For Debate . . .

Bone turnover and trabecular plate survival after artificial menopause

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Abstract

Considerable bone loss often occurs after menopause, particularly if menopause is induced by surgery. For perhaps two years bone formation fails to keep pace with the rapid acceleration of bone resorption that occurs after sex hormone withdrawal. The threat that this poses to the integrity of the skeleton is not clear. Because ethical constraints limit histological studies in normal women existing normal data and statistical modelling techniques were used to explore the dynamics of iliac trabecular bone after menopause. Trabeculae are breached during remodelling when the osteoclasts resorb to a depth equal to the trabecular thickness. Since holes in trabecular plates cannot normally be bridged such defects are probably permanent. Men lose 7% of their vertebral trabecular bone every 10 years; deeper than average resorption of trabeculae at the thin end of the normal range would account for it. The dramatic losses of trabecular bone that are seen in some postmenopausal women, however, require a period of imbalance between bone formation and bone resorption since this leads rapidly to generalised thinning. The statistical model suggested that an imbalance lasting only two years may account for eventual losses of up to half of the iliac trabecular bone. Further understanding is needed of what determines the amount of bone lost in the immediate postmenopause, which varies considerably among women.

A simple means is needed of identifying women who will lose bone most rapidly at the menopause. This must be suitable for

use in general practice because these women should probably be offered long term hormone replacement treatment within a few months of the last menstruation.

Introduction

In women bone loss accelerates at the menopause, which was predicted by Albright *et al* in 1941.¹ Evidence from kinetic² and biochemical^{3,4} studies has shown that resorption first increases then returns slowly to premenopausal levels.⁴ By the time crush fractures occur 10 years or so later bone formation rates are frequently reduced,^{5,7} and the size of individual "packets" of new bone in iliac trabeculae is also low.⁸

Patients who undergo premature or surgical menopause have an increased susceptibility to crush fractures.^{9,10} The results of a large cross sectional study by Stepan *et al*⁶ of women who had ovariectomies showed an immediate rise in indices of bone resorption at castration. Interestingly, an index of bone formation—the plasma activity of bone specific alkaline phosphatase—did not rise rapidly at ovariectomy as would be predicted from the coupling that is known to occur between normal bone resorption and formation.¹¹

Might the one to two years of skeletal imbalance which these results predict lead to an appreciably weakened skeleton? As Wakamatsu and Sissons¹² and Parfitt *et al*¹³ have shown, in women with osteoporosis, as in normal women, bone is lost mainly by complete trabecular plates being removed rather than by the progressive thinning of trabeculae in proportion to the amount of bone lost. The loss of a trabecula must result from the full thickness perforation or fenestration of a trabecular plate by osteoclasts destroying a full trabecular thickness, either by resorption occurring simultaneously on both surfaces of the plate or by deeper than average resorption occurring on a thinner than average trabecula (fig 1).¹³

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Basic multicellular unit of bone

Bone renewal is organised by cooperating groups of cells called "basic multicellular units."¹¹ Each of these units is somewhat analogous to a nephron, and the units function semi-independently. There are 10^5 - 10^6 of these units in the normal skeleton, each consisting of osteoclasts, osteoblasts, and a less well defined population of mononuclear cells. Bone turnover is initiated by osteoclasts, probably prompted by a signal from the lining osteocytes on trabecular bone surfaces. After resorption the osteoclasts disperse and are replaced by mononuclear cells including preosteoblasts (the reversal phase). About six weeks after resorption starts bone formation begins with the development of fully mature osteoblasts. Finally, after the defect in the bone has been filled in many of the osteoblasts become transformed into resting osteocytes, lying in bony lacunae or forming a lining separating the bone surface from the vasculature (lining cells). The whole renewal process takes on average four months in normal human bone and the "packet" of new bone formed is called a basic structural unit.

FIG 1—Three outcomes of bone resorption. (a) Normal resorption to less than half the trabecular thickness, followed by bone formation replacing the bone removed. (b) Fenestration owing to deeper than average resorption on a thinner than average trabecula. (c) Simultaneous remodelling on both surfaces, leading to the same result.

Over the past 25 years many data have accumulated on the statistical distributions of various static and dynamic measurements related to basic multicellular units made on iliac trabecular bone. This prompted the development of a statistical model of the dynamics of individual trabeculae, so that hypothetical trabeculae could be followed through their life history by computer simulation.¹⁴ The normal distributions of trabecular widths,¹² trabecular spacings,¹² thicknesses of completed basic structural units,¹⁵ thicknesses of interstitial bone between structural units,¹⁵ and resorption depths¹⁶ have been directly or indirectly measured, as have indices of effective osteoblast lifespan determined from in vivo tetracycline labelling studies.^{5,8} Incorporating these data into the model has made it possible to examine the effects of various modifications to the dynamics of the basic multicellular unit, such as those which occur at the menopause. This approach was attractive because it was ethically unacceptable to subject large numbers of normal women to bone biopsy before and after menopause to test the effect of hormone withdrawal directly.

THE MODEL

This type of model is called stochastic because it allows for random events to affect the outcome of a modelling simulation. Typically, 100 trabecular "thicknesses" were followed over 20 years. Each initial value was drawn randomly from the appropriate normal distribution (mean (SD) 103 (31.5) μ m). An individual thickness was examined daily; when both surfaces were quiescent each was assumed to have a 1:900 chance of initiating a remodelling cycle. If initiation did occur the evolution of the resulting basic multicellular unit on that surface was followed daily over the three to six months until the cycle was complete. During a basic multicellular unit cycle 12 days' resorption was followed by 27 days of "reversal" and then a longer formative phase (duration 94 (35) days, including resting periods). Unit balance resulted when mean resorption depth was 36.8 (9.2) μ m. Because of the need to fit the observational data and in line with current evidence a positive relation between mechanical strain (which for a given load must affect thin trabeculae more than thick trabeculae) and basic structural unit thickness was incorporated into the model.¹⁴

During the resorptive phase of a basic multicellular unit a fairly rapid thinning occurred, which was followed by a much slower recovery. When this process was completed the trabecula took on a new thickness that might be greater or less than before, depending in part on random fluctuations in the depth of resorption and the lifespan of osteoblasts. Overlap has been found between the distributions of trabecular thicknesses and resorption depths, so occasionally resorption on one surface or both surfaces simultaneously may lead to trabecular fenestration.¹³ In the presence of basic multicellular unit imbalance trabeculae could become thinner and therefore more vulnerable to subsequent fenestration by either mechanism.

An annual census of the fate of the 100 thicknesses studied during each

simulation was carried out. Those that were lost were noted, and the percentage change in trabecular bone volume was calculated from the ratio of the current to the original sums of trabecular thicknesses. Basic structural unit thicknesses were noted at their completion and included in the annual survey of thickness data until replaced on their trabecular surface by a new structural unit. Mean interstitial bone thickness was calculated as mean trabecular thickness minus twice the mean basic structural unit thickness.¹⁷

To determine the effect of the artificial menopause on bone resorption it was necessary to relate urinary hydroxyproline excretion, as determined by Stepan *et al*,⁴ to more direct measurements of bone resorption. We used the linear regression relation obtained in our recent study of hydroxyproline excretion and kinetically determined¹⁸ bone resorption rates in women with osteoporosis¹⁹ to convert Stepan's biochemical data into resorption rates.

Results

Figure 2 shows the calculated mean rates of whole body bone resorption derived from Stepan's data. A doubling of bone resorption seems to occur rapidly after ovariectomy. The rate of return to premenopausal levels is relatively slow and is approximated by an exponential fall with a half life of about five years.

FIG 2—Increments in whole body bone resorption rate after castration, derived from the data of Stepan *et al*,⁴ and evolving with time after operation. Also shown is the transient mismatch between formation and resorption.⁴ The normal rate of whole body bone formation and resorption in premenopausal women is approximately 2 mmol Ca/day, using the methods referred to in the text.

Figure 2 also shows the relative delay in the osteoblastic response implied by the bone specific alkaline phosphatase data. This effect can be approximated as an exponential delay which has a half life of 15 months.

Those transients were simulated with the model (figs 3 and 4). After initial trabecular thinning there were considerable losses of trabeculae. Though loss of trabecular bone volume was apparently permanent, mean trabecular thickness, mean basic structural unit wall thickness, and mean interstitial bone thickness recovered towards normal by 16 years after the menopause.

Discussion

The work of Stepan *et al* suggested that an artificial menopause leads to a short period only of imbalance between bone resorption and bone formation.⁴ It also confirmed previous work that had suggested that at the menopause osteoblastic activity of the whole skeleton gradually rises to almost match osteoclastic activity,² whatever the role of reduced osteoblastic activity in the later evolution of the crush fracture syndrome.^{6,8}

The results of this study show two things. Firstly, the seeds of destruction of the mechanical integrity of axial trabecular bone can be sown in a remarkably short period after the menopause, even

though vertebral collapse may not occur for 10 years. Secondly, the length of the delay in the osteoblastic response to the osteoclast challenge may be crucial in determining whether a woman who suddenly loses ovarian function will develop clinical osteoporosis.

In a series of studies on rate of perimenopausal bone loss using quantitative computed tomography of the vertebrae Ettinger *et al* and Genant *et al* have drawn attention to the speed with which spinal trabecular bone may be lost after a natural²⁰ or, especially, an artificial menopause.²¹ These rates, at 5% and 9% a year respectively, are seven to 15 times faster than those in men, who lose only 7% of spinal trabecular bone in 10 years. These results have been mirrored in studies using dual photon absorptiometry,²²⁻²⁵ although this technique evaluates cortical as well as trabecular bone unsurprisingly the absorptiometry studies showed slower rates of loss than the quantitative computed tomography studies.

FIG 3—Evolution of four trabecular plate thicknesses simulated by the model before and after simulated ovariectomy. The transient thinning induced by normal remodelling will be noted. The frequency of basic multicellular unit activation increases after operation, and the trabeculae tend to become thinner, owing to the transient mismatch between bone resorption and bone formation. This led to permanent loss in the second trabecular plate while it was in a relatively thin state because of coincident remodelling on both surfaces. The third trabecular plate became thin and suffered the same fate owing to the development of a particularly deep resorption pit. Note the effects of statistical variability in the amounts of bone resorbed and formed at individual basic multicellular units. This variability was found to be essential¹⁴ to successful simulation of published¹² observational data on normal subjects of various ages.

This analysis encourages further clinical and laboratory studies on the transient dissociation between rate of bone formation and bone resorption that may occur with sex hormone withdrawal. This dissociation may hold the key to the substantial loss of trabecular bone suffered by many women at menopause. The magnitude of the changes in trabecular bone indices in response to a fairly short perturbation brings into focus the vulnerability of bone to disturbances in basic multicellular unit function, Figure 4, however, also shows the difficulties of relying on data that were obtained 10 years or more after menopause to interpret earlier events. Evidence for a transient menopausal imbalance between formation and resorption of bone at the basic multicellular unit level will be lost 10 years later if a bone biopsy taken at the time of crush fracture is examined for evidence of trabecular and basic structural unit thinning.

Three factors may determine who will suffer fractures: peak bone mass at maturity,²⁶ the rate and duration of the postmenopausal loss considered here, and the apparent failure of osteoblastic bone formation which may occur later.²⁸ The second holds the best

FIG 4—The effects of the transient imbalance between formation and resorption derived from the data of Stepan *et al*⁸ on the evolution of 100 trabecular thicknesses simulated for 20 years from five years before ovariectomy. Also shown are the contrasting effects of a simple increase in activation frequency.

hope of selective prevention with oestrogens, and strategies for identifying the perimenopausal woman at risk, such as that tentatively proposed by Christiansen *et al*,²⁷ deserve urgent investigation.

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Lesson of the Week

Vitamin and energy injections in the Indian subcontinent

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Though it is now common for general practitioners to request immunoglobulin for protection against hepatitis A for travellers visiting the Indian subcontinent, probably more should be done to protect patients against possible exposure to hepatitis B. This is a particular problem with Asian patients who go abroad for extended periods and are therefore more likely to require medical attention while there. Twice during the past year we have diagnosed acute hepatitis B in young women who had received injections while in Pakistan.

Case histories

Case 1—A 26 year old Asian woman normally resident in Britain visited Pakistan for six weeks and returned five weeks before admission. While in Pakistan she was reported to have had an "energy injection." Her two children were in hospital in Birmingham being treated for typhoid when she was taken ill with abdominal pain and fever and admitted with suspected typhoid. She was 16 weeks pregnant. Liver function tests yielded a serum aspartate transaminase activity of 4300 IU/l with a bilirubin concentration of 46 $\mu\text{mol/l}$, and hepatitis B surface antigen was present to a titre of $>1/8000$ by reverse passive haemagglutination (Hepatest, Wellcome Laboratories). Four days after admission to this hospital she was transferred to King's College Hospital, London, with a diagnosis of fulminant hepatic failure due to infection with hepatitis B virus, and she died four days later.

Case 2—A 20 year old Asian woman normally resident in Britain until her marriage in Pakistan in 1984 returned from a visit lasting 12 months in December 1986. During her stay she had suffered persistent diarrhoea which had resulted in weight loss for which she had had several "vitamin" injections from local doctors. She was admitted less than a month after her return with diarrhoea, vomiting, anorexia, and jaundice. On admission her serum aspartate transaminase activity was 1545 IU/l and bilirubin concentration 152 $\mu\text{mol/l}$. Hepatitis B surface antigen was present to a titre of 1/32 (Hepatest, Wellcome). She recovered well and was discharged two weeks later for outpatient follow up.

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Patients should be warned about the infectious risks of "traditional medicines" given by injection while abroad

Discussion

Both patients had IgM class antibody to hepatitis B core antigen, indicating a diagnosis of acute hepatitis B acquired within the previous two to six months. The most likely source of infection in both patients was the injections that they had received. Energy and vitamin injections are presumably not essential to life. Though the local population and family of the visitors may speak highly of such injections, these people have the advantage of probably being naturally immune to hepatitis B through previous exposure. The risk is probably not in the vitamin preparation itself but in the use of inadequately sterilised equipment, especially needles.

Hepatitis is not uncommon in Asians returning from the Indian subcontinent. In a study from East Birmingham Hospital of 50 consecutive cases of acute hepatitis, 16 were in Asians returning from the Indian subcontinent.¹ Three of these were diagnosed as hepatitis B, five were hepatitis A, and eight were non-A non-B.

Cases of hepatitis B such as those described here are avoidable. General practitioners should be especially vigilant in warning their patients of the dangers of accepting traditional medicines given by injection while they are abroad. The risks of infection with the human immunodeficiency virus by the same route may serve to emphasise this message more strongly.

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