

# Bone Loss and Seasonal Variation in Serial DXA Densitometry – A Population-Based Study

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## 1. Introduction

Osteoporosis is a disease of increased skeleton fragility accompanied by low BMD and microarchitectural deterioration. Osteoporosis and bone fragility result in significant morbidity and medical and social costs (Dennison et al., 2005; Cummings et al., 2002). The risk of fractures is greater among women with low BMD although it explains only part of the increased fracture tendency among the elderly (National Osteoporosis Foundation, 1998). The diagnosis of osteoporosis is currently based on axial dual X-ray absorptiometry (DXA) measurements (National Osteoporosis Foundation 1998). In addition to being applicable for fracture prediction, axial DXA has a role in treatment monitoring protocols (Miller et al., 1996; Sowers et al., 1997). Furthermore, serial central DXA measurements have been used for research purposes to evaluate the risk- and preventive factors for postmenopausal bone loss (Burger et al., 1998; Hannan et al., 2000; Sirola et al., 2003). Perimenopausal bone loss rates of over -2 percent /year in spinal and over -1 percent /year in the femoral region have generally been reported (Harris and Dawson-Hughes, 1992; Pouilles et al., 1993; Pouilles et al., 1995; Prior et al., 1998; Ito et al., 1999). In postmenopausal women, age related bone loss continues at age specific rate after the initial fastening during the menopausal transition (Hansen et al., 1995).

There are two forms of vitamin D, ergocalciferol (vitamin D<sub>2</sub>) and cholecalciferol (vitamin D<sub>3</sub>). Cholecalciferol is the metabolically active form of vitamin D. Vitamin D is produced with either the effect of ultraviolet B radiation or ingested with nutrition and the metabolically active form is produced in the kidneys. It has been suggested that there might be a seasonal variation in bone turnover as assessed with both BMD and biochemical markers (Rosen et al., 1994.; Storm et al., 1998; Rapuri et al., 2002). The sun-light related vitamin D production which varies according to season seems to contribute to this phenomenon (Rapuri et al., 2002; Dawson-Hughes et al., 1997). However, other studies have not demonstrated any such effect as measured either by BMD or by levels of bone marker

compounds (Patel et al, 2001; Blumsohn et al., 2003). Consequently, the effect of the season when densitometry was performed on bone density is still unresolved. Furthermore, the role of a seasonal difference between two distant follow-up bone density measurements in postmenopausal bone loss has not been studied and thus, nothing is known about the effect of this phenomenon on treatment monitoring or other longitudinal data collection. If present, such seasonal variation could have significant effect on the evaluation of prospective data.

The purpose of the present study was to investigate the effect of densitometry season on early postmenopausal BMD and bone loss in a subset of 954 Finnish women selected from the population-based Osteoporosis Risk Factor and Prevention (OSTPRE)-study.

## 2. Subjects and methods

### 2.1 Study population

The study population was a randomly selected part of the prospective Kuopio Osteoporosis Risk Factor and Prevention (OSTPRE) study cohort. The OSTPRE cohort was established in 1989 and included all women born in 1932-1941 and who were resident in Kuopio Province, Finland (n=14 220). A postal inquiry including questions about health disorders, medications including HRT, gynaecologic history, nutritional habits, physical activity, lifestyle habits, and anthropometric information was sent to these women at baseline in 1989 (Honkanen et al, 1991). The 5-year (in 1994 follow-up questionnaires were sent to the 13 100 women who responded at baseline and responses were received from 11 954 at 5-year follow-up. The study protocol has been approved by the ethics committee of University of Kuopio and Kuopio University Hospital. Informed written consent from the participants was collected with the postal inquiries.

Of the 13 100 respondents in 1989, 11 055 (84.4 %) were willing to undergo DXA densitometry. A random sample of 2 362 women was selected for densitometry out of which 2025 women actually underwent the procedure during 1989-91. The questionnaire information was updated at the time of bone densitometry. In all, 1 873 women underwent both baseline (1989-91) and follow-up (1994-97) measurements and 1 551 of these had valid serial measurements for both lumbar spine and femoral neck (excluding severe bone deformities, see section *Bone mass measurements*).

For the present study, the following women were additionally excluded: 1) hysterectomized women (for whom it was impossible to define the menopausal status) and bilaterally ovariectomized women (n=445), 2) premenopausal women (n=152). Accordingly, the final study population consisted of 954 women (beginning of menopause either before or during follow-up) aged 48 to 59 years at baseline densitometry. The beginning of menopause was defined as 12 months' amenorrhea (WHO Scientific Group, 1996) and the duration of menopause varied from 1 to 26 years at follow-up densitometry. The duration of follow-up varied from 3.8 to 7.9 years (mean 5.8 years).

### 2.2 Seasonal Difference Index (SDI)

The study population was divided into three equal groups within the year according to month of measurement at baseline and follow-up: Group 1 (from January to April), Group 2 (from May to August) and Group 3 (from September to December). The basis for selecting these cut-

offs was based on the assumption that highest BMDs (reflecting serum vitamin D concentration) would be present at late summer and early fall season (within group 2) and the lowest at late winter (within group 3) whereas group 1 would present an intermediate. Also, in order to reveal the greatest differences in seasonal BMD variation between two successive measurements a numeric value of a Seasonal Difference Index (SDI) was calculated as follows:

$$\text{SDI} = (\text{Group number at baseline} - \text{Group number at follow-up})$$

Accordingly, the numeric value of SDI varied from -2 to +2 (Table 1). In all, 521 (54.6 %) women were measured within the same season at both measurements (SDI group 0). In 212 (22.2 %) women the follow-up measurement was carried out in a later season (SDI groups -1 and -2) and in 221 (23.2 %) women in earlier season (SDI groups +1 and +2) than the baseline measurement. There was a significant variation in distribution of women into measurement seasons between and within baseline and follow-up measurements (Figure 1). Accordingly, any conclusions over differences between specific seasons were considered to be precluded due to this uneven distribution.

**Follow-up season (Group number)**

<b>Baseline season (Group number)</b>	January-April (1)	May-August (2)	September-December (3)
January-April (1)	0	-1	-2
May-August (2)	1	0	-1
September-December (3)	2	1	0

*a) SDI=[Season Group at baseline]-[Season Group at follow-up]*

Table 1. Numeric values for the “Seasonal Difference Index” (SDI)<sup>a</sup> according to baseline and follow-up DXA measurement season

## 2.3 Other variables

### 2.3.1 Hormone therapy

Women were divided into two groups according to their use of hormone therapy (HRT) (tablets and plasters) which was defined as the use of hormonal products for menopausal symptoms. *HRT users* (n=393) had used HRT continuously or occasionally during the follow-up regardless of whether or not they had used hormonal therapy before the baseline (14 women used HRT only before baseline, and were excluded in analysis on HRT effect). *HRT non-users* (n=547) had never used estrogen containing products aimed at postmenopausal therapy. In the OSTPRE cohort, the majority of HRT users were taking estrogen-progesterone combination products (56.2% of all HRT). No data was available on whether HRT was continuous or sequential. Forty-five percent of HRT users (occasional or continuous) had been treated with HRT also prior to baseline. The duration of HRT varied from one month to 7.5 years. The information about the use of hormonal products was obtained from the questionnaires. Comparison between self-reported use of HRT and the national prescription records of The Social Insurance Institution, Finland (KELA), for the whole OSTPRE cohort in 1996–2001 revealed that 97.8% of those who had received an oestrogen drug prescription reported HRT use in inquiries. On the other hand, in 25.5% of the self-reported non-users of HRT some oestrogen use (short-term, median 6.0 months) was recorded (Sirola et al., 2003a).

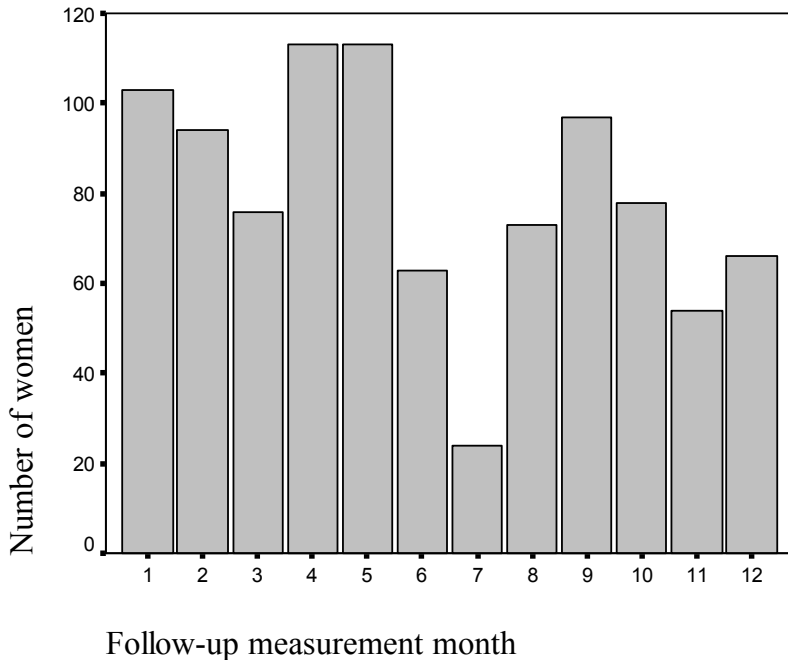
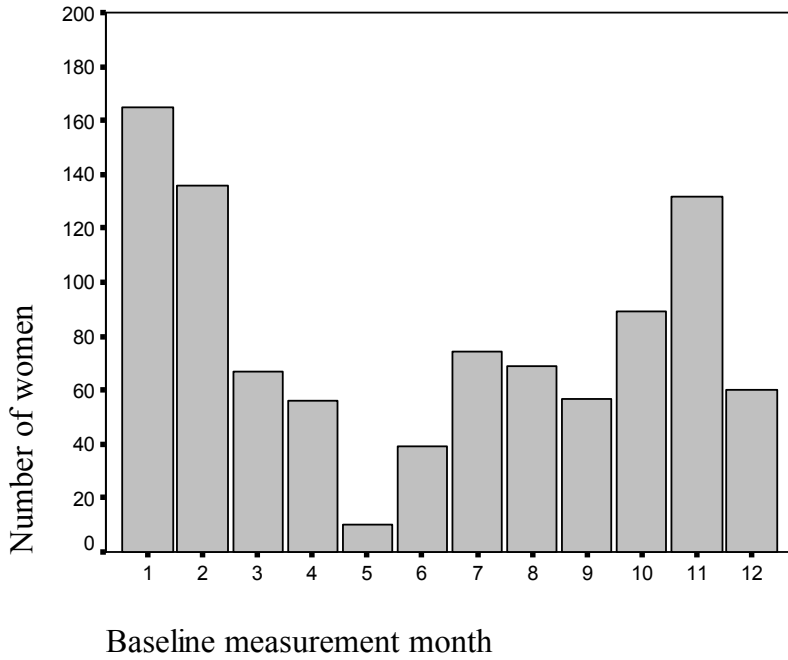


Fig. 1. Distribution of the study population according to DXA measurement month.

### 2.3.2 Adjusting variables

The *height* and *weight* were measured with a stadiometer and calibrated scale by study group nurses at each bone densitometry.

*Nutritional calcium* intake of each participant was estimated according to self-reported ingestion of milk products in postal inquiries. The following questions were asked: "How many deciliters of fluid milk products (milk, sour milk, yoghurt, etc.) do you consume daily?" and "How many slices of cheese do you eat daily?". The amount of calcium was approximated to be 120 mg/dl for fluid milk products and 87 mg/slice for cheese (Sirola et al., 2003b).

Women were divided into two categories (yes, no) according to the presence/absence of *bone affecting diseases or medications* at baseline. Bone affecting diseases/medications have been described previously by Kröger et al. (Kröger et al. 1994). Diseases were: renal disease, liver disease, insulin-dependent diabetes, malignancies, rheumatoid arthritis, endocrine abnormalities (parathyroid/thyroid glands, adrenals), malabsorption (including lactose malabsorption), total/partial gastrectomy, postovariectomy status, premenopausal amenorrhea, alcoholism and long-term immobilisation. Medications were: corticosteroids, diuretics, cytotoxic drugs, anticonvulsive drugs, anabolic steroids, calcitonin, bisphosphonates, vitamin D.

*Physical activity level* was calculated based on combined physical activity in work and leisure based on self-reports in the postal inquiries. The physical activity was categorised into low, moderate and high (Kröger et al., 1994).

### 2.4 Bone mass measurements

The bone mineral density of lumbar spine (L2-L4) and left femoral neck was determined using the same dual X-ray absorptiometry (DXA) (Lunar DPX, Madison, Wisconsin, USA) equipment at both the baseline and five year measurements. The measurements were carried out in Kuopio University Hospital by specially trained personnel. The short term reproducibility of this method has been shown to be 0.9 % for lumbar spine and 1.5 % for femoral neck BMD measurements (Kröger et al., 1992). The long-term reproducibility (coefficient of variation) of the DXA instrument for BMD during the study period, as determined by regular phantom measurements, was 0.4 % (Komulainen et al., 1998). Each DXA measurement print was reviewed and women with bone deformities (osteoarthritis, osteophytes, scoliosis and compression fractures) in either area were excluded from the analyses. At the time of densitometry, also the weight and height of each participant was measured in a controlled situation.

### 2.5 Statistical methods

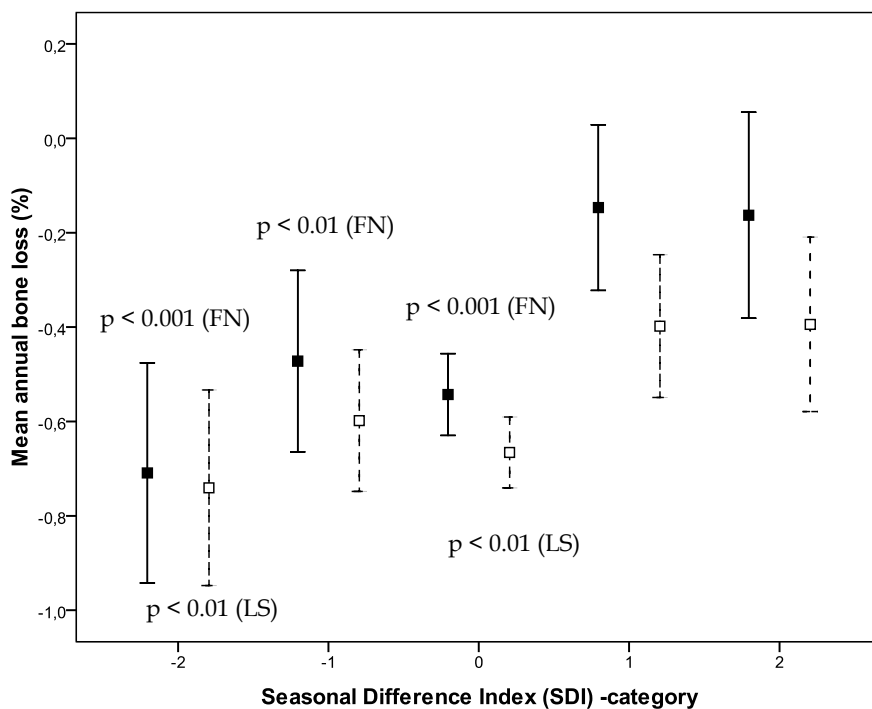
Statistical analyses were carried out with the Statistical Package for Social Sciences (SPSS) for Windows, version 17. The annual BMD changes for both measurement sites were calculated as follows:  $[(\text{BMD at the 5-year follow-up} - \text{BMD at baseline}) / \text{duration of follow-up in years}]$  and reported as percentage of baseline BMD. In categorical analyses, uni- and multivariate analysis of variance was used and Tukey (crude models) and Least Significant Difference (adjusted models) -post hoc tests were utilized to study differences between multiple groups when applicable. Adjustment for age, height, weight, months since menopause, mean calcium intake, use of HRT (no, occasional, continuous), physical activity level (low, moderate, high),

duration of follow-up (years) and the use of bone affecting medications or diseases (yes/no) (including vitamin D supplements) as covariates was performed.

### 3. Results

The baseline data revealed that there were no significant differences between the three season groups with respect to age, duration of menopause or HRT use (Table 2). There were no differences in the cross-sectional BMD between the season groups at baseline or at the five year follow-up (Table 2).

In order to evaluate a possible contribution of seasonal differences to the follow-up BMD values, the association of SDI with mean annual bone loss was investigated (Figure 2). The bone loss rate in SDI categories -2 and 0 was greater than in SDI categories +1 and +2 ( $p < 0.01$ ) in both lumbar and femoral regions (Figure 2). In lumbar spine, the difference in bone loss rate between SDI categories -1 and +1 was also significant ( $p = 0.015$ ). In femoral neck there was no significant difference between SDI category -1 and the other categories. These effects were independent of any adjustments.



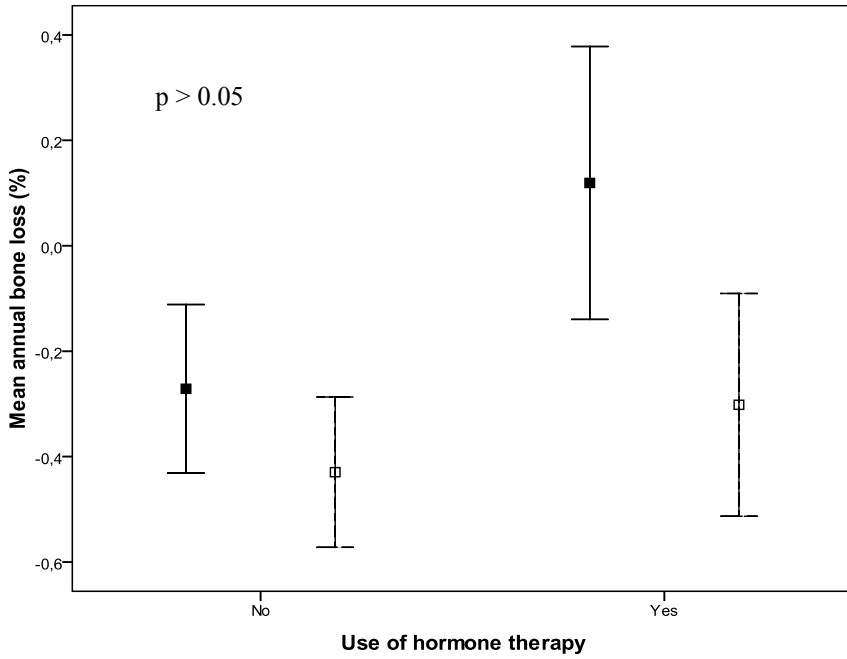
*p*-values refer to the differences of the respective SDI group in comparison to SDI 1 and 2 for lumbar spine (LS=full line) and femoral neck (FN=dotted line). a) adjusted for age, height, weight, months since menopause, calcium intake, use of HT (no, occasional, continuous), overall physical activity level (low, moderate, high), duration of follow-up (years) and use of bone affecting medications or diseases (yes/no)

Fig. 2. Effect of Seasonal Difference Index (SDI) on mean annual bone loss rate (%) in early postmenopausal women (n=954). Analysis of covariance<sup>a</sup>

Variable	Season group <sup>a</sup>			
	Group 1 (n=424)	Group 2 (n=192)	Group 3 (n=338)	Total (n=954)
<b>Means (SD) of continuous variables</b>				
Duration of follow-up yrs	5,9(0,5)	5,8(0,4)	5,8(0,5)	5,8 (0,5)
Duration of menopause, months	89,4(54,1)	86,2(48,1)	106,6(50,4)	95,0(52,3) <sup>d</sup>
Baseline age, yrs	53,5(3,0)	53,5(2,9)	54,2(2,7)	53,7(2,9) <sup>d</sup>
Baseline height, cm	160,9(5,1)	161,6(5,5)	160,8(5,3)	161,0(5,3)
Baseline weight, kg	69,1(12,4)	68,6(11,4)	69,3(11,9)	69,1(12,0)
Weight change (%)	2,9(5,2)	2,9(5,6)	3,0(5,3)	2,9(5,3)
Grip strength, kPa	62,0(16,5)	62,2(15,6)	62,4(16,0)	62,2(16,1)
Mean calcium intake, mg/day	789(343)	813(311)	799(319)	797(328)
Baseline lumbar BMD, g/cm <sup>2</sup>	1,13(0,17)	1,13(0,16)	1,11(0,16)	1,12(0,16)
Baseline femoral neck BMD, g/cm <sup>2</sup>	0,93(0,13)	0,93(0,13)	0,92(0,12)	0,92(0,13)
FU lumbar BMD, g/cm <sup>2</sup>	1,07(0,17)	1,09(0,15)	1,07(0,16)	1,08(0,16)
FU femoral neck BMD, g/cm <sup>2</sup>	0,88(0,12)	0,90(0,13)	0,89(0,12)	0,89(0,12)
<b>B. Distribution of category variables (%)</b>				
Use of HRT during follow-up				
No use	55,8	56,0	64,7	59,0 <sup>c</sup>
Occasional (<90 % of FU)	32,2	34,6	28,2	31,2
Continuous (>90 % of FU)	12,1	9,4	7,2	9,8
No bone affecting disease/ medication	60,4	64,1	64,7	62,7
Any previous fracture at baseline	20,9	23,3	20,6	21,3
Previous wrist fracture at baseline	7,2	7,7	5,5	6,7
Alcohol >1 drink/week	35,1	34,4	32,3	33,9
Smoking	9,4	11,1	8,8	9,5
High overall physical activity level <sup>b</sup>	29,9	37,8	32,8	32,5

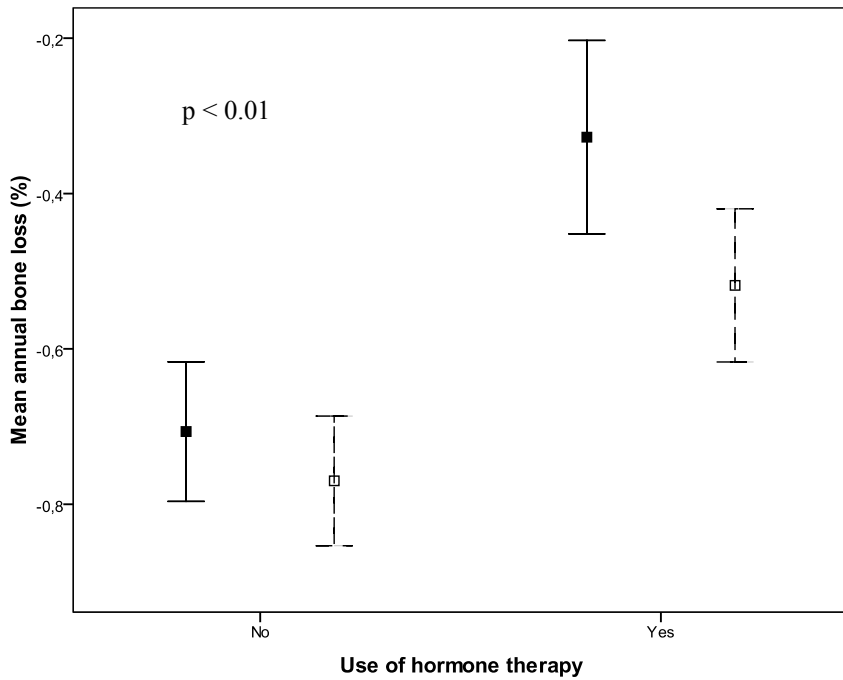
a) Season Groups: Group 1 (from January to April), Group 2 (from May to August) and Group 3 (from September to December). b) Three categorical variable: low, moderate, high. c)  $p < 0.05$  / d)  $p < 0.001$

Table 2. Baseline characteristics of the study population according to season group<sup>a</sup> (n=954).



A: SDI category -2 to 0





B: SDI category 1 and 2

Fig. 3. Effect of hormone therapy on lumbar spine (full line) and femoral neck (dotted line) bone loss according to seasonal difference index (SDI) category. Analysis of variance (ANOVA, n=954).

In ANOVA, SDI explained 2.3 percent ( $R^2=0.023$ ) and 1.3 percent ( $R^2=0.013$ ) of the bone mass changes in lumbar spine and femoral neck, respectively. Furthermore, in linear regression models, SDI was positively associated with both lumbar spine and femoral neck bone loss ( $p<0.001$ ) independent of all adjustments.

In order to mimic the possible effect of SDI on a treatment monitoring protocol, we investigated the effects of HRT on bone loss according to SDI (Figure 3). HRT users had significantly lower bone loss rate in SDI categories -2 to 0 in comparison to HRT non-users (lumbar spine and femoral neck) ( $p<0.01$ ). In SDI categories 1 to 2, there was no statistically significant difference between HRT users and non-users. These results were not affected by adjustments.

#### 4. Discussion

The present study evaluated the effect of season on BMD and bone loss with a randomly selected population-based sample of 954 Finnish women. The seasonal difference between two successive axial DXA measurements, estimated with the "Seasonal Difference Index" (SDI), influenced the evaluation of postmenopausal bone loss rate. In addition, this factor interfered with the evaluation of protective bone effects of HRT. The exact direction of these relationships, in terms of specific seasons, was found to be complex partly due to the study setting.

The present study sample was large and randomly selected. There were few differences in the baseline variables between the three season groups. The DXA measurements were carried out with same equipment and measurement staff and all bone deformities were excluded. In addition, phantom calibration was performed regularly which should exclude any significant seasonal changes attributable to equipment performance. Furthermore, comprehensive adjustment for any potential confounders was used in the analyses. Hence, it is most unlikely that any major confounding could have occurred in the present study.

Some weaknesses of the present study should also be considered. The follow-up time was relatively long with considerable inter-individual variation. Although the results were adjusted for duration of follow-up and reported in annual percent changes the follow-up period in treatment monitoring is usually only one or two years. However, the long follow-up and large sample probably facilitated the detection of bone mass changes in the present study. Furthermore, the present study assumed that the pattern of bone loss between the approximately five-year follow-up was linear, masking any possible short-term non-linear patterns. However, adjusting for these changes would have required DXA measurement at the very least at 1 year intervals. Lastly, the lack of information of serum vitamin D levels precluded causal conclusions. However, adjustment for bone affecting medication (including vitamin D containing products, medications and supplements) and calcium intake was performed eliminating bias due to these factors.

The results of the present study have two major applications. Firstly, in treatment monitoring, an attempt should be made to measure bone density within the same season. Naturally, the seasonal limits depend on amount of seasonal variation in sunlight exposure of the study population and should be closely studied. The sub-division used in the present study provides one example in DXA measurements suitable for Scandinavian latitudes.

Secondly, in prospective studies, possible distortion in results due to seasonal differences in risk-factor analyses and treatment effects on bone loss rate should be closely considered. Accordingly, it could be worthwhile to create a “seasonal difference index” for each population based on the respective DXA measurement data. The effect of seasonal differences on densitometry-based risk-factors for postmenopausal bone loss remains to be resolved. It might be that seasonal dependency could interact with certain factors lessening their true impacts. Also, the impact of these differences on fracture prediction remains to be resolved in future studies.

The interpretation of seasonal indices needs to be undertaken with caution. In the present study, the differences between the SDI categories do not necessarily provide information of the exact season that each participant was measured (e.g. SDI +1 could represent a difference between baseline season category 3 and a 5-year season category 2 or a baseline category 2 and a 5-year category 1). Also, the baseline and follow-up season groups (groups 1, 2, 3) itself included quite heterogeneous population. For example, the women measured during the first months of group 1 (January-February) were likely to have significantly lower BMDs in comparison to women presenting the other end (March-April) during which sun light exposure would be higher. We used this season categorisation order to categorize the measurement months into three equal groups within a year (i.e. four months per group: Spring (January-April), Summer (May-August) and Fall/Winter (September-December). This was based on the rapid changes in sun light exposure in the northern latitudes: sun light hours rapidly increase during may and decrease rapidly during september. Furthermore, the distribution of women into measurement months within each group was found to differ between baseline and follow-up densitometry. However, the goal of the present study was not to identify specific “risk seasons”, but only to assess variability in the estimated bone loss rate attributable to the seasons when the two successive measurements had been done. Thus, some arbitrary cut-offs, in terms of season groups, were forced to be decided. Accordingly, the hypothesis of high or low bone density according to sun exposure (and vitamin D levels) in baseline and follow-up was precluded by skew distribution of women into different measurement months. Before adaptation for wider use, these indices would need further testing and refinement in order to optimize the categorisation for local purposes.

This is the first long-term population-based study investigating the contribution of seasonal difference between two successive DXA measurements on postmenopausal bone loss. Previous studies have shown significant alterations in vitamin D and PTH levels attributable to season (Rapuri et al., 2002; Dawson-Hughes et al., 1997).which could also provide a pathophysiological mechanism for the seasonal bone effects. Some studies have also found seasonal variation in cross-sectional BMD data, bone markers and bone loss rate (Rosen et al., 1994.; Storm et al., 1998; Rapuri et al., 2002; Dawson-Hughes et al., 1997). However, other studies have failed to find any evidence on altered bone metabolism related to seasons as measured with either BMD or bone markers (Patel et al, 2001; Blumsohn et al., 2003). The ability to observe seasonal effects is likely to depend on geographical location. Another study conducted in northern latitudes (Gerdhem et al, 2004) failed to demonstrate any seasonal variation in cross-sectional study design in Sweden. The present large study population, living at northern latitudes (latitude 63 degrees), might facilitate the detection of seasonal differences. However, the present study also showed no significant cross-sectional variation in BMD.

The present study also attempted to evaluate the possible bias in treatment monitoring resulting from seasonal difference via an investigation of how the SDI could affect the bone effect of HRT. In fact, variability in the protective effect of HRT between the SDI groups was detected. This serves as preliminary example of the extensiveness of the distortion in estimation of bone loss rate caused by seasonal difference. The inclusion of occasional use of HRT and less-than-perfect validity of HRT use in “non-users” probably modified the results (Sirola et al., 2003a) but the trend was clear. Also, the effects may have been affected by lack of power due to small group sizes. However, seasonal densitometry difference may help in the identification of “non-responders” to HRT and be included in the list of other contributing factors (Sirola et al., 2003c; Komulainen et al., 1999). Previously, it has been suggested that calcium may flatten the seasonal differences in bone loss rate among elderly women<sup>8</sup> and that there might be seasonal variation in the bone response to vitamin D (Dawson-Hughes et al, 1991). The present study also showed abolition in the difference in the bone loss rate between SDI categories in HRT users.

In summary, seasonal differences should receive closer attention in treatment monitoring protocols and longitudinal risk factor studies. In future studies, the seasonal densitometry difference should be considered as a potential confounder and its effect on risk factor and treatment monitoring data should be assessed. In addition, factors that might lessen the seasonal changes in the bone loss rate, such as calcium, vitamin D and other bone drugs, should be identified. Our study raises, for the first time, the question of whether the results of longitudinal DXA measurements might be significantly distorted by seasonal differences especially in northern latitudes.

## 5. Acknowledgements

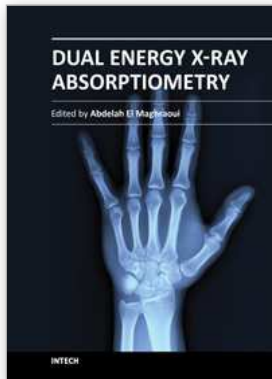
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## **Dual Energy X-Ray Absorptiometry**

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The World Health Organization (WHO) has established dual-energy x-ray absorptiometry (DXA) as the best densitometric technique for assessing bone mineral density (BMD) in postmenopausal women and has based the definitions of osteopenia and osteoporosis on its results. DXA enables accurate diagnosis of osteoporosis, estimation of fracture risk and monitoring of patients undergoing treatment. Additional features of DXA include measurement of BMD at multiple skeletal sites, vertebral fracture assessment and body composition assessment, including fat mass and lean soft tissue mass of the whole body and the segments. This book contains reviews and original studies about DXA and its different uses in clinical practice (diagnosis of osteoporosis, monitoring of BMD measurement) and in medical research in several situations (e.g. assessment of morphological asymmetry in athletes, estimation of resting energy expenditure, assessment of vertebral strength and vertebral fracture risk, or study of dry bones such as the ulna).

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