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Differential Effects of Hormone Replacement Therapy on Bone Mineral Density and Axial Transmission Ultrasound Measurements in Cortical Bone

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Abstract

The menopause has a large effect on bone density and hormone replacement therapy (HRT) has been shown to be an effective treatment for preventing postmenopausal bone loss. The aim of this study was to investigate the effects of HRT use on speed of sound (SOS) measurements at the radius, tibia, phalanx and metatarsal in comparison to bone mineral density (BMD) measurements of the lumbar spine and proximal femur.

The study population consisted of 278 healthy premenopausal women, 194 healthy postmenopausal women and 126 healthy postmenopausal women currently receiving HRT for one or more years. SOS measurements were taken at the radius, tibia, phalanx and metatarsal using the Sunlight Omnisense™ and BMD measurements at the lumbar spine and proximal femur using Hologic QDR-4500 densitometers. Z-scores were calculated using the postmenopausal control group and Z-score differences between the postmenopausal controls and HRT group for the entire group and with the HRT group subdivided into three groups, based on duration of HRT usage, were calculated.

Significant postmenopausal bone loss was found for all SOS and BMD measurements. *A positive effect of HRT usage was found for all measurement sites, although only the radius and tibia SOS and lumbar spine BMD reached statistical significance. The Z-score differences between the two groups were 0.44, 0.37, 0.15 and 0.26 for*

the radius, tibia, phalanx and metatarsal SOS respectively and 0.28, 0.00 and -0.03 for the lumbar spine, femoral neck and total hip BMD respectively. A clear effect of the duration of HRT use was seen for the radius measurements, the differences being less marked elsewhere.

In conclusion, these results demonstrate a positive effect of HRT on SOS measurements at the radius and tibia and *BMD measurements of the lumbar spine.*

Key words: Hormone Replacement Therapy, Multisite Ultrasound, Age-related change.

Osteoporosis has been recognised as a major health problem due to the increased morbidity and mortality associated with the common osteoporotic fractures of the wrist, spine and hip. Both bone mineral density (BMD) and quantitative ultrasound (QUS) measurements of bone have been shown to be predictive of fracture risk [1-6]. Hormone replacement therapy (HRT) has been shown to be an effective treatment for preventing postmenopausal bone loss [7-12], and there is some evidence that it can reduce the incidence of vertebral fractures [13, 14].

QUS has had limited use in the monitoring of patients undergoing treatment with HRT, primarily due to its poor precision compared with Dual X-ray Absorptiometry (DXA), leading to long time intervals being required to detect changes in bone [9, 15, 16]. In addition to this, bone gain at the calcaneus in response to treatment is less than that at axial sites [16] and the majority of QUS devices use the calcaneus as a measurement site. Most QUS devices are currently limited to a single measurement site. Previous studies have found limited or no effect of HRT measurable by QUS [17-19], whilst other studies have found a positive influence detectable with QUS [9, 20]. The Sunlight Omnisense™ is the first QUS device with the ability to measure multiple sites using hand held probes. In a previous preliminary study using this device, significant positive T-score differences were detected at the radius and tibia in a group treated with HRT compared to postmenopausal controls [21]. *Weiss et al also reported positive effects of HRT on SOS measurements using the Omnisense™ in a similar study* [22]. This study is the first to use axial transmission ultrasound at the phalanx, radius, tibia and metatarsal and DXA in a large group of HRT users to compare to normal postmenopausal controls.

The aim of this study was to investigate the effects of HRT on SOS measurements at the radius, phalanx, tibia and metatarsal in comparison to DXA measurements at the lumbar spine and proximal femur.

Materials and Methods

The Sunlight Omnisense™

The Sunlight Omnisense™ (Omnisense, Sunlight Ltd, Tel-Aviv, Israel) is the first quantitative ultrasound system with the ability to perform SOS measurements at multiple skeletal sites. To accomplish this it uses a number of hand held probes designed for specific sites. The probes contain an array of transducers, some acting as transmitters and others as receivers and measure the path of the sound wave taking the shortest propagation time between the transmitting and receiving transducers. The time taken for the signal to travel between the transmitting and receiving transducers is used to infer the SOS in bone [23]. The Omnisense also corrects for soft tissue thickness, giving a true SOS measurement of bone [24, 25].

Subjects

The study population consisted of three groups: ***1. healthy premenopausal women (n=278); 2. healthy postmenopausal women (n=194); and 3. healthy postmenopausal women receiving HRT (n=126).*** The exclusion criteria for the healthy premenopausal and postmenopausal controls included: a menopause before the age of 45;

amenorrhoea for greater than six months; a history of drugs of diseases known to affect bone metabolism; or a history of low trauma fracture [26]. The same criteria were applied to the HRT group except the menopause age was not restricted to greater than 45 and all patients in this group were currently receiving HRT which they had taken for at least 12 months. The patients were recruited from a number of sources: *1. patients referred for DXA at Guy's Hospital by their general practitioner (GP)*; 2. hospital personnel; 3. volunteers from the general population and; 4. twin volunteers attending the Twin Research Unit at St Thomas' Hospital. For each mono zygotic pair of twins, only one randomly selected twin was included in the study population. However, for dizygotic pairs, due to a much lower correlation between twin pair measurement values, both twins were included. *The study was approved by the Guy's and St Thomas' Hospitals research ethics committees.*

Subject Measurement

Speed of sound (SOS) measurements were performed at the non-dominant third proximal phalanx, medial aspect of the 1/3 radius, the antero-medial aspect of the midshaft tibia and the lateral aspect of the fifth metatarsal using the Sunlight Omnisense™. The Omnisense uses a total of three different probes to perform measurements at these four sites. One probe measures both the radius and tibia, whilst the phalanx and metatarsal use individual probes. Fewer subjects had measurements of the phalanx and metatarsal because these probes were not available at the start of the study. Two Omnisense devices were used based at Guy's Hospital and St Thomas' Hospital. In addition to the SOS measurements all subjects also had BMD measurements of the lumbar spine and proximal femur using one of four Hologic DXA densitometers (Hologic Inc, Bedford, MA). *The **two Omnisense devices** and **four QDR densitometers** were **cross-calibrated using in vitro** and **in vivo cross calibration**. The in-vitro cross calibration was performed using 10 repeated phantom scans with repositioning between scans. The in-vivo cross calibration involved 25 subjects who had BMD measurements of the spine and hip on four Hologic QDR densitometers and SOS measurements of the radius, tibia, phalanx and metatarsal on the two Sunlight Omnisense devices.* The data were corrected where appropriate using the slope and intercept from linear regression analysis.

Statistical analysis

The mean and standard deviations (SD) of the SOS and BMD measurements and the anthropometric data were calculated for each group and the difference between groups was tested using a t-test. T-scores were calculated using a subset of premenopausal controls aged between 20-40 years. The short-term precision (CV%) for the Omnisense as measured by duplicate scans in 37 subjects, mean age 42 y (± 13.2 y) and calculated using equations A1 and A2 in the appendix. These results were standardized as T-score units using Equation A3. Z-scores were calculated using the slope and intercept from linear regression analysis of the postmenopausal women. An unpaired two-tailed student's t-test was used to test the differences between the postmenopausal control group and the HRT users group. The effect of duration of HRT usage was evaluated by stratifying the HRT group into three groups, ≥1-<4 years, 4-8 years and >8 years HRT usage. A one-way ANOVA was then used to test the significance of differences between these groups. Finally the correlation between SOS and SOS and BMD measurement sites was evaluated using linear regression on 250 subjects from the pre- and postmenopausal control group who had measurements at all four SOS measurement sites and spine and hip BMD.

Results

Table1 shows the anthropometric data for the young normals aged 20-40, used to calculate T-scores, the postmenopausal control group and the HRT group. Statistically significant differences were found between the pre- and postmenopausal control groups for body mass index (BMI), height and all SOS and BMD sites. The postmenopausal group had a significantly higher BMI and reduced height compared to the premenopausal controls. All SOS

measurement sites demonstrated a significantly reduced SOS in the postmenopausal controls compared to the premenopausal control group. The same trend applied to BMD, where all sites were significantly lower in the post- than the premenopausal women. The HRT group was significantly younger than the postmenopausal controls and had a significantly younger age at menopause. All mean SOS and BMD values in the HRT group were significantly greater than found for the postmenopausal control group, however this may be a factor of the age difference between the two groups. There was however, no significant difference in height, weight or BMI between these two groups.

The RMSSD (CV%) was 22.8 m/sec (0.55%) for the radius, 17.7m/sec (0.45%) for the tibia, 44.8m/sec (1.11%) for the phalanx and 27.8m/sec (0.76%) for the metatarsal. Standardized as T-score units they became 0.21, 0.16, 0.28 and 0.13 for the radius, tibia, phalanx and metatarsal respectively. The short term precision RMSSD was divided by the postmenopausal control group annual loss to estimate the mean years required for follow-up measurements at each site. This resulted in follow-up periods of 3.4y, 3.8y, 2.9y and 3.6y for the radius, tibia, phalanx and metatarsal respectively.

Table 2 shows the age-related changes for the control and HRT groups. Highly significant age related bone loss was found for the postmenopausal women at all SOS and BMD sites. These ranged from –4.7 to -15.4$ms^{-1}y^{-1}$ for the SOS measurements and –0.006to -0.007$gcm^{-2}y^{-1}$ for the BMD measurements. When these rates of change were standardised as T-score units by dividing by the young adult population SD, the results found for the tibia and metatarsal SOS were slightly less than for the BMD results, whilst the radius and phalanx SOS had slightly increased rates of bone loss.

Table 3 shows the mean Z-score differences between the postmenopausal control and HRT users groups. The mean Z-score for the control group was zero for all the SOS and BMD sites. This is because the postmenopausal control population was used to calculate the Z-scores. The mean Z-scores for the HRT group ranged from 0.15 to 0.44 for SOS measurements, however, only the radius and tibia reached statistical significance and -0.03 to 0.28 for the BMD sites, with only the lumbar spine being statistically different from the postmenopausal controls.

Figure 1 shows the Z-score difference for the HRT group compared to the postmenopausal control group once the HRT group was stratified into three groups based on the duration of HRT usage. The first group comprises of individuals whom received HRT for between one and less than four years. Radius SOS was the only measurement in this group to reach a significantly different Z-score from the controls. The second group included individuals receiving HRT for between four and eight years. The Z-score differences in this group were greater than found for the previous group for all SOS measurement sites and reached statistical significance at the radius, and tibia. The final group included subjects taking HRT for greater that eight years. The Z-score differences were slightly greater than in the previous group for radius SOS. The Z-score difference for lumbar spine BMD was greater than found for the 4-8yr group and statistically significant, whilst the femoral neck and total hip had no significant differences. When the Z-score differences between the three different groups were tested for statistical significance using a one-way ANOVA the trend of increasing Z-score difference with increasing duration of HRT failed to reach statistical significance between the three groups for any SOS or BMD measurement site.

Table 4 shows the correlation between SOS and SOS and BMD measurement sites. The correlations between SOS measurement sites are weak to moderate, ranging from 0.14 to 0.48, with the best correlation between the radius and phalanx. The correlation between SOS and BMD measurements are again weak to moderate, ranging from 0.00 to 0.38, with the best correlation between metatarsal SOS and femoral neck BMD.

Discussion

This study evaluated the effects of HRT treatment on axial transmission SOS measurements at the radius, tibia, phalanx and metatarsal using the Sunlight Omnisense™ and compared them with the effect on spine and hip BMD. When the postmenopausal controls and HRT group were compared, the HRT group had positive Z-score differences for all SOS and BMD measurement sites. These were statistically significant for radius and tibia SOS and lumbar spine BMD and a similar trend was observed for metatarsal SOS. However, the phalanx, femoral neck and total hip failed to demonstrate a significant difference between the postmenopausal control and the HRT user groups. Weiss et al in a similar study also found a positive effect of HRT on SOS measurements [22]. When the HRT group was stratified based on years of usage, radius SOS demonstrated a trend of increasing Z-score differences with an increasing duration of HRT usage. Tibia SOS demonstrated a similar trend, although the results in the ≥1-<4 year group failed to reach significance. These results demonstrate a general positive effect of HRT usage on all SOS measurement sites, and this effect is greater than found for BMD at the proximal femur in the same group. These data are not consistent with the reported effects of HRT on calcaneal QUS parameters, which have found limited or no effect [17-19]. However, Sahotal et al found a positive effect of HRT at the calcaneus in a longitudinal study over a four year period, although the individual increases for broadband ultrasound attenuation (BUA) and SOS were not as great as found for lumbar spine and total hip BMD [9]. Lehmann et al also found positive effects of HRT use in a cross sectional study using SOS measurements at the patella [20]. De Aloysio et al reported AD-SOS measurements at the phalanx using the DBM sonic 1200 to significantly increase after 1 year HRT usage in 32 women in a prospective study [27].

The lumbar spine BMD performed better than the femoral neck and total hip in this study, which is not unexpected as the lumbar spine contains approximately 66% of the more the metabolically active trabecular bone than does the femoral neck which contains approximately 25% [28]. This has also been observed in previous studies [7, 9].

The Omnisense measures predominantly cortical bone, with a SOS measurement in the range of 3800-4200m/s. However, it is possible that if the cortex of the bone is less than the wavelength of the ultrasound, the measurement may contain a mixture of cortical bone and the medullary cavity [29]. The positive effects found in this study of HRT on SOS measurements in predominantly cortical bone, which were considerably greater than those found on BMD measurements at the femoral neck and total hip, were unexpected.

The most important limitation of this study was that it was cross-sectional with self-selecting volunteers, which can create study biases. Additionally, longitudinal studies are warranted to prove the ability of SOS to monitor the effect of HRT treatment. Other limitations include the fact that many subjects within the HRT group had been receiving HRT for a number of years, with the mean duration of HRT usage being 6.5y ± 4.4y. The HRT and postmenopausal groups were not matched for age, although this was accounted for in the analysis by age adjusting the data. The subjects receiving HRT were put onto HRT by their General Practitioners for clinical reasons. These may have included having a low BMD result on a previous DXA scan, as well as other reasons, such as an early menopause, or for reducing menopausal symptoms. Whilst every effort was made to match the control and HRT populations by excluding risk factors, the study groups may still contain biases, and may not be representative of the general population.

In conclusion, a significant positive effect was found for HRT usage on SOS measurements at the radius and tibia and on BMD of the lumbar spine, with the same trend being found for phalanx and metatarsal SOS. No significant effect of HRT was found for the proximal femur BMD in this study.

References

1 WHO Study Group. Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis. 1994 Geneva WHO

2 Eastell R, Riggs B L, Wahner H W et al. Colles' Fracture and Bone Density of the Ultradistal Radius. 1989 J Bone Miner Res 4;4:607-613

3 Jergas M & Glüer C-C. Assessment of Fracture Risk by Bone Density Measurements. 1997 Sem Nucl Med 27;3:261-275

4 Hans D, Dargent-Morina P, Schott A M et al. Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. 1996 Lancet 348: 511-514

5 Bauer D C, Glüer C-C, Cauley J A et al. Broadband Ultrasound Attenuation Predicts Fractures Strongly and Independently of Densitometry in Older Women - A prospective study. 1997 Arch Intern Med 157:629-634

6 Porter R W, Miller C G, Grainger D et al. Prediction of Hip Fracture in Elderly Women: A Prospective Study. 1990 Br Med J 301:638-41

7 Duan Y, Tabensky A, DeLuca V et al. The Benefit of Hormone Replacement Therapy on Bone Mass is Greater at the Vertebral Body than Posterior Process or Proximal Femur. 1997 Bone 21;5:447-451

8 Riggs B L, Melton L J III & O'Fallon W M. Drug Therapy for Vertebral Fractures in Osteoporosis: Evidence That Decreases in Bone Turnover and Increases in Bone Mass Both Determine Antifracture Efficacy. 1996 Bone 18;3:197S-201S

9 Sahota O, San P, Cawte S A et al. A Comparison of the Longitudinal Changes in Quantitative Ultrasound with Dual-Energy X-ray Absorptiometry: The Four-Year Effects of Hormone Replacement Therapy. 2000. Osteoporosis Int 11:52-58

10 Hillard T C, Whitcroft S J, Marsh M S et al. Long-Term Effects of Transdermal and Oral Hormone Replacement Therapy on Postmenopausal Bone Loss. 1994 Osteoporosis Int 4:341-348

11 Christiansen C, Christensen M S, McNair P et al. Prevention of Early Postmenopausal Bone Loss: Controlled 2-Year Study in 315 Normal Females. 1980. Eur J Clin Invest 10:273-279

12 Stevenson J C, Cust M P, Gangar K F et al. Effects of Transdermal Versus Oral Hormone Replacement Therapy on Bone Density in Spine and Proximal Femur in Postmenopausal Women. 1990. Lancet. 336:265-69

13 Lufkin E G, Wahner H W, O'Fallon W M et al. Treatment of Postmenopausal Osteoporosis with Transdermal Estrogen. 1992 Ann Intern Med 117:1-9

14 Lindsay R. Prevention of Spinal Osteoporosis in Oophorectomised Women. 1980 Lancet 1151-1153

15 Ravaud P, Reny J L, Giraudeau B et al. Individual Smallest Detectable Difference in Bone Mineral Density Measurements. 1999 J Bone Miner Res 14;8:1449-1456

Stewart A & Reid D M. Precision of Quantitative Ultrasound: Comparison of Three Commercial Scanners. 2000 Bone 27;1:139-143

17 Stegman M R, Heaney R P & Recker R R. Comparison of Speed of Sound Ultrasound with Single Photon Absorptiometry for Determining Fracture Odds Ratios. 1995. J Bone Miner Res 10;3:346-352

18 Rosenthall L, Caminis J & Tenehouse. Calcaneal Ultrasonometry: Response to Treatment in Comparison with Dual X-ray Absorptiometry Measurements of the Lumbar Spine and Femur. 1999. Calcif Tissue Int 64:200-204

19 Balikian P, Burbank K, Houde, J et al. Bone Mineral Density and Broadband Ultrasound Attenuation with Estrogen Treatment of Postmenopausal Women. 1998 J Clin Densitometry 1;1:19-26

Lehmann R, Wapniarz M, Kvasnicka H M et al. Velocity of Ultrasound at the Patella: Influence of Age, Menopuase and Estrogen Replacement Therapy. 1993 Osteoporosis Int 3:308-313

21 Knapp K M, Singal C, Blake G M et al. Quantitative Ultrasound Measurements Detect Skeletal Changes in Cortical Bone Following HRT Use. In: Program and Abstracts, 11th International Workshop on Calcified Tissues 1999 p31.

22 Weiss M, Ben Shlomo A, Hagag P et al. Effect of Estrogen Replacement Therapy on Speed of Sound at Multiple Skeletal Sites. 2000 Maturitas 30;35(3):237-43

23 Barkmann R, Kantorovich E, Singal C et al. A New Method for Quantitative Ultrasound Measurements at Multiple Skeletal Sites. 2000 J Clin Densitometry 3,1:1-7

Sunlight. Sunlight Omnisense™ User Manual 1998 Sunlight

25 Njeh C F, Hans D, Fuerst T et al. Quantitative Ultrasound Assessment of Osteoporosis and Bone Status. Martin Dunitz Ltd, London, 1999. pp156-161.

26 Ryan P J, Blake G M & Fogelman I. Postmenopausal Screening for Osteopenia. 1992. Br J Rheumatology. 31:823-828

27 de Aloysio D, Rovati L C, Cadossi R et al. Bone Effects of Transdermal Hormone Replacement Therapy in Postmenopausal Women as Evaluated by Means of Ultrasound: an Open One-year Prospective Study. 1997 Maturitas 27:61-8

28. Bonnick S L & Lewis L A. Bone Densitometry for Technologists. Humana Press Inc 2002. pp 9.

29. Njeh C F, Hans D, Wu C, Kantorovich E, Sister M, Fuerst T & Genant H K. An In Vitro Investigation of the Dependence on Sample Thickness of the Speed of Sound Along the Specimen. 1999 Med Eng Phys 21:651-659

30. Gluer C-C, Blake G M, Lu Y et al. Accurate Assessment of Precision Errors: How to Measure the Reproducibility of Bone Densitometry Techniques. 1995 Osteoporosis Int 5:262-270

31. Blake G M, Wahner H W & Fogelman I. The Evaluation of Osteoporosis: Dual X-ray Absorptiometry and Ultrasound in Clinical Practice. 1999 Martin Dunitz Ltd.

Table 1 Patient Characteristics

	Young Normals (20-40)	Premenopausal	Postmenopausal	HRT
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Number	135	278	194	126
Age	31.73 (5.96)	37.77 (9.31)	59.91 (7.27)	55.91 (6.52)**
BMI (kg/m^2)	24.23 (4.76)	24.60 (4.58)	25.37 (3.74)*	25.52 (3.93)
Weight (kg)	66.73 (14.12)	66.04 (13.07)	66.67 (11.97)	66.49 (12.39)
Height (cm)	164.64 (6.87)	163.76 (6.51)	161.20 (9.64)*	160.72 (15.34)
Menopause Age	-	-	50.24 (3.28)	47.02 (6.23)**
YSM	-	-	9.65 (7.18)	8.42 (6.73)
SOS				
Radius (m/sec)	4105 (111)	4115 (103)	4020 (118)**	4128 (97)**
Tibia (m/sec)	3917 (110)	3904 (112)	3822 (142)**	3893 (118)**
Phalanx (m/sec)	4053 (160)	4053 (156)	3856 (194)**	3954 (187)**
Metatarsal (m/sec)	3748 (222)	3779 (207)	3580 (190)**	3663 (221)**
BMD				
Lumbar Spine (g/cm^2)	1.029 (0.123)	1.036 (0.126)	0.930 (0.142)**	0.999 (0.147)**
Femoral Neck (g/cm^2)	0.851 (0.122)	0.846 (0.119)	0.757 (0.114)**	0.785 (0.114)
Total Hip (g/cm^2)	0.920 (0.120)	0.926 (0.129)	0.877 (0.122)**	0.917 (0.129)

* p=<0.05 ** p=<0.001 when compared to the premenopausal group for the postmenopausal group and to the postmenopausal group for the HRT group

Table 2 Controls group age related changes

	Controls Annual Loss	Annual Loss/SD	% Annual Loss	Correlation Coefficient
SOS	(m/sec)	(m/sec)		
Radius	-6.8	0.061	0.16	0.39**
Tibia	-4.7	0.037	0.12	0.22*
Phalanx	-15.4	0.096	0.38	0.54**
Metatarsal	-7.7	0.035	0.21	0.26*
BMD	(g/cm^2)	(g/cm^2)		
Lumbar Spine	-0.007	0.058	0.68	0.34**
Femoral Neck	-0.007	0.057	0.82	0.42**
Total Hip	-0.006	0.050	0.63	0.37**

* p=<0.05, ** p=<0.001 significance of linear regression

Table 3 Z-score differences between the Postmenopausal Controls and HRT users group

Site	n Controls	n HRT	Mean Z-Score difference HRT Users	SE
SOS				
Radius	185	124	0.44**	0.10
Tibia	192	126	0.37*	0.10
Phalanx	130	100	0.15	0.13
Metatarsal	118	91	0.26	0.17
BMD				
Lumbar spine	190	125	0.28*	0.13
Femoral Neck	190	125	0.00	0.13
Total Hip	190	125	-0.03	0.14

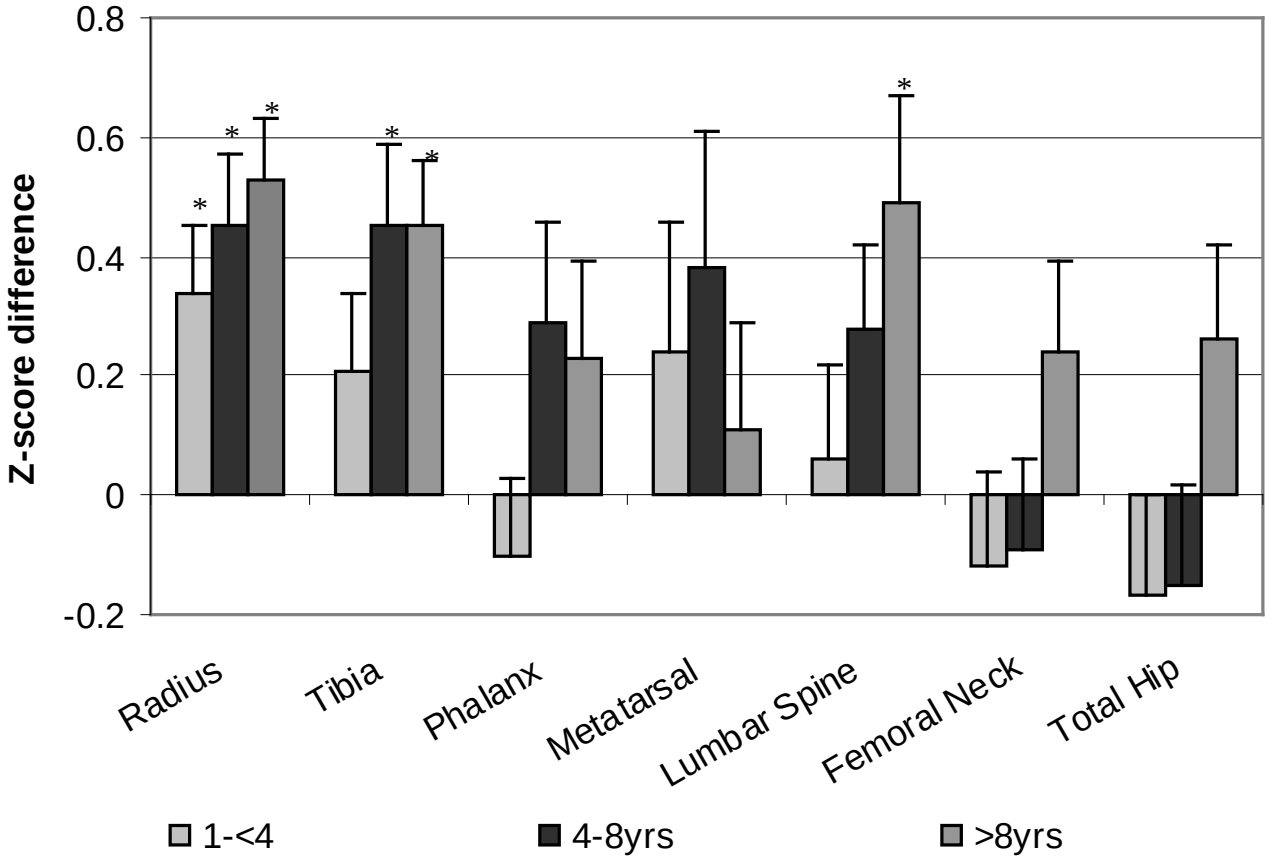
* p=<0.05, ** p=<0.001 when comparing means of the control and HRT group.

Table 4 Correlation between SOS measurement sites and between SOS and BMD measurements

	Tibia SOS	Phalanx SOS	Metatarsal SOS	L Spine BMD	Fem Neck BMD	Total Hip BMD
Radius SOS	0.35**	0.48**	0.37**	0.21**	0.15*	0.09
Tibia SOS	-	0.21**	0.14**	0.18**	0.03	0.00
Phalanx SOS	-	-	0.38**	0.26**	0.25**	0.18*
Metatarsal SOS	-	-	-	0.37**	0.38**	0.33**

* $p < 0.05$, ** $p < 0.001$.

Figure 1 Z-score differences associated with the duration of HRT use, 1-4 years, 4-8 years and > 8 years.



Error bars demonstrate SEM, * $p < 0.05$ when compared to the control group

Appendix 1

A 1: Calculation of the RMSSD using duplicate scans [30]

Where duplicate measurements are made for m individuals, the precision error can be calculated from the difference d_j between the first and the second result using the following equation:

A 2: When expressing the precision as a percentage the following formula is

$$RMSSD = \sqrt{\sum_{j=1}^{m} d_j^2 / 2m}$$

used

$$RMSCV_{SD} = \left(RMSSD / \sum_{j=1}^{m} \bar{x}_j / m \right) \bullet 100\%$$

A 3: When expressing the precision as a T-score unit the following formula is used [31]

$$\mathrm{T-score\,precision} = \left(\frac{RMS\ SD}{SD_{\mathrm{young\ adult}}} \right)$$