone enters a phase of plastic deformation. Being a composite material, the internal stresses within a bone are relieved by the generation of microscopic cracks, which it is as well for the animal's health should remain microscopic [1]. If the bone requires a very large additional load to be applied before it finally breaks, after the elastic limit has been passed, it is described as not being brittle. If the reverse is the case it is brittle. It is a characteristic of aged bones that they are more brittle than young bones. To the extent that bone's material properties have been investigated as a cause of this, it seems likely that microscopic cracks in elderly bone are likely to be longer than in young bone, implying that the physical mechanisms that normally 'capture' developing cracks and prevent then spreading further or coalescing into macrocracks work less well [2].

There has been much consideration given to the purpose or purposes of bone remodelling. Remodelling is the process by which bone is renewed in microscopic packets where old bone has preceded it. Modelling is

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the process by which bones alter their macroscopic shape by adding bone in one place and removing it in another. Remodelling requires that removal and formation of bone should be sequential as well as spatially co-ordinated [3]. Bone that has been killed cannot remodel; although blood-born osteoclasts may gain access, given a blood supply, osteoblasts are derived locally from adjacent bone surfaces or bone marrow. It is known clinically that dead bone (osteonecrosis) suffers the gradual degradation of its mechanical properties and eventually collapses (cancellous bone) or fractures (cortical bone). It seemed natural therefore to assume that remodelling of live bone prevents this by replacing bone before it experiences too many cycles of loading. However it was only quite recently that Mori and Burr were able to demonstrate that bone loaded to the point of accumulating microdamage abnormally quickly was preferentially remodelled [4]. The relevance of oestrogen to this process remains speculative and is discussed later.

It has long been known that with the onset of puberty and exposure of the skeleton to adult levels of oestradiol, bone density increases rapidly, bone turnover declines and resistance to fracture increases remarkably. So Albright's discovery [5] that the most common form of osteoporosis results from oestrogen deficiency led naturally, when oestrogen preparations became available, to their use in osteoporosis prevention. Much later it was discovered that men without functioning aromatase, who therefore cannot convert testosterone into oestradiol [6], or who are insensitive to oestrogens because they have dysfunctional oestrogen receptors [7], become osteoporotic. In the case of the man with oestrogen deficiency, his osteoporosis could be treated with low-dose oestrogen replacement [6]. This illuminated and simplified our understanding of the central role of oestrogens in the maintenance of the adult skeleton. It also helped redefine our thinking about the appropriate dose of oestrogen for replacement therapy in older women.

An apparent consequence of the adaptability of the skeleton to its loading environment is its propensity to remove bone that does not experience loading. If the skeleton is unloaded generally, this can lead to 'disuse osteoporosis' [8]. The young skeleton can of course add bone where overload is perceived, giving rise to the concept of a homeostatic 'mechanostat' [9]. This ability to add bone becomes much attenuated in the elderly and even the merely mature. There has been much speculation that oestrogen is a crucial tonic modulator of the mechanostat [10]; but so far the mechanostat has been visualised in non cellular and non biochemical abstraction; and the usefulness of this concept requires that it be defined in more biological detail.

In this article, what is known about the epidemiology of osteoporosis will be reviewed succinctly in relation to our knowledge of the epidemiology of oestrogen 'deficiency'. The role of oestrogen deficiency as a risk factor for osteoporosis being fairly well defined, the remainder of the chapter will be devoted to an attempt to reconcile our epidemiological knowledge with our rapidly developing understanding of how oestrogens regulate the biology of bone as a tissue, within the context of the remarkably complex specification in self regulation demanded of each individual bone by the body's need for strength with lightness.

2. Osteoporotic fractures: their epidemiology in relation to bone strength

Space does not permit a discussion of the role of trauma and its frequency in the generation of osteoporotic fractures. Here the discussion will focus on the decline in bone strength after menopause, the differences in the effects of age between genders and the interactions of other risk factors with oestrogen deficiency in determining whether a hip or a spine fracture will occur.

The most devastating osteoporotic fracture is the hip fracture, the incidence of which rises exponentially with age in elderly women and men [11]. The impact and importance of vertebral fractures has been rather more controversial; but new evidence of their impact on quality of life [12] (whether or not the patient presents clinically as a case of vertebral fracture [13,14]) as well as better techniques for their unequivocal identification[15] are likely to emphasise their importance in degrading the benefits of longer life for vast numbers of men and women.

Men have hitherto been comparatively neglected in studies of osteoporosis, particularly of the spine. The European Prospective Osteoporosis Study of vertebral fractures (EPOS), an age-stratified population-based cohort study and the first to study both sexes, has recently been completed. The study employed novel, rigorous and well-validated methods for the radiological identification of both prevalent and incident vertebral fractures. Men at 65 had vertebral fracture rates which were 60% of those seen in women [15]. As in women [15,16], age, bone density and the existence of a previous fracture [17,18] were the principal determinants of fracture risk. The main statistical determinant of the gender difference in risk appears to be the faster rate of bone loss in women once they had been through the menopause [15].

After allowing for methodological differences in identification, the vertebral fracture rates in the female EPOS participants, who were drawn from 18 different European countries from Russia to Portugal and from Sweden to Greece, could not be distinguished clearly from rates [16] in Rochester, MN. The strong effect of

previous fracture to increase risk independently of bone density in both sexes had been previously reported for US women [17,18], as had the independent effect of age to approximately double fracture risk with the passage of each decade; this was seen in both genders in EPOS. A novel finding in EPOS was that the volumetric size of an incident fracture, an important determinant of its impact on the patient, could be predicted by the shape of any previous vertebral fracture [19]. Overall, EPOS suggested that in Europe two women in five and one man in four who lives to be 80 will suffer at least one vertebral fracture by that age.

Lifestyle, gynaecological, anthropomorphic and dietary risk factors for vertebral fracture have been studied intensively in prevalence studies. The European Vertebral Osteoporosis Study, upon which EPOS was based, gave results which, when not unique, are typical of other studies of vertebral fracture. Physical activity (with the exception of the most intensive physical activity in men) was protective in women especially [20] as was a long fertile period [21]. Dietary calcium only had a weak effect on bone density, through a statistical interaction with physical activity [22]. A higher body mass index (BMI, weight/height^2) was protective [23], an effect that appeared to be mediated through higher bone density in individuals with high BMI [24]. In a much smaller study of normal women followed for 11 or more years, higher BMI was a positive determinant of a low post-menopausal rate of bone loss [25], an effect seen also in a more obese population [26]. Two possible explanations for these concordant findings concerning anthropometric variables are discussed below.

A great deal is now known about the epidemiology of hip fracture in white European and American women. A major component of risk can be attributed to variables that may be loosely regarded as markers of physical frailty or inactivity [27,28], while poor eyesight and dementia or other degenerative neurological disorders contribute to the risk of falling. Also, after 20 years of controversy, it is now firmly established that in some settings a mild form of vitamin D-related osteopathy leads to accelerated bone loss, which can be reversed partially with replacement therapy with a variable but potentially very valuable effect on fracture risk [29]. A likely very important new discovery, which remains to be confirmed, concerns the role of endogenous oestrogens as determinants of hip fracture risk. When these are very low, so that oestradiol was undetectable in a modern, high sensitivity assays, the increment in risk of hip and vertebral fracture was very substantial [30]. After menopause, most endogenous oestrogens are synthesised in adipose tissue. However, obesity is also associated in most women with increased muscle bulk and larger forces applied to the skeleton in consequence, particularly in the lower limbs. So there is an important need to disentangle the possibly partly independent effects of relative obesity and endogenous oestrogens on both hip and vertebral fracture.

3. Trial evidence

Remarkably, there have been no randomised controlled trials of ERT (except in very high risk groups) so we infer that ERT is effective in preventing a large proportion of hip and spine fractures from studies on women who selected their own treatment. There could be a large effect of selection bias to artificially enhance the apparent effect of treatment in these observational studies, because women who start ERT appear from at least one trial pilot study to have higher bone density than the rest of the population [31]; and they also have less risk of cancers unconnected with oestrogen exposure. These observations suggest that ERT-takers are healthier than average women.

The trial evidence for the anti-fracture efficacy of activators of oestrogen receptors has recently been notably enhanced by the demonstration that raloxifene, a selective (o)estrogen receptor modulator (SERM) is efficacious in preventing vertebral fractures [32], while simultaneously reducing risk of breast cancer. This trial was remarkable for showing that about 50% of vertebral fractures could be prevented with an almost negligible effect on bone density in the spine and other agents which reduce bone formation have in many cases a larger beneficial effect on fractures than can be explained by their effect on bone density alone [33].

4. The biological basis for oestrogen's actions on bone

Until recently it was thought that oestrogen acted entirely by suppressing the excessive bone resorption that develops at menopause. The biology underlying the anti-resorptive effects of oestrogens, as understood until recently, have been regularly reviewed (see e.g. Riggs and Spelsberg [34]). That, it emerges, is only part of the story. We studied women with endometriosis treated with stringent oestrogen suppression using GnRH agonists. As bone turnover (measured histologically in biopsies) increased, the osteoclasts, which resorb bone in the process of bone renewal, excavated much more deeply than usual. This led to the perforation of the plate-like structures that form cancellous bone and the formation of very large haversian cavities in the bony cortices [35]. Most striking of all however was the effect of oestrogen suppression on osteocytes, the cell type in bone generally considered to act as mechano-sensors (and the most numerous specialised bone cell type) which are found in their individual lacunae deep within the bone matrix. Rates of osteocyte

apoptosis were elevated to 10–15% as measured by several complementary techniques [36]; presumably their inaccessibility preserved these apoptotic osteocytes from immediate phagocytic destruction by other cells.

After confirming these results in an ovariectomised rat model [37] attention focussed on the role of the dying osteocyte as a potential source of the unknown homing signal which regulates the architectural response of bone to the changing needs of its mechanical loading environment. In collaborative studies with Brendon Noble, Lance Lanyon and Tim Skerry it was found that externally applied mechanical loading partially prevents osteocyte apoptosis, providing the loading is non-destructive [38]. However, if the loading is increased to the point where the matrix develops microcracks, osteocyte apoptosis rates rise enormously [39]. Some 7 days later, bone damaged by microcracks begins to be resorbed by osteoclasts, which are not seen initially when the osteocyte apoptosis is first demonstrated. It is not as yet known whether the observed U-shaped curve relating the loading environment and osteocyte apoptosis is modified by oestrogen exposure [40].

The osteocyte as a target for the actions of oestrogen is of increased interest for two further reasons. First, as described elsewhere in this volume, it expresses both known receptors (ERα and ERβ) [41] and secondly it expresses proteins, such as osteopontin, containing RGD sequences which attract osteoclasts to sites that are being targeted for destruction in the processes of bone modelling and remodelling [42]. Whereas the dying (but not yet dead) osteocyte has been implicated in the process of targetting remodelling to bone that has been recently damaged by plastic deformation [39], osteocytes that have previously died and disappeared from their lacunae without provoking the destruction of their surrounding bone are clearly incapable of later expressing molecules that act as attractants to osteoclasts. It is likely, therefore, that osteocytes which die 'un-noticed', as it were, cannot inaugurate the process of replacement of bone that potentially has been damaged by new bone with more favourable mechanical properties.

The second reason why the living non-apoptotic osteocyte is of interest in this context is because there is gathering evidence that the osteocyte network provides the cellular system which underlies the perception of mechanical strain and therefore potentially of the mechanostat. Osteocytes are very sensitive to mechanical deformation and especially to shear stress induced by fluid flow and other stimuli. In response to these signals, there is an increased activation of glucose-6-phosphate dehydrogenase within minutes [43] and subsequently potentially exportable signalling molecules such as the prostaglandins [44] and nitric oxide [45] are generated by the cell. Such molecules are readily permeable through the lacunar fluid to surfaces accessible to osteoblasts and osteoclasts. It has now been claimed that, in vitro, osteoblast-like cells, which are of the same lineage as osteocytes, may be capable of generating the specific osteoclast inhibitor, osteoprotegerin [46]. It seems possible that, under stimulation by estrogen [47], enough additional osteoprotegerin may be generated by cells of the osteoblast lineage to neutralise in part osteoclast differentiation factor [48,49] (acronyms ODF/OPGL/TRANCE/RANKL) and thereby reduce the capability of osteoclasts to resorb neighbouring bone. There are, potentially, also clear parallels with the actions of activated oestrogen receptors to increase NO synthesis in vascular endocytes. Cells of the osteoblast/osteocyte lineage appear generally to synthesise NO through activation of eNOS [50]. In moderate concentrations NO tends, like prostaglandins to favour the stimulation of bone formation during bone modelling.

4.1. Observations relevant to explaining the increased fragility of the skeleton with age in light of loss of exposure to oestrogens

Let us return to the subject of the unexplained increase in susceptibility of the hip to sustain osteoporotic fractures as the subject grows older even after adjusting for the effects of reduced bone density [51]and the increased impact of other risk factors. Different mechanisms might operate in the spine and hip to explain a possible difference in this respect between the two sites, since the effect of increasing chronological age to increase vertebral fracture rates became statistically non-significant in EPOS when an adjustment was made for falling bone density with age [15]. First, in the cancellous bone of the spine remodelling of bone is a much more rapid process than in the cortical bone of the hip. In the latter, it has been known for at least a decade that rates of bone matrix microcracking increase exponentially with age in women, even more steeply than in men [1]. Microcracks are not a well-recognised feature of the cancellous or cortical bone in the ageing vertebral body. Instead, the individual trabeculae become less well connected and it has been suggested that the directional organisation, orientation or anisotropy of the cancellous lattice may become less optimal [52].

Attention should focus, therefore, on two types of mechanism that might counteract the effects of increasing age: those for preserving trabecular structures and those for preventing brittleness developing in the bony cortices. Concerning the proximal femur, we have studied bone remodelling in complete cross-sections of the distal femoral neck bone discarded at hemiarthroplasty for intracapsular femoral fracture to try to determine what special features were associated with hip fractures which might explain the fragility of the femoral neck in

osteoporotic patients. We found that loss of bone strength was concentrated in the antero-inferior cortex and to some extent in the inferior cortex [53,54]. It has been suggested, based on studies of in vivo loading studies in volunteers followed by some somewhat idealised finite element analyses that in the typical sideways fall associated with fracture, it is the antero-inferior cortex that experiences the maximal tensile load [55]. The contra-lateral postero-superior cortex would conversely experience the maximal compressive load. In the fractured femoral neck, the appearances of increased cortical thinning [53] and porosity [54] in the antero-inferior cortex was associated with locally increased rates of bone resorption indices [56] and, further, appeared to be secondary to two factors distinguishing fracture patients from controls. The first was the anatomical clustering of remodelling activity in the cortex, which by inference might have led to the coalescence of resorbing osteons [57]; and the second was the very marked inhibition of bone formation in these complex osteons leading to the conversion of compact to more cancellous bone [58]. We also found a high prevalence of dead osteocytes in all zones of the cortex, but this was as true of the controls as the cases; and zones with higher remodelling as suggested by higher bone porosity or a higher frequency of osteoid on the haversian canal surfaces were affected less by osteocyte death than other zones [59].This is consistent with previous suggestions that the prevalence of osteocyte death might be dependant on the age of the bone tissue concerned.

Pending further studies of the candidate signalling pathways between osteocytes and the destructor osteoclasts, what further light can be shed on the possibility that post-menopausal bone loss might be averted even without taking ERT? Could increased physical activity levels avert the loss of fracture resistance associated with ageing in post-menopausal women? Asprey [60] and Adebajo [61] have studied respectively post-menopausal bone density and hip fractures in post-menopausal West Africans. In Asprey's study the subjects were peasant farmers; they appeared in this cross-sectional study to lose bone at rates which were if anything faster than British rates and, as could be in part at least explained by their lean-ness their BMD values were lower than typical for age-matched British women. Yet in the Gambia, in centres provided with good if basic hospital care, hip fracture was almost unknown while falls were frequent. In Adebajo's Nigerian study hip fracture was confirmed to be much less common than in Caucasians and there was an absence of either an effect of gender of an increase in risk with age [61]. The possibility exists that part at least of this contrast between Caucasians and black Africans may be attributable to genetic differences.

To investigate the effects of very high levels of physical activity after the menopause in Causasian women, we contrasted bone density values in women over 40, who were both pre and post menopausal, who were also running competitively and training for 15 or more hours a week. The premenopausal women had higher than average bone density in the hip and spine after adjusting for body size, whereas the post-menopausal women had bone density values which were merely appropriate for British sedentary post-menopausal women after adjusting for body size [62]. These data make it understandable why Asprey's Gambian women, who were highly physically active peasant farmers, had bone density values which were certainly no better than British values.

4.2. Conclusions

Oestrogens have many effects on the skeleton, such as limiting childhood growth through fusing of the epiphyses, increasing bone strength at puberty, protecting against osteoporosis in older subjects of both genders and probably reducing the risk of death by apoptosis in the bone stromal cell lineage (osteoblasts, osteocytes, lining cells and their precursors). It seems possible that the molecular mechanisms of actions of oestrogens on bone stromal cells may have parallels to those seen in the vascular system in particular, but this field is developing rapidly and many advances are expected in the near future. From a general biological perspective it is necessary that investigators interested in oestrogens' actions should be cognisant of the skeleton's primary function, which is to provide a rigid jointed structure against which the musculature can pull to induce movement. Other functions are to protect the internal organs and to provide a reserve of minerals such as calcium. In light of this it is vital that all bones are constructed so as to provide an adequate safety factor against breakage in the event of excessive and abnormal loads being applied. Nor should bone fracture through repeated loading, the so-called fatigue fracture. It is insufficiently recognised that as a composite material, bone tissue can absorb significant amounts of plastic deformation, but that this capacity (called toughness) declines with age, so that old bones become brittle. As investigators probe the molecular and cellular basis of oestrogens' actions on bone, they will equally study the effects, long and possibly short term, of oestrogens on the toughness of bone as a tissue as well as on the amounts of bone formed and maintained under their influence.

References

[1] D. Burr, M. Forwood, D. Fyhrie, R. Martin, M. Schaffler, C. Turner, Bone microdamage and skeletal fragility in osteoporotic and stress fractures, J. Bone Miner. Res. 12 (1997) 6–15.

[2] P. Zioupos, J.D. Currey, Changes in the stiffness, strength and toughness of human cortical bone with age, Bone 22 (1998) 57–66.

[3] A. Parfitt, Skeletal heterogeneity and the purposes of bone remodelling. Implications for the understanding of osteoporosis, in: R. Marcus, D. Feldman, J. Kelsey (Eds.), Osteoporosis, Academic Press, New York, 1996, pp. 315–329.

[4] S. Mori, D. Burr, Increased intracortical remodelling following fatigue damage, Bone 14 (1993) 103–109.

[5] F. Albright, P.H. Smith, A.M. Richardson, Postmenopausal osteoporosis: its clinical features, JAMA 116 (1941) 2465–2474.

[6] J.P. Bilezikian, A. Morishima, J. Bell, M.M. Grumbach, Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency, N. Engl. J. Med. 339 (1998) 599–603.

[7] E.P. Smith, J. Boyd, G.R. Frank, H. Takahashi, R.M. Cohen, B. Specker, T.C. Williams, D.B. Lubahn, K.S. Korach, Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man, N. Engl. J. Med. 331 (1994) 1056–1061.

[8] P. Minaire, P.J. Meunier, C. Edouard, J. Bernard, P. Courpron, J. Bourret, Quantitative histological data on disuse osteoporosis. Comparison with biological data, Calcif. Tissue Res. 17 (1974) 57–73.

[9] H.M. Frost, The mechanostat: a proposed pathogenetic mechanism of osteoporoses and the bone mass effects of mechanical and non-mechanical agents, Bone Miner. 2 (1987) 73–85.

[10] L. Lanyon, Why does a mechanism that successfully matches bone strength to bone loading before the menopause fail when oestrogen levels decline?, in: J.E. Compston (Ed.), Osteoporosis: new perspectives on causes, prevention and treatment, Royal College of Physicians of London, London, 1996, pp. 135–150.

[11] C. Cooper, G. Campion, L.J. Melton III, Hip fractures in the elderly: a world-wide projection, Osteoporos. Int. 2 (1992) 285–289.

[12] P. Lips, C. Cooper, D. Agnusdei, F. Caulin, P. Egger, O. Johnell, J.A. Kanis, S. Kellingray, A. Leplege, U.A. Liberman, E. McCloskey, H. Minne, J. Reeve, J.-Y. Reginster, M. Scholz, C Todd, M.-C. deVernejoul, I. Wiklund, Quality of Life in patients with vertebral osteoporosis. Validation of the quality of life questionnaire of the European Foundation for Osteoporosis (QUALEFFO), Osteoporos. Int. 10 (1999) 150–160.

[13] S.L. Silverman, M.E. Minshall, W. Shen, K.D. Harper, S. Xie, The inpact of incident vertebral fracture on health-related quality of life in established postmenopausal osteoporosis: results from the multiple outcomes of Raloxifene evaluation study, J. Bone Miner. Res. 14 (suppl 1) (1999) s159.

[14] A. Oleksik, W. Shen, A. Dawson, M. Minshal, P. Lips, The impact of incident vertebral fractures on health-related quality of life in women with prevalent vertebral fractures, J. Bone Miner. Res. 14 (Suppl 1) (1999) s262 abstract.

[15] D. Felsenberg, M. Lunt, G. Armbrecht, L. Benevolenskaya, A. Bhalla, J. Bruges Armas, J. Cannata, J. Dequeker, C. Fabian, B. Felsch, S. Havelka, K. Hoszowski, I. Jajic, O. Johnell, A. Lopes Vaz, G. Lyritis, P. Masaryk, T. Miazgowski, G. Parisi, H.A.P. Pols, G. Poor, A. Rapado, H. Raspe, D.M. Reid, W. Reisinger, C. Scheidt-Nave, C. Todd, C. Weber, A. Woolf, W. Gowin, C. Cooper, J.A. Kanis, T.W. O'Neil, A. Ismail, A.J. Silman, J. Reeve, Rates and determinants of vertebral fracture incidence in European men and women, J. Bone Miner. Res. 14 (Suppl 1) (1999) s159 errata (abstract).

[16] L.J. Melton III, A.W. Lane, C. Cooper, R. Eastell, W.M. O'Fallon, B.L. Riggs, Prevalence and incidence of vertebral deformities, Osteoporos. Int. 3 (1993) 113–119.

[17] P.D. Ross, J.N. Davis, R.S. Epstein, R.D. Wasnich, Pre-existing fractures and bone mass predict vertebral fracture incidence in women, Ann. Intern. Med. 114 (1991) 919–923.

[18] P. Ross, H. Genant, J. Davis, P. Miller, R. Wasnich, Predicting vertebral fracture incidence from prevalent fractures and bone density among non-black, osteoporotic women, Osteoporos. Int. 3 (1993) 120–126.

[19] M. Lunt, D. Felsenberg, G. Armbrecht, A. Aroso, K. Abendroth, D. Banzer, L. Benevolenskaya, A. Bhalla, J. Bruges Armas, J. Cannata, J. Dequeker, M. Diaz Curiel, S. Grazio, O. Johnell, C. Kiss, R.P.M. Lorenc, T. Miazgowski, H. Pols, H. Raspe, D.M. Reid, W. Reisinger, C. Scheidt-Nave, V. Scoutellas, E. McCloskey, C. Cooper, T.W. O'Neill, A.J. Silman, J. Reeve, Incident vertebral fractures: determinants of size and location, J. Bone Miner. Res. 14 (Suppl 1) (1999) s259 errata (abstract).

[20] A. Silman, T. O'Neill, C. Cooper, J. Kanis, D. Felsenberg, the EVOS Study Group. Influence of physical activity on vertebral deformity in men and women: results from the European Vertebral Osteoporosis Study. Journal of Bone and Mineral Research (1997), 12, 813–819.

[21] T.W. O'Neill, A.J. Silman, M. Naves Diaz, C. Cooper, J. Kanis, D. Felsenberg, et al., the European Vertebral Osteoporosis Study Group. Influence of Hormonal and Reproductive Factors on the Risk of Vertebral Deformity in European Women, Osteoporos. Int. 7 (1997) 72–78.

[22] P. Masaryk, M. Lunt, J. Dequeker, D. Felsenberg, C. Scheidt-Nave, G. Poor, H. Pols, J. Falch, D. Reid, L. Benevolenskaya, K. Weber, J. Cannata, C. Dodenhof, T. O'Neill, A. Silman, J. Reeve, Influences of historical and current lifestyle and dietary factors on BMD in men and women in the EVOS study, Journal of Bone and Mineral Research. 12 (Suppl. 1) (1997) s485.

[23] O. Johnell, T. O'Neill, D. Felsenberg, J. Kanis, C. Cooper, A. Silman and the EVOS Study Group. Anthropometric measurements and vertebral deformities. American Journal of Epidemiology (1997), 146, 287–293.

[24] M. Lunt, D. Felsenberg, J. Reeve, L. Benevolenskaya, J. Cannata, J. Dequeker, C. Dodenhof, J. Falch, P. Masaryk, H. Pols, G. Poor, D. Reid, C. Scheidt-Nave, K. Weber, J. Varlow, J. Kanis, T. O'Neill, A. Silman, Bone density variation and its effects on risk of vertebral deformity in men and women studied in 13 European centres: the EVOS study, J. Bone Miner. Res. 12 (1997) 1883–1894.

[25] J. Reeve, J. Walton, L.J. Russell, M. Lunt, R. Wolman, R. Abraham, J. Justice, A. Nicholls, B. Wardley-Smith, J.R. Green, A. Mitchell, Determinants of the first decade of bone loss after menopause at spine, hip and radius: the Harrow postmenopausal bone loss study, QJM 92 (1999) 261–273.

[26] F. Tremollieres, J.M. Pouilles, C. Ribot, Vertebral postmenopausal bone loss is reduced in overweight women, J. Clin. Endocrinol. Metab. 77 (1993) 683–686.

[27] S.R. Cummings, M.C. Nevitt, W.S. Browner, K. Stone, K.M. Fox, K.E. Ensrud, J. Cauley, D. Black, T.M. Vogt, for the Study of Osteoporotic Fractures Research Group. Risk factors for hip fracture in white women, N. Engl. J. Med. 332 (1995) 767–773.

[28] R.G. Cumming, M.C. Nevitt, S.R. Cummings, Epidemiology of hip fractures, Epidemiol. Rev. 19 (1997) 244–257.

[29] M.C. Chapuy, M.E. Arlot, P.D. Delmas, P.J. Meunier, Effect of calcium and cholecalciferol treatment for 3 years on hip fractures in elderly women, BMJ 308 (1994) 1081–1082.

[30] S.R. Cummings, W.S. Browner, D. Bauer, K. Stone, K. Ensrud, S. Jamal, B. Ettinger, Endogenous hormones and the risk of hip and vertebral fractures among older women, N. Engl. J. Med. 339 (1998) 733–738.

[31] N. Crabtree, J. Wright, A. Walgrove, J. Rae, L. Hanratty, M. Lunt, I. Fogelman, R. Palmer, M. Vickers, J.E. Compston, J. Reeve. Vertebral morphometry: repeat scan precision using the Lunar Expert-XL and the Hologic 4500A. A study for the

'WISDOM' RCT of Hormone Replacement Therapy. Osteoporos. Int. 11 (2000) 537–543.
[32] B. Ettinger, D.M. Black, B.H. Mitlak, R.K. Knickerbocker, T. Nickelsen, H.K. Genant, C. Christiansen, P.D. Delmas, J.R. Zanchetta, J. Stakkestad, C.C. Glüer, K. Krueger, F.J. Cohen, S. Eckert, K.E. Ensrud, L.V. Avioli, P. Lips, S.R. Cummings, Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene, JAMA 282 (1999) 637–645.
[33] S. Cummings, D. Black, T. Vogt, For the FIT Research Group. Changes in BMD substantially underestimate the anti-fracture Effects of Alendronate and other antiresorptive Drugs, J. Bone Miner. Res. 11 (1996) S102.
[34] B.L. Riggs, T.C. Spelsberg, Mechanisms of estrogen action on bone cells, in: S.E. Papapoulos, P. Lips, H.A.P. Pols, C.C. Johnston, P.D. Delmas (Eds.), Osteoporosis'96, Elsevier, Amsterdam, 1996, pp. 241–250.
[35] J.E. Compston, K. Yamaguchi, P.I. Croucher, N.J. Garrahan, P.C. Lindsay, R.W. Shaw, The effects of gonadotrophin-releasing hormone agonists on iliac crest cancellous bone structure in women with endometriosis, Bone 16 (1995) 261–268.
[36] A. Tomkinson, J. Reeve, R.W. Shaw, B.S. Noble, The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone, J. Clin. Endocrinol. Metab. 82 (1997) 3128–3135.
[37] A. Tomkinson, E. Gevers, J.M. Witt, J. Reeve, B.S. Noble, The role of oestrogen in the control of rat osteocyte apoptosis, J. Bone Miner. Res. 13 (1998) 1243–1250.
[38] B.S. Noble, H. Stevens, J.R. Mosley, A.A. Pitsillides, J. Reeve, L. Lanyon, Bone loading changes the number and distribution of apoptotic osteocytes in cortical bone, J. Bone Miner. Res. 12 (Suppl 1) (1997) s111 abstract.
[39] B.S. Noble, H.Y. Stevens, N.M. Peet, G. Reilly, J.D. Currey, T.M. Skerry, Matrix microdamage and osteocyte apoptosis: a mechanism for targeting of bone resorption, Bone 23 (Suppl 5) (1998) s179 abstract.
[40] B.S. Noble, J. Reeve, Osteocyte function, osteocyte death and bone fracture resistance, Mol. Cell. Endocrinol. 159 (2000) 7–13.
[41] O. Vidal, L.-G. Kindblom, C. Ohlsson, Expression and localisation of estrogen receptor-β in murine and human bone, J. Bone Miner. Res. 14 (1999) 923–929.
[42] K. Terai, T. Takano-Yamamoto, Y. Ohba, K. Hiura, M. Sugimoto, M. Sato, H. Kawahata, N. Inaguma, Y. Kitamura, S. Nomura, Role of osteopontin in bone remodelling caused by mechanical stress, J. Bone Miner. Res. 14 (1999) 839–849.
[43] T.M. Skerry, L. Bitensky, J. Chayen, L.E. Lanyon, Early strain-related changes in enzyme activity in osteocytes following bone loading in vivo, J. Bone Miner. Res. 4 (1989) 783–788.
[44] S.C.F. Rawlinson, J.R. Mosley, R.F.L. Suswillo, A.A. Pitsillides, L.E. Lanyon, Calvarial and limb bone cells in organ and monolayer culture do not show the same early responses to dynamic mechanical strain, J. Bone Miner. Res. 10 (1995) 1225–1232.
[45] A.A. Pitsillides, S.C.F. Rawlinson, R.F.L. Suswillo, S. Bourrin, G. Zaman, L.E. Lanyon, Mechanical strain-induced NO production by bone cells: a possible role in adaptive bone (re)modelling, FASEB J. 9 (1995) 1614–1622.
[46] W.S. Simonet, D.L. Lacey, C.R. Dunstan, M. Kelley, M.S. Chang, R. Luthy, H.Q. Nguyen, S. Wooden, L. Bennett, T. Boone, G. Shimamoto, M. DeRose, R. Elliott, A. Colombero, H.L. Tan, G. Trail, J. Sullivan, E. Davy, N. Bucay, L. Renshaw Gegg, T.M. Hughes, D. Hill, W. Pattison, P. Campbell, W.J. Boyle, et al., Osteoprotegerin: a novel secreted protein involved in the regulation of bone density, Cell 89 (1997) 309–319.
[47] L.C. Hofbauer, S. Khosla, C.R. Dunstan, T.C. Spelsberg, B.L. Riggs, Estrogen stimulates production of the anti-resorptive cytokine receptor osteoprotegerin in human osteoblastic cells, Bone 23 (1998) s172 abstract.
[48] B.R. Wong, J. Rho, J. Arron, E. Robinson, J. Orlinick, M. Chao, S. Kalachikov, E. Cayan, F.S. Bartlett, W.N. Frankel, S.Y. Lee, Y. Choi, TRANCE is a novel ligand of the tumour necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells, J. Biol. Chem. 272 (1997) 25190–25194.
[49] H. Yasuda, N. Shima, N. Nakagawa, K. Yamaguchi, M. Kinosaki, S. Mochizuki, A. Tomoyasu, K. Yano, M. Goto, A. Murakami, E. Tsuda, T. Morinaga, K. Higashio, N. Udagawa, N. Takahashi, T. Suda, Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL, Proc. Natl. Acad. Sci. USA 95 (1998) 3597–3602.
[50] M.V.J. Hukkanen, L.A.M. Platts, I. Fernandez de Marticorena, M. O'Shaughnessy, I. MacIntyre, J.M. Polak, Developmental regulation of nitric oxide synthase expression in rat skeletal bone, J. Bone Miner. Res. 14 (1999) 868–877.
[51] C. DeLaet, B. can Hout, H. Burger, A. Hofman, H. Pols, Bone Density and risk of hip fracture in men and women: cross-sectional analysis, BMJ 315 (1997) 221–225.
[52] K.S. Jensen, L. Mosekilde, L. Mosekilde, A model of vertebral trabecular bone architecture and its mechanical properties, Bone 11 (1990) 417–423.
[53] K. Bell, N. Loveridge, J. Power, N. Garrahan, M. Lunt, B. Meggitt, J. Reeve, Structure of the femoral neck in hip fracture: cortical bone loss in the infero-anterior to supero-posterior axis, J. Bone Miner. Res. 14 (1999) 111–119.
[54] K.L. Bell, N. Loveridge, J. Power, M. Stanton, B.F. Meggitt, J. Reeve, Regional differences in cortical porosity in the fractured femoral neck, Bone 24 (1999) 57–64.
[55] J.C. Lotz, E.J. Cheal, W.C. Hayes, Stress distributions within the proximal femur during gait and falls: implications for osteoporotic fracture, Osteoporos. Int. 5 (1995) 252–261.
[56] K.L. Bell, N. Loveridge, J. Power, N. Rushton, J. Reeve, Intracapsular hip fracture: increased cortical remodelling in the thinned and porous anterior region of the femoral neck, Osteoporos. Int. 10 (1999) 248–257.
[57] G. Jordan, N. Loveridge, K.L. Bell, J. Power, N. Rushton, J. Reeve, Spatial clustering of osteonal remodelling: a cause of focal weakness in the femoral neck cortex in hip fracture, Bone 26 (2000) 305–313.
[58] K.L. Bell, N. Loveridge, G.R. Jordan, J. Power, C.R. Constant, J. Reeve, A novel mechanism for the induction of the increased cortical porosity in cases of intra-capsular hip fracture, Bone 27 (2000) 297–304.
[59] J. Power, B.S. Noble, N. Loveridge, N. Rushton, J. Reeve, Osteocyte viability in the femoral neck cortex, Calcif. Tissue Int. 64 (suppl 1) (1999) s60 abstract.
[60] T.J. Aspray, A. Prentice, T. Cole, Y. Sawo, J. Reeve, R.M. Francis, Low bone mineral content is common but osteoporotic fractures are rare in elderly rural gambian women, J. Bone Miner. Res. 11 (1996) 1019–1025.
[61] A.O. Adebajo, C. Cooper, J.G. Evans, Fractures of the hip and distal forearm in West Africa and the United Kingdom, Age Ageing 20 (1991) 435–438.
[62] J.H. Gibson, M. Harries, A. Mitchell, R. Godfrey, M. Lunt, J. Reeve, Determinants of bone density and prevalence of osteopenia among female runners in their second to seventh decades of age, Bone 26 (2000) 591–598.