Increasing Mineral Density After Menopause in Individual Lumbar Vertebrae as a Marker for Incident Degenerative Disease: A Pilot Study for the Effects of Body Composition and Diet

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ABSTRACT. ***Objective.*** To investigate the potential utility of dual x-ray absorptiometry (DXA) in incidence studies of lumbar spinal spondyloarthropathy.

Methods. Fifty-eight women recruited after menopause to a study of spinal bone loss were measured every 2 years for over a decade. Five developed scan image evidence of patchy calcification and 10 developed statistically significant ($p < 0.05$) nonparallelism of their bone loss (or gain) in L2, L3, and L4. The number of years since menopause at which these abnormal calcification trends (ACT) occurred was made the outcome in Cox proportional hazard modeling. At baseline, diet was assessed twice using 3-day weighed intakes. Nutrients estimated included carbohydrate, fat, protein, fiber, calcium and other minerals, and 6 vitamins. Measurements at baseline of fat mass and other anthropometric variables were made.

Results. The best single explanatory variable for developing ACT was whole body fat mass. Dietary fat was also predictive ($p = 0.05$) and adding dietary vitamin D (obtained substantially from oily fish) as a second predictor improved the diet model further (to $p = 0.006$ for model). These 2 dietary variables remained significantly predictive when fat mass was adjusted for ($p = 0.0003$ for model).

Conclusion. Serial DXA measurements of the lumbar spine have the potential to provide a new, low radiation-dose approach to early identification of localized abnormal spinal calcification in epidemiology and trials. Alongside body fat, dietary fat intake and its components may warrant further investigation as risk factors for incident degenerative disease of the spine. (J Rheumatol 2004;31:1986–92)

Key Indexing Terms:
LUMBAR SPINE OSTEOARTHRITIS DUAL X-RAY ABSORPTIOMETRY
DIETARY NUTRIENTS OBESITY COHORT STUDY

Degenerative osteoarthritic (OA) changes in the spine developing typically from the 6th decade of age are exceedingly common and cause much morbidity. Indeed, it is arguable that most of the population becomes to some degree affected given a long enough lifespan. The study of the epidemiological risk factors for spinal OA that might retard or accelerate its development and also trials of treatment have been impeded because of the absence of low risk, acceptable methods for accurately determining the time of onset of the abnormal calcification that allows radiological diagnosis. Plane radiographs of the lumbar spine enable both the identification and localization of abnormal calcification in the spine. But they are a relatively high radiation-dose procedure that has the disadvantage of being difficult to justify in prospective cohort studies, for example, at annual intervals, over long intervals such as 5 or more years.

The highly precise localization of where in the spine the abnormal calcification has taken place may sometimes be only of secondary interest and can be achieved with a single exit radiograph (perhaps combined with an entry radiograph). In the last 15 years, new radiological techniques have been developed for measuring mineral density that discard some precision in anatomical localization in favor of the benefits of low dose and hence safe repeatability. Dual x-ray absorptiometry (DXA) also has the great advantage over plane radiography of being able to measure the amount of calcified mineral within a given region of the spine reproducibly and accurately. Thus, changes of 3–5% within a group of 3–4 lumbar vertebrae or 6–10% within a single vertebra may be identified routinely using 2 interval DXA scans[1].

In women as in men, bone mineral density (BMD) can

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increase rather than decline from the 6th decade of age onwards[2]. This is generally attributed to degenerative joint disease with its associated juxtaarticular endplate sclerosis or osteophytosis[3-7]. Indeed, from the 7th decade, bone densitometry of the lumbar spine is used less commonly than at younger ages for the diagnosis of osteoporosis and bone loss simply because degenerative change affecting BMD measurements is so common. However, the longitudinal development of this class of BMD changes has never been studied systematically.

The Harrow Postmenopausal Bone Loss Study was begun in 1984 with the aim of documenting, in a community setting, rates of spinal bone loss in healthy women beginning within 3 years of the menopause[8]. We have published descriptive data on spinal bone loss rates over the first 11–14 postmenopausal years in that part of the same representative population sample that did not take hormone replacement therapy[9]. We were also 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 cooperate in weighing and recording all food and fluid intake for 3 days. From these 3-day data on food intake, we calculated nutrient intakes including those micronutrients for which there was evidence that they might influence the biological pathway(s) responsible for postmenopausal bone loss. In a previous report, we demonstrated that dietary intakes of calcium were relatively stable over 2.5 years[10].

We describe a possible new and practical method for studying the incidence of spinal degenerative disease. The onset of localized increases in BMD in one or more spinal vertebrae (L2–L4) are classed as abnormal calcification trends (ACT), and include significant deviations from expected trends in spinal bone loss in women expected to lose bone after the menopause. This was not anticipated to be an outcome of interest when the study was designed, but became a practical proposition when the study acquired a DXA densitometer to replace its old isotope-sourced machine. We anticipated that among 60 women, about 12 would develop spinal osteoarthrosis by age 65[11]; thus we could pilot the detection of abnormal calcification trends by DXA and (if we could detect them) perform a preliminary investigation of their anthropometric and dietary determinants as an aid in the design of future studies. We describe preliminary findings concerning risk determinants for incident unexpected localized accretion of mineral by the spine against a background of more prevalent bone loss. It seems likely that using DXA every 12–24 months in prospective studies of spinal OA could be valuable in the future study of its epidemiology and in trials of treatment.

MATERIALS AND METHODS

Subjects. Women between 42 and 52 years of age from 4 primary care practices who had not had a hysterectomy were approached concerning participation in a study of how women lose spinal bone at the menopause, following a visit for a routine cervical smear. At that time all women were called up routinely for a smear at regular intervals provided by the National Health Service, so non-attenders were ineligible for our study. Women who had a history of malignancy were not interviewed and the remainder were asked about the date of their last menstrual period. Those consenting to the study 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 undertaken on a parallel sample to the smear[12]. All those showing parabasal cells, which are a marker of diminished ovarian hormone stimulation, and a random 50% of those not showing parabasal 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. The study was approved by the Harrow District Ethical Committee.

Weighed diet intakes. The women were instructed on the use of a portable digital scale (Soehnle) accurate to 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 checked with each subject. Nutrient intake was analyzed[13] using a computerized data entry system recording weights and categories of food consumed, based on Paul and Southgate's tables of food composition[14,15] extended to include foods not already on the computer database. The nutrients analyzed included carbohydrates, protein, and fat as well as energy (kcal/day). Among micronutrients, those that could be estimated and were considered potentially to influence bone metabolism were as follows: 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; fiber (associated with phyto-estrogens); and a group of B vitamins that are essential cofactors in certain energy-utilizing processes: thiamine, riboflavin, and niacin.

Weighed dietary intakes were done 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 accepted; the remaining 33 weighed their food intake for 3 days. For this study we used all the data from each subject (Thursday, Friday, and Saturday for the first assessment and again on the second occasion in those weighing for only 3 days on the second occasion) to examine the power of selected nutrients to predict subsequent DXA changes.

Bone densitometry. Six-monthly measurements of the lumbar spine over the first 2 years were made using the Novo BMC Lab 22a dual photon absorptiometer (DPA)[16], but as soon as it became available (at 3–5 years postmenopause in most subjects) measurements were transferred to a Hologic QDR-1000. This occurred at about the same time as the second dietary assessments. Spine density data were expressed in g/cm^2. Subjects were measured on both machines on the same day at the time of commissioning of the Hologic and the data used to individually cross-calibrate the bone density data[9]. At a later date, the original Hologic QDR 1000W scanner was replaced by one of the same brand and type, which was adjusted by Hologic to give the same readings on both the Hologic Spine and European Spine Phantoms at the time it was commissioned.

The images from the bone density scans (Hologic) were reviewed as individual series. If the spine image showed unequivocal evidence of degenerative spondylopathy, with patchy increases in bone density, irregularity of vertebral body outline, and loss of intervertebral disc spaces, the time at which this was first seen was noted.

To determine objectively whether there were dissimilar rates of bone loss (or gain) between individual vertebrae L2, L3, and L4, they were compared using repeated measures multiple analysis of variance (MANOVA) with time as the independent variable. The time (if ever) at which the effect of time on BMD became significantly different between the 3 vertebrae ($p < 0.05$) was noted. At their last DXA measurement, the

subjects were a mean of 65.2 years of age. Two subjects were noted to have consistent spinal deformity on serial DXA images consistent with long-standing scoliosis. The remaining subjects were judged to have undeformed spines on their initial DXA images.

Anthropometric variables. Height was always measured on the same stadiometer and weight on the same balance. Weight was remeasured at each densitometry visit and height at least once every 3 years. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Weight gain or loss coefficients (kg/yr) over the course of the study were calculated by linear regression of each subject's body weight against time.

At the beginning of the study and at yearly intervals for 2–3 years, each woman had a computer tomographic scan of the trunk at the L3 level for measurement of BMD by quantitative CT scan[17]. Because of the reported association of psoas muscle weight with bone density[18], each scan was analyzed for the mean of the cross-sectional areas of the left and right belly of the psoas muscle by manually tracing around the images of the 2 muscles, which were then averaged. The anteroposterior and lateral diameters of the L3 vertebral body were also measured.

In addition, the subjects submitted to a number of other measurements related to fitness and muscle mass or function, including oxygen consumption and grip strength, as described[9]. Total body potassium (mmol) was measured in a whole body counter equipped with 8 sodium iodide crystals using the technique described by Smith, *et al*[19]. The standard error on a value for total body potassium was approximately 4%. On the assumption that there is an average of 60 mmol potassium/kg of fat-free mass[20] in healthy adult women, lean body mass was calculated. Fat mass in kilograms was obtained by subtracting lean body mass from body weight. The women also answered a simple questionnaire concerning smoking, exercise, and alcohol consumption.

Statistical modeling. Cox proportional hazard modeling was employed, using the JMP statistical package (v.4, SAS Institute, Cary, NC, USA). The time in years since menopause at which each woman developed signs of ACT, based on the results of the DXA scans as defined above, was entered onto the spreadsheet and the women without ACT were censored after noting their total followup time in years since menopause. The modeling strategy was to include factors related to body composition in Cox models based on single independent variables. Next, dietary variables related to calorie intake were examined individually in Cox models, before combining significant body composition and dietary determinants of ACT risk in multivariate Cox models. It was the intention to remove any determinants from multivariate models that were no longer significant at $p > 0.05$.

RESULTS

Four subjects who might have been eligible for inclusion dropped out of the study before DXA measurements started. Two others had scoliosis of the lumbar spine so were also excluded, leaving 58 subjects in the study. Table 1 shows their characteristics at recruitment. One subject could not tolerate whole body counting because of claustrophobia, so did not have measurements of body fat. There was a median of 11.3 years DXA followup (maximum 12.3 yrs) with 6 measurements achieved per subject (median). In all, 15 subjects were identified who showed evidence of abnormal calcification trends.

The mean interval between dietary assessments was 2.50 (SD 0.21, range 1.69–2.82, median 2.47) years, 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

Table 1. Characteristics of subjects at first measurement/recruitment.

Continous Variables	Degenerative Changes in Spine Detected, n = 15 Mean	SD	No Degenerative Changes Detected, n = 43 Mean	SD
Time between recruitment and first DXA, yrs	4.44	1.20	4.01	0.83
Age at first DXA, yrs	56.8	2.03	55.6	2.72
YSM at first DXA	6.24	1.46	5.94	1.07
DXA followup time, yrs	9.20	3.57	9.28	4.08
Height, cm	163.6	7.3	161.1	5.4
Weight, kg	68.8	7.8	62.3	7.9
BMI, kg/cm^2	25.8	2.6	24.0	3.3
Psoas area, mm^2	646	104	599	132
VO_{2max}, ml/kg·min	26.2	4.2	27.9	4.0
Grip strength, kg	26.0	3.1	24.9	3.8
Body fat, %	40.4	5.2	35.5	5.2
Body fat, kg	28.2	6.3	22.2	5.3
Weight gain, over followup, kg/year	0.45	0.45	0.40	0.48

YSM: years since menopause.

Categorical Variables	Degenerative Changes in Spine Detected (percentage of 15)	No Degenerative Changes Detected (percentage of 43)
Hormone replacement therapy (ever use during study)	20	28
Smoker	27	15
Ever alcohol consumption	80	74
Regular exercise	53	61

in energy intake, which fell from a mean of 1840 to 1746 kcal ($p < 0.05$). This was due principally to a decline 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 3 other examples, were also stable ($p > 0.05$). Table 2 shows the means and SD of the nutrient intakes in those judged positive and negative for DXA signs of ACT, estimated from the weighed 3-day and 7-day diets averaged over both periods of assessment.

Figure 1 shows trends in lumbar spine BMD in a subject whose individual vertebral BMD trends were significantly nonparallel. Figure 2 shows the years after menopause when significant nonparallelism in BMD trends was first identified in the 15 affected subjects. The scan image became unmistakably irregular in a way that is characteristic of severe degenerative disease in 5 of the 15 subjects, in addition to revealing large differences between the BMD of individual vertebrae together with nonparallelism in their rates of change of BMD (Figure 3).

In the Cox proportional hazard models, percentage body fat ($p < 0.005$) and fat mass (kg; $p < 0.003$) were both significantly predictive of the development of densitometric signs of ACT, whereas neither lean body mass ($p = 0.4$) nor BMI ($p = 0.16$) were significantly predictive. Also nonpredictive were height, VO_{2max}, use of hormone replacement therapy, smoking, and use of alcohol. However, there was a significantly adverse effect of baseline body weight ($p = 0.015$), although not body weight rate of increase. Psoas muscle and vertebral body dimensions were not predictive ($p > 0.1$).

Among the dietary variables, only intake of fat was predictive of ACT at $p = 0.05$; when dietary vitamin D (as a fat soluble vitamin) was added to the model, prediction of ACT improved considerably, with total fat being a positive risk factor and vitamin D apparently protective against the

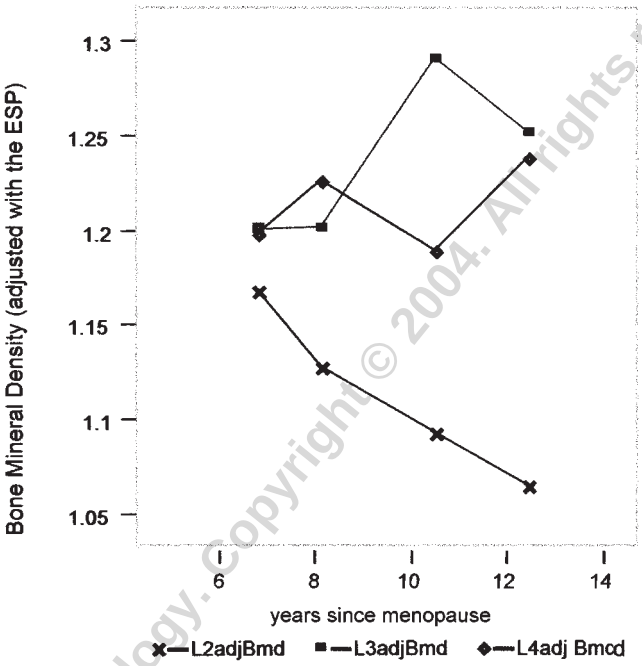

Figure 1. Bone mineral densities of the L2–L4 lumbar vertebrae in a subject with nonparallel rates of BMD change. L2 shows normal postmenopausal bone loss, whereas the other 2 vertebrae show irregular BMD gains suggestive of early endplate sclerosis or developing osteophytosis. Because the Hologic scanner was changed mid-study for one of the same brand and type, the data were cross-calibrated using the European Spine Phantom (adjBmd).

Table 2. Estimated nutrients in subjects' diets at study entry.

Variable	ACT Absent, n = 43		ACT Present, n = 15	
	Mean	SD	Mean	SD
Carbohydrate, g/day	209	50	212	39
Protein, g/day	69.1	12.4	71.2	13.1
Fat, g/day	73.6	19.1	82.4	15.9
Energy, kcal/day	1759	341	1866	308
Calcium, mg/day	976	233	949	229
Phosphate, mg/day	1160	235	1154	233
Magnesium, mg/day	268	67	265	60
Iron*, mg/day	11.8	3.2	12.5	4.4
Zinc, mg/day	8.64	1.94	9.14	2.27
Vitamin A* (retinol equivalents/day)	1512	1204	1483	1197
Vitamin C, mg/day	81.6	37.7	82.7	42.7
Vitamin D*, µg/day	3.35	3.27	2.11	0.87
Thiamine, mg/day	1.15	0.27	1.14	0.29
Riboflavin*, mg/day	1.85	0.54	171	0.55
Niacin, mg/day	30.7	5.9	29.8	6.0
Copper*, mg/day	1.56	0.58	1.67	0.75
Fibre, g/day	19.6	5.6	19.2	4.0

* The distributions of these variables were significantly non-normal (Shapiro-Wilk W test, $p < 0.05$) and positively skewed, but could be normalized with a logarithmic transformation. ACT: abnormal calcification trends.

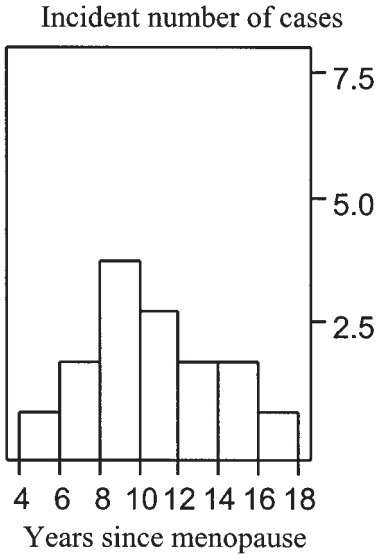

Figure 2. Distribution of times of onset of signs of abnormal calcification trends/osteophytosis on the DXA scan, in years since menopause.

development of ACT. Dietary vitamin D is quite closely related to consumption of oily fish in Britain. At the time the dietary estimates were done, vitamin D supplementation was uncommon and throughout the study fortification of food with vitamin D has remained confined to margarine. These statistical effects of diet were not changed when body fat (Table 3) or alternatively percentage body fat were added to the dietary Cox model. The effect of body fat in particular was quite steep, with an estimated 17% increase in risk for each kg increase in body fat.

DISCUSSION

The technique of dual x-ray absorptiometry (DXA) was developed as a method for quantitating the amount of calcified mineral in the trunk and upper legs with accuracy and precision. It is not so precise at localizing calcified tissue as plane radiographs, which remain the gold standard for diagnosis of spinal OA. However, plane radiographs are unsuitable for repeated use in research because they deliver too high a radiation dose, particularly to the lumbar spine, and they do not quantify the amount of mineral gained. There is therefore a place for a tool complementary to plane radiology that is particularly sensitive to the early detection and quantitation of abnormal calcification in the spine. It appears from our results that DXA is a promising new approach for this task that could be used repeatedly. It could then be assessed alongside a single exit-plane radiograph to

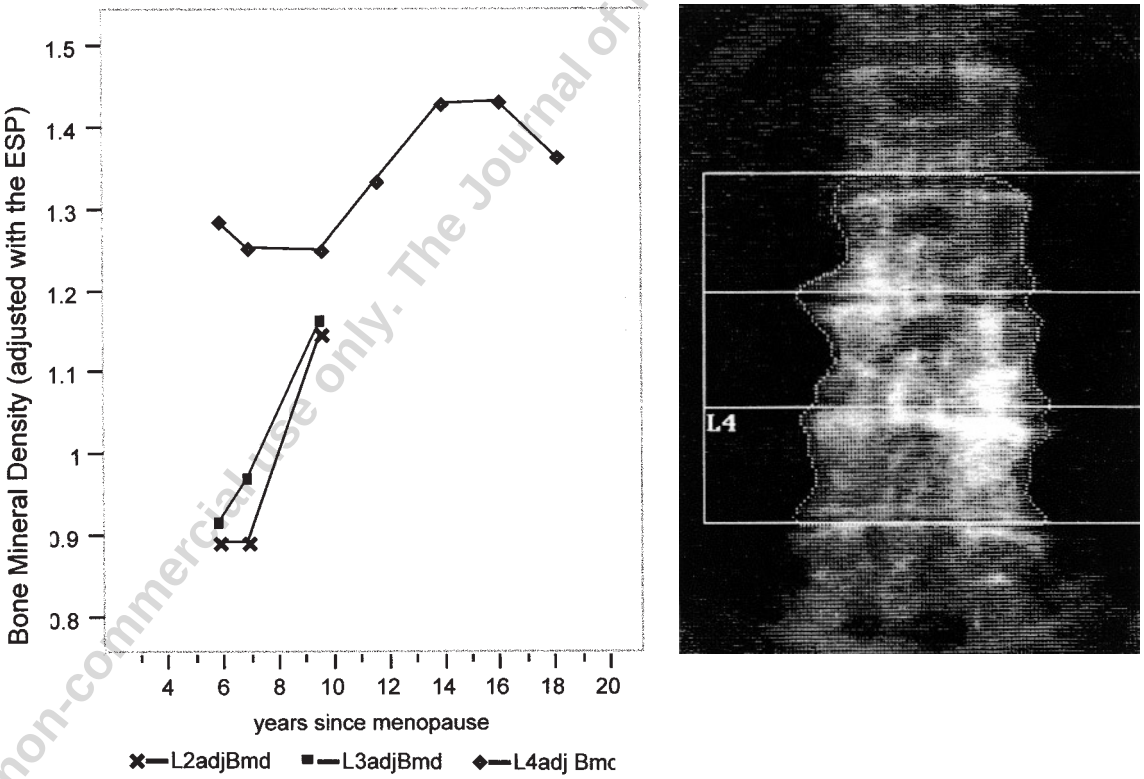

Figure 3. BMD in L2–L4 in a subject judged to have severe degenerative disease on the scan image. It was found to be difficult by the scan operator to separate L2 and L3 reproducibly on successive scans after the 3rd measurement, because of the effects of the apparent osteophytes and reduction of the intervertebral disc space combined with the growing irregularity of the outlines of all 3 vertebrae. The image shown is from the penultimate scan.

Table 3. Cox model results for risk ratios for developing abnormal calcification trends (ACT) according to fat mass and dietary fat at study entry (multivariate model).

Effect	Risk Ratio ACT per Unit Increase* Yes vs No	95% CI	p
Body fat, kg	1.17	1.07–1.27	< 0.0005
Diet vitamin D	0.61	0.32–0.95	< 0.025
Diet fat	1.07	1.02–1.11	< 0.002

* Units as per Tables 1 and 2.

derive longitudinal data of diagnostic value in the investigation of the causes of spinal OA. We present these results in part to alert others interested in spinal OA, who may need to plan (or secure consent) for DXA and exit radiographs in cohort studies on the determinants of this common and poorly understood condition.

It is generally recognized that the abnormal calcification associated with spinal OA can vary from being extremely severe and instantly recognizable from the DXA image to an experienced densitometrist to being so subtle that it is not always recognized routinely. This spectrum of severity parallels the wide spectrum of severity recognized in the Kellgren grades. It is well recognized that spinal DXA bone density is increased, by comparison with what is found in the hip or forearm, when the spine is affected by endplate sclerosis or osteophytosis[3-7]. On the other hand, it is generally held that in a spine unaffected by OA, bone loss after menopause proceeds fairly uniformly. Therefore our interest and attention was also aroused by a minority of women in this study who showed significantly non-uniform changes in density in their individual 2nd, 3rd, and 4th lumbar vertebrae over time. There were 10 women in this category who did not have the florid changes on the scan image that were instantly recognizable as severe degenerative disease. In light of no other pathology becoming evident to explain these findings, we postulated that they were due to lesser degrees of abnormal and localized calcification, increasing in some locations. These preserved or actually increased the total amount of mineral in a lumbar vertebra by comparison with what was expected from the losses seen in its neighbors. We lacked statistical power to properly test for differences in risk factors between the more severe, visually obvious, form of spinal OA (5 cases) and the others; but no effect of this nature was so large or uniform that it was statistically significant.

Our present data using DXA support the concept that in our combined group of 15 cases body fat is an important risk factor for the onset of lumbar spinal degenerative disease or OA, as we[21] and others have observed repeatedly in prevalence studies on Western populations. However, obesity is not invariably a risk factor for spinal degenerative disease; a contrast in this respect between British and Japanese subjects was clearly demonstrated by Yoshimura, *et al*[22]. Therefore the search for the fundamental mechanisms underlying this effect of body fat on spinal degenerative disease is necessary, and future studies of the interactions of genes, lifestyle, and diet will be essential for understanding the true pathogenesis of spinal OA.

Our finding of an effect of body fat on risk of ACT, while consistent with prevalence data and data on risk of OA in various other joints, provides only an approximate estimate for quantifying risk according to the degree of excess body fat a woman may be carrying. It was also interesting to observe the possible effects of dietary fat on the incidence of spinal OA. This may justify followup in a larger study in view of other work[23,24] pointing to the modifying effects of dietary unsaturated fats on disease mechanisms.

At a time when large-scale studies of diet, genes, and chronic disease are under way, it would be comparatively easy to collect data on the dietary determinants of spinal OA given an improved, nonhazardous method of detecting the incidence of this important cause of chronic morbidity. This opportunity should be considered carefully. That interest is high in the possible role of dietary lipids and other nutrients in reducing the impact of OA is another argument favoring new prospective studies of the onset of spinal OA in relation to dietary and other risk factors.

Our study had certain favorable features. Retention of our cohort was high and initial compliance was also good and followup long. Because the dietary data were collected about a decade and a half ago, not only have the diets of British women changed somewhat in the ensuing time, but we were unable to explore in a more focused way some newer hypotheses concerning the prevention of either postmenopausal bone loss (the original purpose of the study) or the origins of OA. This study therefore has considerable limitations associated with its opportunistic origins. It was designed to investigate the distribution of loss rates of spinal bone density in a group of healthy postmenopausal women at a time in the early 1980s when little was known about variation in loss rates between individuals. Nor was it anticipated at the design stage that spinal OA would be an outcome of interest in this study. In consequence, no spine radiograph was taken at baseline, nor was ethics permission requested for a radiograph at study exit so none could be taken. However, our results provide a useful basis for designing more definitive studies of the effects of diet and other factors on risk of spinal degenerative disease.

In conclusion, serial DXA measurements might become a new and valuable way to identify the initiation of incident spinal OA in future prospective studies. Our preliminary results suggest that body fat is a significant risk factor for the incident development of the condition in the first 15 years after menopause; but we failed to find an additional element of risk associated with postmenopausal weight gain. This might indicate that another factor associated with

body fat, possibly genetic or constitutional rather than dependent on diet or lifestyle, is responsible for this body fat effect. The independently predictive effect of dietary fat, pointing perhaps toward an adverse effect of non-marine or saturated fat intake on spinal OA risk, is interesting but preliminary; it warrants further exploration in more powerful population based cohort studies in women and men.

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