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ORIGINAL ARTICLE

Dietary determinants of post-menopausal bone loss at the lumbar spine: a possible beneficial effect of iron

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Abstract *Introduction:* Previous studies suggesting different effects of diet on post-menopausal bone loss may have given conflicting results because they sometimes failed to exclude confounding conditions or used imprecise methodology. *Design:* To identify dietary determinants of bone loss from the lumbar spine after menopause in women not taking hormone replacement who developed no evidence of spondylotic or sclerotic degenerative disease, forty-three women were followed with repeated (mean = 12) measurements of bone mineral density (BMD) at L2–4 for 11–14 years. Eleven developed evidence suggestive of degenerative disease and were excluded. Diet was assessed at the beginning of the study and 2.5 years later using 3-day and 7-day periods of weighed intakes. Nutrients estimated were: carbohydrate, fat, protein, fibre, calcium, magnesium, iron, phosphorus, copper, zinc and six vitamins. We tested the ability of diet to predict post-menopausal bone loss using stepwise regression. *Results:* Each woman's BMD change was described by a single coefficient after log transformation of the BMD data. The best model for BMD loss including dietary factors alone had two significant determinants: daily energy or protein (p=0.0003) intake was adverse, while dietary iron (p=0.002) was predictive of bone maintenance, an effect that persisted if iron was expressed as a ratio to energy intake. Adding body mass index to the model increased the goodness of fit (R^2adj rose from 0.33 to 0.42) without affecting the statistical significance of the dietary determinants. *Conclusions:* Diet may influence bone loss after menopause, with dietary iron (or an associated factor) possibly having a protective effect on bone at the spine.

Keywords Bone loss · Dietary iron · Dual X-ray absorptiometry · Menopause · Nutrition · Osteoporosis

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Introduction

It is well established that women not taking hormone replacement therapy (HRT) lose bone from the spine after the menopause at rates that are several-fold faster than those seen in men of the same age or in women before menopause. Furthermore, after age 50, rates of vertebral fracture increase faster in women than in men of the same age [1]. This difference in fracture rates can be largely attributed to the faster rate of decline in spinal bone mineral density (BMD) in women than men [2]. The subject of differential bone loss has once again become a major public health issue since publication of the results of the Women's Health Initiative randomised trials of HRT and the reduction in HRT use that rapidly followed.

There has been much interest in the possibility of preventing osteoporotic fractures by reducing post-menopausal bone loss. Future fracture risk is in part dependent on pre-menopausal or peak bone density. However, as a woman grows older, the risk of fracture becomes increasingly dependent on the rate of bone loss subsequent to menopause. Black [3] has calculated that by age 70 each of these components contributes about one-half of the between-individual variance in fracture risk. Hence, interest has centred on developing methods for predicting rates of bone loss during the two decades following menopause. It would be an ideal solution if reduced bone loss

might be achievable in this period of a woman's life by means of a safe modification of the diet and/or lifestyle.

While there have been a number of long-term prospective studies of forearm bone density in peri- [4] and post-menopausal women [5, 6], the technique of dual photon absorptiometry, which became available more than one decade after that of single photon absorptiometry, has made it possible to make precise spinal measurements [7]. Thus, it is only now becoming possible to analyse results from long-term DXA cohort studies on the spine and hip lasting more than a decade. The Harrow post-menopausal bone loss study was begun in 1984 with the aim of documenting, in a community setting, rates of spinal bone loss in normal women beginning within 3 years of the menopause [8]. Because rates of bone loss after the menopause attenuate quite rapidly, with women more than 5 years after the menopause losing bone less quickly than women within 2 years of the menopause [8, 9], we related bone loss to years since menopause rather than chronological age.

We have recently published descriptive data on spinal bone loss rates over the first 11–14 post-menopausal years (referred to as rates of BMD change in this paper) in the same representative population sample [10]. Among various measures of muscle strength, endurance, body dimensions and composition, we showed that a significant statistical determinant of low rates of bone loss from the lumbar spine was Body Mass Index (BMI: weight/height^2). A second but not independent determinant was the cross-sectional area of the psoas muscles at the level of the third lumbar vertebra. The psoas muscles are the principal flexors of the femur on the trunk [10]. It seemed likely, therefore, that either spinal loss was faster in more asthenic individuals (due to lower body fat, less body muscle, or both) or that larger (and presumably therefore stronger) muscles acting on the lumbar spine might protect it to some extent from post-menopausal bone loss. However, a considerable proportion of the inter-individual variability in spinal loss rates remained unaccounted for, even when a measurement of imprecision was taken into account.

At this study's inception we were interested in measuring the dietary determinants of bone loss from the spine. Initially, and again some 2.5 years later, we asked all participants to co-operate in weighing and recording all food and fluid intake. Based on weighed food intakes for 6- to 10-day time spans, we calculated nutrient intakes, including those micro-nutrients for which there was evidence of an influence on the biological pathway(s) responsible for post-menopausal bone loss. In a previous report [11], we demonstrated that dietary intakes of calcium were relatively stable over 2.5 years.

The purpose of this paper is to report on the dietary and other determinants of spinal bone loss in those women who did not take HRT and who showed no evidence of developing degenerative disease of the vertebrae (leading to localised increases in mineralisation). Our long-term aim is to develop hypotheses concerning possible dietary modifications after menopause that might be tested for their ability to reduce bone loss and hence risk of fractures due to post-menopausal osteoporosis.

Methods

Subjects

Women between 46 and 55 years of age from four primary care medical practices who had not had a hysterectomy were requested to participate in a study on the loss of spinal bone after the menopause. Women who had a history of malignancy were not interviewed, and the remainder were asked about the date of their last menstrual period. Those giving written informed consent (as required by the Harrow District Ethical Committee) and who were between 9 and 36 months of their last menstrual period and in good general health were assessed by additional cytology of their vaginal cells at the same time as a routine cervical smear [12]. All those women showing para-basal cells, which are a marker of diminished ovarian hormone stimulation, and a random 50% of those not showing para-basal cells were invited to join the study. Sixty-four accepted, giving an 80% response rate. Over the ensuing years up until their final bone density measurement, 17 received hormone replacement therapy for 3 months or more at some stage. None had cervical cancer. This left 47 potentially available for study.

Weighed diet intakes

The women were instructed on the use of a portable digital scale (Soehnle) which is accurate to within 1 g and were asked to weigh all food and fluid intake during the subsequent Thursday, Friday and Saturday. Food eaten outside the home was recorded in household measures and with the aid of the portion sizes in the diagrams illustrated in the weighed intake record booklet. Supervision was done by telephone and home visits, and the records were checked with each subject. Nutrient intake was analysed [13] using a computerised data entry system recording weights and categories of food consumed, based on Paul and Southgate's tables of food composition [13–15] extended to include foods not already on the computer database. To these intakes were added those arising from dietary supplements, calculated on the basis of the manufacturers' data. No analysis of food groups was undertaken, although the subjects' records (except for their reported consumption of dietary supplements) have been retained. The nutrients analysed included carbohydrates, protein, fat as well as energy (kcal/day). Among the micro-nutrients, we analysed those which could be estimated and which were considered as having the potential to influence bone metabolism: calcium; iron, copper, magnesium, phosphorus and zinc (in part as markers for total or animal protein and therefore acid ash consumption); total vitamin A; vitamin C (essential for collagen cross-linking), vitamin D; fibre (associated with phyto-oestrogens); a group of B vitamins which are essential co-factors in certain energy-utilising processes (thiamine, riboflavin and niacin). Nutrient intakes were also adjusted for daily calorie intakes.

Weighed dietary intakes were carried out at baseline for 3 days and again at 2.5 years after recruitment. When asked to weigh their food on the second occasion for 7 days, 27 women accepted; the remaining 33 weighed their food intake for 3 days. The days chosen for the 3-day weighings were Thursday, Friday and Saturday, and as far as possible weighing was undertaken in a continuous run of consecutive days.

Bone densitometry

All measurements were made and analysed by AM or BW-S who worked together. Six-monthly measurements of the lumbar spine over the first 2 years with further measurements at 3.5 years and in all but 15 women at 5 years were made using the BMC Lab 22a dual photon absorptiometer (DPA; Nova Industries, Bagsvaerd, Denmark) [7]. Measurements at 3.5 years were also made on most subjects, and 5-year measurements were made on all subjects using a Hologic QDR-1000W, which had replaced the Novo BMC Lab 22a. All duplicate measurements on the Hologic and on the Novo at the 3.5- and 5-year time-points were made on the same day. Spine density data from the Novo and Hologic densitometers were expressed in grams per square centimetre. At the time of transfer from the Novo to the Hologic, individual conversion factors were calculated from the ratios of the paired measurements to allow an individual's Hologic data as well as her Novo data to be expressed in grams per square centimetre (Hologic units). A previous investigation which included a Bland and Altman plot and a measurement of the ratio of imprecisions for the Novo and Hologic techniques confirmed that the Novo and Hologic data could be validly combined for the purpose of individual longitudinal analyses of the bone density data – after application of these conversion factors – and has been published as an appendix to a previous paper [10]. There were subsequently two further changes of Hologic machine, the effects of which were minimised by physical adjustment of each new machine to match the exact performance of the old when measuring the Hologic spine and European Spine phantoms (HSP and ESP, respectively) followed by regular quality assurance by daily phantom measurements of the HSP and periodic checks of the ESP to ensure consistency and stability.

The images from the bone density scans (Hologic) were reviewed as individual series. In four cases there was unequivocal evidence of degenerative spondylopathy, with patchy increases in bone density, irregularity of vertebral body outline and loss of intervertebral disc spaces. These subjects were excluded. Another exclusion was a woman with severe adolescent scoliosis. Six others showed significantly ($p<0.05$) dissimilar rates of *BMD change* when the rates of *change* in the vertebrae L2, L3 and L4 were compared using multiple analysis of variance (MANOVA) with at least one vertebra showing a trend reversal towards bone gain. Since the purpose of the study was to identify consistent determinants of lumbar spine *BMD change*, and it was suspected that these women were developing early degenerative disease, these subjects were also excluded [16].

Anthropometric variables

Height was always measured on the same stadiometer and weight on the same balance. Weight was re-measured at each densitometry visit and height at least once every 3 years. BMI was calculated as weight in kilograms divided by the square of height in metres.

On up to four occasions at the beginning of the study and at yearly intervals, each woman had a CT scan of the trunk at the L3 level for measurement of BMD by QCT [17]. Because of the reported association of psoas muscle weight with bone density [18], each scan was analysed for the mean of the cross-sectional areas of the left and right belly of the psoas muscle by manually tracing round the images of the two muscles, which were then averaged. The QCT trabecular bone density data are not reported on because we did not standardise table height on our GE machine; failure to do this leads to substantial inaccuracies.

In addition, the subjects submitted to a number of other measurements related to fitness and muscle mass or function, including predicted maximum oxygen consumption (VO_2max), grip strength, and total body potassium, which, as we have reported previously, are predictors of observed rates of spinal *BMD change* [10]. However, fat mass calculated as the difference between lean body mass and body weight is associated positively with BMI. The women also answered a simple questionnaire on whether they were smokers, whether they ever drank alcohol and whether they ever took recreational exercise.

Analysis of densitometry time-trend data

The present analysis centred on those women who never took HRT and who showed no evidence of osteorthrosis (OA) or dissimilar patterns of *BMD change* between individual vertebrae. The bone density data for vertebrae L2–L4 were plotted against years since menopause and fitted by means of regression analysis. In a number of previous studies rates of bone loss were related to initial bone mass. In order to eliminate this effect of bone size, our statistical models incorporated bone density values that had been log-transformed before analysis. The guiding principles in the choice of statistical model were that it should be biologically plausible and mathematically simple and that it only incorporated additional terms to describe differences between individuals on good biological grounds or on grounds of goodness of fit. Lastly, computational tractability was taken into account. For the spine we chose a mathematically linear model. The simplest such model which adequately fitted the data incorporated individual starting values for the logarithm of BMD at menopause, individual rates of loss of log (BMD) for each woman and a final term representing an initially slow rate of attenuation in the rate of loss of BMD. This last term was the same for

each woman and proportional to (time since menopause)^2; consequently, it contributed quite a small proportion of loss in the first 10 years after menopause. We have previously shown that if BMD loss was modelled without being first logarithmically transformed, the model needed to include a larger number of terms representing independent variables to adequately fit the data [10].

Statistical modelling of the data

For the bone density data, it was possible to calculate one coefficient per woman that would represent her individual rate of fractional spinal BMD change, which in turn described her BMD change in the first decade post-menopause. This coefficient was treated as the dependent variable, and simple regression analysis was used to determine the significance of relationships between the BMD change coefficient and continuous variables representing dietary determinants. Based on the extensive documentation in the literature relating bone loss to many dietary variables, after relating the dietary nutrients individually to spinal BMD change by means of simple regression, we applied a stepwise multiple regression approach. Initially, all diet-related variables were available, with or without one of the body composition-related variables (BMI, psoas area or, as an alternative to BMI, fat mass), and mixed (forwards and backwards) stepwise regression was applied using an initial entry and leaving criterion for each variable of $p<0.05$ and $p>0.05$, respectively. Due to the variable amount of BMD data available from each subject and its effect on the precision of the estimation of rates of BMD change, the data were weighted statistically in proportion to the number of BMD measurements (with each DPA measurement counting for statistical weighting purposes as 0.3 of a DXA measurement, as justified previously [10]). The range of the weighting factors calculated in this manner extended from 2.8 to 9.1 (inter-quartile range: 7.8–9.1). Adjustments were made for projected bone area in the statistical modelling because of its potential role as a confounding variable representing body size [19]. For other non-dietary variables that were measured repeatedly and entered into the models, we either took their initial values or entered the measurements as rates of change over time. Finally, all nutrients that showed significant relationships with BMD change were re-investigated after a so-called energy correction (i.e. after re-calculating in units per kilocalorie energy consumed). All statistical calculations were implemented on JMP v4.0 (SAS Institute, Cary, N. C.). *The term 'statistical effect' is used here to indicate the statistical dependence of an outcome variable on another variable, without inputting direct causality.*

Results

Table 1 shows the characteristics at recruitment of the 32 compliant subjects who did not take HRT nor show evidence of degenerative disease of the spine. These women had a mean of 12 (range: 7–13) DXA measurements each. The mean initial rate of loss of spinal BMD at–L2-4 for this group, calculated from the individual loss rate coefficients shown in Table 1, averaged 1.2% per year (inter-quartile range: 1.0–1.5%; overall range: −0.3 to 2.4% lost per year).

The mean interval between dietary assessments was 2.5 years (SD: 0.21, range: 1.69–2.82, median: 2.47 years), with the first assessment occurring within the first 6–9 months of recruitment. Overall, the dietary intakes of nutrients over this period were relatively stable, with a small downward trend in energy intake, which fell from a mean of 1840 to 1746 kCal ($p<0.05$). This was due principally to a fall in fat intake from 79.3 to 72.8 g/day ($p<0.02$) rather than any significant change in protein or carbohydrate intake ($p>0.05$ in each case). Calcium, vitamin C and riboflavin intakes, to take three other examples, were also stable ($p>0.05$). Table 2 shows the means, medians and distributions of the nutrient intakes estimated from the weighed 3-day and 7-day diets averaged over both periods of assessment.

Table 3 shows the simple correlations of the dietary nutrients with spinal loss rate. Noteworthy are the statistically significant inverse associations of a number of nutrients, notably fat, protein and carbohydrate, as well as total energy, with spinal BMD change rate. However, only iron showed a significant relationship with preservation of bone density after a correction for energy density. Stepwise regression was then carried out with spinal BMD change rate as the dependent variable using the uncorrected nutrients as independent variables without including data on body composition. In the resulting model,

Table 1 Characteristics of subjects

Variable	Median	Mean	Inter-quartile range	Extreme range
Age at menopause (years)	50.5	50.1	48.8, 51.8	43.9, 54.0
Height (cm)[a]	160.7	160.6	155.4, 163.6	151.5, 173.6
Weight (lg)[a]	63.6	63.1	57.6, 67.6	50.1, 79.9
BMI (kg/cm^2)[a]	24.9	24.5	22.1, 26.2	17.4, 31.4
Fat mass (kg) (n=30)[b]	22.0	22.3	19.1, 25.6	13.6, 33.9
Rate of weight gain (kg/year)	+0.6	+0.7	+0.3, +1.2	−0.6. +2.8
BMD T-score L2-4[a,c]	−1.1	−1.2	−1.7, −0.6	−3.0, +0.5
Exponent describing BMD loss from L2-L4×10^2	−1.31	−1.30	−1.53, −1.31	−0.25, +0.01
Vo_2max (ml/min/kg)[a]	28.6	27.8	24.8, 30.7	20.8, 36.5
Psoas area (mm^2)[a]	573	590	490–702	330–919

[a]Within 4 years of recruitment
[b]Two subjects found the whole body counter too claustrophobic to agree to this measurement
[c]BMD T-score of the lumbar spine was calculated for L2-L4 using the data of Dequeker et al. [38] as the referent

Table 2 Estimated nutrients in subjects' diets

Variable (expressed on a per-day basis)	Median	Mean	Inter-quartile range	Median (as % of the UK norm)[a]	Extreme range
Carbohydrate (g)	209	212	177–236	103	102–401
Protein (g)	70.6	70.1	63.0–75.3	106	42.9–104.7
Fat (g)	72.3	76.3	62.5–94.6	89	37.5–110.8
Energy (kCal)	1727	1802	1594–2049	106	1006–2683
Calcium (mg)	974	991	771–1169	115	457–1578
Phosphate (mg)	1136	1167	1044–1291	97	683–1845
Magnesium (mg)	257	267	228–292	107	170–388
Iron[b] (mg)	11.1	12.0	10.2–13.2	101	6.2–24.7
Zinc (mg)	8.5	8.8	7.6–9.9	105	4.8–13.0
Vitamin A[b] (mg retinol equivalents)	0.94	1.59	0.77–2.198	92	0.41–4.79
Vitamin C (mg)	77.9	82.7	54.0–104.1	126	20.6–196.2
Vitamin D[b] (μg)	2.20	3.15	1.48–3.21	85	0.06–15.09
Thiamine (mg)	1.12	1.17	0.93–1.29	88	0.72–1.95
Riboflavin[b]* (mg)	1.82	1.91	1.37–2.30	112	1.15–3.13
Niacin (mg)	29.5	31.0	27.1–35.6	108	21.8–45.2
Copper[b] (mg)	1.39	1.57	1.21–1.75	138	0.70–3.61
Fibre (g)	18.4	19.3	16.7–21.9	N/A	11.5–34.1

[a]From the UK National Health and Nutrition Survey 2000/2001 using data from the mid-1980s reported therein where possible [39]
[b]The distributions of these variables was significantly non-normal (Shapiro-Wilk W test, $p<0.05$) and positively skewed, but could be normalised with a logarithmic transformation

dietary protein was a highly significant determinant (being positively associated with loss of bone, $p=0.0003$), and dietary iron was associated with the retention of bone ($p=0.043$). Because of the large statistical effects of energy correction on the associations with BMD change, energy consumption was then forced into the stepwise model; this resulted in the substitution of diet protein by a combination of energy ($p=0.008$) and zinc ($p=0.006$) intakes, which had inverse statistical effects. This was unsurprising, since together energy and zinc predicted protein intake with an R^2 of 0.71. Iron remained the single nutrient with a positive statistical effect ($p=0.002$).

Adding the BMI measured at the start of the study to the diet model increased the model's goodness of fit without substantially changing the statistical significance of the effects of protein, energy or iron (Table 4). Similar results were obtained when body fat was substituted for BMI (body fat and BMI correlated quite closely: r^2adj 0.72). Furthermore, energy-corrected iron remained a significant determinant of bone retention after adjusting for BMI or body fat (Table 4).

However, when the rate of body weight change (calculated by linear regression from the weights measured at each DXA attendance) was forced into this final model, it did not have a significant independent statistical effect ($p=0.24$). Neither psoas area nor lean body mass contributed independently to the prediction of BMD change rate when dietary variables were included in either of the dietary models; neither did the other candidate determinants we measured (VO_2max, qualitative measures of alcohol, smoking, exercise) have a significant independent effect. Adding the bone area for L2–4 measured by the densitometer at study entry, exit or both into the model had no significant effect ($p>0.67$).

Table 3 Simple correlation coefficients (r) between nutrients and the coefficient of vertebral *BMD rate of change* and the mean total dietary energy intake

Nutrient	BMD loss rate coefficient	Energy
Energy	−0.46**	
Protein	−0.53**	0.70***
Fat	−0.45**	0.88***
Carbohydrate	−0.30	0.87***
Calcium	−0.34	0.76***
Magnesium	−0.45**	0.64***
Phosphate	−0.47**	0.82***
Iron	0.00[a]	0.58***
Vitamin A	0.06	0.15
Thiamine	−0.24	0.52**
Niacin	−0.24	0.60***
Riboflavin	−0.23	0.67***
Vitamin C	−0.14	0.34
Zinc	−0.49**	0.55**
Copper	−0.34	0.35*
Vitamin D	−0.25	0.30
Fibre	−0.17	0.38*

$p<0.05$, **$p<0.01$, ***$P<0.001$
[a]$r=0.42$, $p=0.02$ for energy corrected relationship

Table 4 Regression models for the effects of dietary protein, energy, iron, zinc and body mass index (BMI) on the exponent describing the rate of BMD change from the lumbar spine (L2–L4) in grams/centimetre/year

	Estimate×10^{2}[a]	*t*- Ratio	*p*= (2-tailed)	Change for a 1 SD change[b]
Term; overall R_2adj 0.52				
Intercept	−1.3	−2.07	0.0480	
Dietary protein	−0.028	−4.64	<.0001	−0.32
Dietary iron	0.062	3.43	0.0019	0.23
BMI	0.050	3.02	0.0054	0.17
Term; overall R_2adj 0.57				
Intercept	−1.1	−1.82	0.08	
Dietary energy	−0.0006	−2.56	0.17	−0.22
Dietary zinc	−0.126	−3.15	0.004	−0.24
Dietary iron	0.077	3.72	0.0009	0.28
BMI	0.046	2.55	0.017	0.16
Term; overall R_2adj 0.35				
Intercept	−3.9	−6.44	0.0480	
Dietary iron×10^{3}/kcal	0.141	2.99	<.0001	0.22
BMI	0.068	3.26	0.0054	0.24

[a]Estimated effects are shown per effect unit of measurement
[b]Shows change in the exponent describing BMD loss per year in L2-L4 for a 1 SD increase in the independent variable. These changes translate into approximately the same reductions in percentage BMD losses per year for a positive value (or increases for a negative value). For units and ranges, see Table 2

Discussion

These data suggest that a substantial part of the variance in the individual rates of post-menopausal spinal BMD change in this representative group of normal women was associated with variations in diet and variations in body composition that were independent of each other. Previous studies of the statistical effects on the skeleton of dietary variation after menopause have focussed primarily on protein and calcium intakes, with variable results [20–23]. The present study has not identified with certainty which nutrient or nutrients were the prime determinants (in a statistical sense) of BMD change rates in the spine. This is partly a consequence of the fact that multiple nutrients were studied and that there were the high levels of correlation between them. However, we have demonstrated a substantial association between diet and spinal BMD change independently of body mass index (or body fat content). This encourages future research on the dietary determinants of spinal bone density regulation after menopause. If one or more of these nutrients, or the food groups from which they were derived, were to be found to be acting somewhere along the causative pathway, this would encourage researchers to hold future trials on osteoporosis prevention through modification of the diet.

The association of greater iron intake with a beneficial effect on vertebral BMD change remained significant whether it was included as a co-variate in models that included energy or protein intake or whether it was expressed as a ratio to energy intake. This statistical effect of iron was not attenuated by including either of the significant protective determinants BMI or body fat in the model. Therefore, it seems that iron intake (or any of its close correlates) would be a worthwhile nutrient to choose for further investigation into its potentially protective effect against post-menopausal bone loss from the spine. The importance of an adequate iron intake for bone might be explained by iron's indirectly beneficial effect on oxygen transport to bone through avoidance of clinical anemia, since hypoxia [24] and local acidosis [25] interact as major determinants of osteoclast activity. In a recent epidemiological study, there was an inverse association between the haemoglobin concentration and bone density as well as bone mass in elderly subjects [26]. The statistical effect of total protein intake was dependent in large measure on the energy density of the diet, so our study cannot be used to argue specifically for or against protein as a determinant of bone loss. Non-haem iron is found mainly in cereals, pulses, vegetables, beans and many other foods, whereas zinc is found in association with animal protein, maize and wholemeal wheat to name only a few. Only about 15% of UK dietary iron is supplied by meat, perhaps explaining the modest correlation between these nutrient intakes (r=0.46, p<0.01). Furthermore, at least one study has found that a high meat diet was not beneficial for iron retention [27].

In the elderly there is an accumulating body of evidence indicating that protein provides a protective effect against hip fracture and a faster recovery from the effects of fracture [28]. However, the women we studied were initially in the early menopause and had by conventional standards excellent diets, unlike those incriminated in more elderly individuals as contributing to the risk of osteoporosis. There is a substantial body literature available on the effects of protein intake on bone, and increasing protein intakes may have anabolic effects on bone both by providing building bone matrix and by an amino acid-stimulatory effect that increases insulin-like growth hormone (IGF1) production, while high – and specifically animal – protein intakes also increase endogenous acid production, which can have offsetting catabolic effects [29, 30]. Our subjects also had good calcium intakes, which reduced the power of this study to identify any statistical effect of low calcium intake.

With respect to the estimated biological variation in post-menopausal loss of vertebral bone density, a statistically significant but still modest proportion (about 20%) of this variation in the women participating in this study had been previously shown to be attributable to BMI or, in an alternative analysis, the cross-sectional area of the psoas muscles at the mid-point of the region measured by DXA

[10]. BMI is moderately protective against vertebral deformity in European women and men [31], an effect that is probably mediated through increasing bone density [2]. Our data suggests that, for the spine, BMI protects the skeleton against post-menopausal bone loss in the first decade after menstruation ceases. This is consistent with two alternative or possibly complementary concepts. The first of these is that following menopause adipose tissue is the main source of endogenous oestrogens and, as such, may contribute usefully to slowing bone loss through elevated levels. The second is that a higher BMI, through its association with higher muscle mass as well as fat mass, might reduce bone loss through the greater mechanical loads imposed on the skeleton by larger muscles. There is evidence in young athletes who develop athletic amenorrhoea that increased mechanical loading can provide some site-specific if incomplete protection against spinal bone loss [32].

Given the relatively large number of candidate dietary determinants of lumbar spine *BMD change*, there was clearly scope in such a relatively small study as the one reported here for the identification of determinants in the wrong order of precedence – for purely statistical reasons. In particular, it has to be borne in mind that most of the nutrients we estimated were positively correlated with each other. Furthermore, the diet data were collected early in the study; consequently, the statistical effects of diet, if continued as the study progressed, must have been through the tendency of dietary patterns to remain consistent with ageing.

In our analysis of the subjects in this study who developed scan image evidence of degenerative arthritis in the lumbar spine, or of non-parallel rates of loss or gain in BMD in the three lumbar vertebrae studied, we found that body fat content was a significant risk factor for these potential manifestations of spinal degenerative disease [16]. Therefore, it would seem that there may be an inverse relationship between risk of fast vertebral bone loss and risk of degenerative disease in the lumbar spine, based on body fat content at study entry.

This study has limitations. As already mentioned, it was relatively small with respect to cohort participation. It was not designed to measure precisely attributable risks in populations, but rather to identify any common determinants of fast bone loss which might prove useful in managing individual patients. There were a change in the equipment utilized, which were inevitable in light of the limited lifespan of such equipment in relation to the study's intended duration. However, we had shown in a previous study that this did not invalidate or distort our analysis of rates of spinal *BMD change* calculated from these data [10]. There is no accepted biomarker for iron intake, and our study design did not include the measurement of haematinic indices such as haemoglobin (Hb) concentrations. A retrospective clinical note search revealed Hb and mean cell volume results were retained from the study period for only 50% of the sample and that neither correlated significantly with the rate of spinal *BMD change* ($p>0.26$). The Novo DPA densitometer is now obsolescent and gave comparatively modest precision. However, with over a decade's worth of data on the participants, we calculated that less than 25% of the estimated between-subject variance in spinal *BMD change* was attributable to measurement imprecision, leaving over 75% to be accounted for by biological determinants [10]. Our study has other favourable features. Retention of our cohort has been high and initial compliance was also good. This is the first longitudinal study that has followed spinal bone loss after menopause for so long – three quarters of our subjects were over 15 years post-menopause at exit, having been recruited within 3 years of menopause. The technique used for collecting dietary data is well-regarded compared to food frequency questionnaires and some other methods [33]. Because, however, the dietary data were collected about a decade and a half ago, not only have the diets of British women inevitably changed somewhat in the ensuing period of time, but we were unable to explore in a more focussed way some newer hypotheses concerning the prevention of post-menopausal bone loss, such as the potential role of dietary oestrogens.

While this was a small study with respect to numbers of subjects, it was carried out over an unusually long interval of time and involved many measurements on each woman. The precise measurement of rates of post-menopausal *BMD change* in the spine is not straightforward. It seems likely that future improvements in the precision of DXA measurements will be modest unless ways can be found to improve the reproducibility of positioning. The reason for this is because photon fluxes generated by current generation densitometers allow very precise measurements in vitro, which are difficult to improve upon meaningfully. Consequently, there is a considerable advantage to studying women for a prolonged period of time so that a high degree of measurement precision can be obtained in the measurement of *BMD change* rates in individuals.

In conclusion, bone loss can be considerable in normal women after menopause, with the quartile of women showing the fastest rate of loss having a 27% decline in their spinal BMD over 16 years of observation. Our results have confirmed our earlier finding of the potential importance of body leanness, even within the normal range, in predisposing to vertebral bone loss and deformity. Several previous studies have found a protective effect of both frank obesity and higher than average BMI against spinal bone loss [10, 34, 35], and the question has arisen as to whether this effect could be attributed to increased food consumption. However, this effect of BMI or percentage body fat does not seem likely to represent a surrogate marker for high food intake because in our study high energy intake was associated with BMD loss and not bone retention. In our study more than 75% of the subjects had a BMI under 26 at entry, indicating that this was not an obese population. It is well known that patients with hypothyroidism are protected from osteoporosis and that patients with hyperthyroidism frequently suffer from the condition [36]. Physical activity is associated with a modest protective effect against spinal osteoporosis, so it seems unlikely that the adverse effect of high protein (or possibly caloric)

intake we saw was acting as a surrogate for high physical activity. These women were not particularly physically fit as evidenced by their rather modest VO_2max results in comparison with a contemporary population of their athletic peers [37]. We conclude therefore that there is a reasonable likelihood that some aspect of diet linked to protein intake is an important co-determinant of the rate of spinal bone loss. At the same time, the potentially beneficial effect of a good intake of iron is of considerable interest. These data justify larger cohort studies of diet (and basal metabolic rate) as possible key determinants of spinal bone loss in the two decades after menopause, in women carefully screened to exclude developing osteoarthritis, with the aim of identifying beneficial dietary constituents which could be the subject of future intervention trials.

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