Genetic effects on bone loss in peri- and post-menopausal women: A longitudinal twin study

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Micro Abstract

This longitudinal twin study was designed to assess the heritability of bone loss in peri- and post-menopausal women. A sample of 724 female twins was studied. Baseline and repeat BMD measurements were performed. Results of genetic model-fitting analysis indicated genetic effects on bone loss account for around 40% of the between individual variation in bone loss at the lumbar spine, forearm and whole body.

Abstract

Background: Bone mineral density (BMD) and bone loss are important predictors of fracture risk. While the heritability of peak BMD is well documented, it is not clear whether bone loss is also under genetic regulation. The present study was designed to assess the heritability of bone loss in peri- and post-menopausal women.

Subjects and Methods: A sample of 724 female twins (177 monozygotic (MZ) and 185 dizygotic (DZ) pairs), age 45-82 was studied. Each individual had baseline BMD measurements at the lumbar spine, hip, forearm and total body by dual energy x-ray absorptiometry and at least one repeat measure, on average 4.9 years later. Change in BMD (ΔBMD) was expressed as percent gain or loss per year. Intraclass correlation coefficients for ΔBMD were calculated for MZ and DZ pairs. Genetic model-fitting analysis was conducted to partition the total variance of ΔBMD into three components: genetic (G), common environment (C), and specific environment, including measurement error (E). The index of heritability was estimated as the ratio of genetic variance over total variance.

Results: The mean ($\pm$ SD) of annual ΔBMD was –0.37% ($\pm$1.43) per year at the lumbar spine, -0.27 % ($\pm$ 1.32) at the total hip, -0.77% ($\pm$ 1.66) at the total forearm, -0.36% ($\pm$ 1.56) at the femoral neck and -0.16 % ($\pm$ 0.81) at the whole body. Intra-class correlation coefficients were significantly higher in MZ than in DZ twins for all studied parameters, except at the hip sites. Results of genetic model-fitting analysis indicated that the index of heritability for ΔBMD was 0.38, 0.49, and 0.44 at the lumbar spine, total forearm and whole body, respectively. However, the genetic effect on ΔBMD at all hip sites was not significant.

Conclusions: These data suggest that although genetic effects on bone loss with ageing are less pronounced than on peak bone mass, they still account for around 40% of the between individual variation in bone loss at the lumbar spine, forearm and whole body in peri- and post-menopausal women. The finding is also relevant for studies aimed at identification of genes that are involved in the regulation of bone loss.

Key Words: BMD, Bone loss, Genetics, Twins, HRT

Introduction

Osteoporosis is a common multifactorial disorder of reduced bone mass associated with micro-architectural deterioration of bone tissue leading to bone loss and increased bone fragility. The major consequence of bone loss is fracture. Typical osteoporotic fractures involve the proximal femur, thoraco-lumbar vertebral bodies and distal forearm, although many bones may be affected [1-4].

Low bone mineral density (BMD) is considered the hallmark of osteoporosis. Bone density measurements are valuable predictors of the risk of low-trauma fractures [5]. Bone loss is highly variable among individuals, with the typical standard deviation being between 2 and 3-fold higher than the mean rate of loss [6-8]. BMD at any given age is determined by the relative contributions of peak bone mass achieved and subsequent bone loss. It has been suggested that the relative contributions of peak bone mass and bone loss to BMD at age 70 are equal [9]. This implies that BMD in older women is significantly influenced by the rate of bone loss and bone turnover with advancing age. In support of this hypothesis, a cross-sectional twin study specifically selected for older female subjects (mean age 68 years) estimated additive genetic effects accounted for 75% of residual variation in spine and hip BMD after adjusting for environmental factors like smoking and alcohol intake [10].

While the heritability of peak BMD is well documented in many family [11-19] and twin [20-26] studies, there is no clear evidence that the variation of bone loss is under genetic regulation [27]. A number of studies have attempted to dissect the genetic effect on rate of bone loss [28-30]. Only a few prospective studies on heritability of bone loss have been reported in humans [17, 31-34]. No evidence of a genetic component to loss of BMD at the midshaft of the radius was found by Christian and colleagues in 25 MZ and 21 DZ older twin men (mean age 63 years) followed over a 16-yr period [31]. However, although the length of the study period was sufficient to detect significant bone loss, the sample size was small and the skeletal site measured is not a typical site of osteoporotic fracture in men or women. Moreover, in a study of older female twins there was no significant heritability of BMD at the distal forearm, suggesting that environment could be more powerful than genetic effects at this site with ageing [10]. Indeed, although the study of Christian found significant within-pair correlations for both identical (monozygotic or MZ) and non-identical (dizygotic or DZ) twins at the mid-shaft of the radius (intra-class correlations of 0.62 and

0.48 respectively), bone loss correlated with environmental factors such as smoking and alcohol intake and, after adjustment for these covariates, it was concluded that changes in bone density were more influenced by factors common within twin pairs such as common environmental influences rather than genetic factors [31]. There were other limitations to this study apart from the small sample size. Although the period of follow-up was long (16 years), bone loss at the mid-shaft site was low (0.45% per year) with an overall loss of 6.9%, one half the rate reported in postmenopausal women at the same site [35]. In contrast, another small twin study observed genetic influences on change in BMD at the spine and hip in 21 MZ and 19 DZ twins measured over a mean three year period [33]. However, the period of study was relatively short, ranging from only 1–5 years, the subjects were a mixture of men, premenopausal and postmenopausal women who were largely not losing bone, and the age range was wide, extending from 25–65 years.

Thus, there are no current studies in the literature that adequately address the important question of whether the rate of bone loss is heritable at skeletal sites where osteoporotic fractures are common. The present study was designed to assess the heritability of bone loss in peri- and post-menopausal women, an age group in which bone loss is more readily measured.

Materials and methods

Subjects

Study subjects were female twin pairs with multiple visits, recruited as part of the Sydney Twin Study, which has been running at the Department of Rheumatology of Royal North Shore Hospital, and the Twin and Sisters Study at the Royal Melbourne Hospital. The twins were recruited through the Australian National Health and Medical Research Council (NHMRC) Twin Registry and from local media campaigns. Twins were invited to participate in an investigation into the genetic and environmental determinants of various diseases including osteoarthritis, cardiovascular disease, asthma, and osteoporosis. The hospitals' Human Research Ethics Committees approved the study. After providing written informed consent, each twin was interviewed separately in accordance with a standard questionnaire to collect demographic, lifestyle and medical history data. Except for hormone therapy, twins who used medications or who had medical conditions that could interfere with bone metabolism were excluded from the analysis. Hormone therapy use was

recorded and included as a covariate in the analyses. Zygosity in like-sex twins was determined from the twins' self-report using questions from a validated questionnaire [36]. DNA fingerprinting was used to determine zygosity in twin pairs in which their zygosity was either unknown or disputed.

Bone Mineral Density Measurements

Baseline characteristics included age, height (m), weight (kg), BMI (Wt/Ht^2), and menopausal status for women. Lumbar spine (L1-L4), and hip were scanned by fan beam dual-energy X-ray absorptiometry (DEXA) machine (QDR 4500W or QDR 1000W, Hologic, Waltham, MA. USA). Twins within each pair were always scanned using the same densitometer. Bone mineral density (BMD) of lumbar spine, total hip, forearm and whole body were obtained from DEXA scans using standard protocols as previously described [37, 38]. Change in BMD (ΔBMD) was expressed as percent gain or loss per year.

Statistical Analysis

Twin resemblance for a variable trait was assessed for MZ and DZ twin pairs separately by intra-class correlation analysis. In this method, the total variation (about the mean) of a trait was partitioned into two sources: between-pairs (B) and within-pairs (W). The correlation was estimated as the difference between the two sources over their sum, i.e., (B-W)(B+W). The test for significant difference between the coefficients of MZ and DZ was based on the modified Fisher's z-transformation procedure [39].

To estimate the heritability (proportion of variance of a trait attributable to genetic factors), we analysed the data according to the classical twin model [40]. In this model, the variance of a variable trait is partitioned into genetic and environmental components. The genetic variance may be due to additive (A) or dominant (D) genetic influences. The environmental variance may be due to environmental factors shared by twins (common environment, C) and to the non-shared environmental factors (E). Shared environmental effects and dominant genetic effects cannot be assessed simultaneously as they are completely confounded in the classical twin models. Additive genetic factors are the effects of genes taken singly and added over multiple loci, whereas dominant genetic factors

represent genetic interaction between loci. The classical twin model assumes that additive genetic factors and dominant genetic factors are perfectly correlated in MZ pairs, while DZ pats, like ordinary siblings, share only one half of the additive genetic effects and one quarter of the dominant genetic effects (Figure 1). The model also assumes that shared environmental effects are perfectly correlated in both MZ and DZ twins; that the effects of assortive mating, epistasis, and the genotype-environmental interaction and/or correlation are negligible; and that shared environmental influences are similar for MZ and DZ twins. The influences of A, D, C, and E on the phenotype are represented by the parameters *a, d, c* and *e*, respectively, which are equivalent to the standardized regression coefficients (Figure 1). The amount of variance due to each source is the square of these parameters. To estimate *a, d, c* and *e*, for each variable trait, the data were summarized into 2 x 2 variance-covariance matrices. The matrices were then subject to analysis specified by five possible models incorporating different combinations of these factors, namely, E, CE, AE, ACE, and ADE. The maximum likelihood method was used to estimate model parameters. Selection of the best model was based on the difference between likelihood ratio chi-square goodness-of-fit tests. The index of heritability was obtained as the square of the parameter *a* from the most parsimonious model. Modelling was performed with adjustment for HRT use as well as a separate analysis excluding these subjects.

Results

The characteristics of the 724 peri- and postmenopausal female twins who participated in the study are presented in Tables 1 and 2. There were 177 MZ and 185 DZ pairs. There were no significant differences between MZ and DZ twins in age, baseline BMD or dietary calcium intake or exercise (data not shown). There were significant differences in height and body mass index which were adjusted for in the analysis. The age of the twins ranged from 45 to 82 years at the initial visit (mean age 56.2, SD 8.0). The average time between two BMD measurements was 4.9 years (range 1 –10 years). There were 121 peri-menopausal and 603 postmenopausal female twins. During follow-up, the mean percent ($\pm$ SD) overall BMD loss was -1.46 $\pm$ 4.93 at the lumbar spine, -1.30 $\pm$ 4.35 at the total hip, -1.61 $\pm$ 4.90 at the femoral neck, -2.98 $\pm$ 4.07 at the forearm, and -0.77 $\pm$ 3.34 at the whole body. There was no significant difference in ΔBMD between MZ and DZ twins. Bone loss was greater in twins aged 40-60 than those aged greater than 60 at most sites (data not shown). The most rapid bone loss was observed for the total and distal forearm -0.77 $\pm$ 1.66 % per year and the slowest rate of change was present in whole body BMD -0.16 $\pm$ 0.81 % per year. Proximal site of the forearm did not show any bone loss. ΔBMD was weakly correlated with baseline BMD at most sites (r = –0.140; -0.135; -0.135 for lumbar spine, femoral neck and whole body, respectively). Women taking HRT for greater than 12 months (265 subjects) showed significantly (p < 0.05) slower rates of bone loss (-0.89 $\pm$ 5.06 at the lumbar spine, -0.42 $\pm$ 4.29 at the total hip, -1.13 $\pm$ 4.94 at the femoral neck, -2.71 $\pm$ 4.82 at the forearm, and -0.37 $\pm$ 3.37 at the whole body) compared to those who had never taken it (-1.76 $\pm$ 4.31 at the lumbar spine, -1.79 $\pm$ 4.31 at the total hip, -1.89 $\pm$ 4.87 at the femoral neck, -3.14 $\pm$ 3.57 at the forearm, and -0.98 $\pm$ 3.30 at the whole body).

The results of intra-class correlation analysis for MZ and DZ twins are presented in Figures 2a-c. The correlations were higher in MZ pairs than in DZ pairs for all measured parameters, consistent with significant genetic influence on these traits. No significant intraclass correlations were found for annual changes in any of the hip or proximal forearm BMD measurements.

The results of the twin model analyses are presented in Table 3. The maximum likelihood method was used to estimate model parameters. Selection of the best model was

based on the difference between likelihood ratio chi-square goodness-of-fit tests. For lumbar spine, total forearm and whole body, AE models gave the best fit. The indices of heritability (A) were 38%, 49% and 44% for annual changes in lumbar spine, total forearm and whole body ΔBMD, respectively when all twins were included. Adjustment for hormone therapy use did not significantly alter these results (Table 3). When HRT users were excluded from the analysis, only lumbar spine heritability remained significant but the power was reduced at the forearm and whole body sites in this analysis.

Discussion

The results of the present study suggest that although genetic effects on bone loss with ageing are less pronounced than those on peak bone mass, they still account for around 40% of the between individual variation in bone loss at the lumbar spine, forearm and whole body in peri and postmenopausal women.

Susceptibility to osteoporosis is largely genetically determined, and it is likely that many genes are involved, each having a small effect [41]. Bone density after young adulthood is determined by peak bone mass and subsequent bone loss [9]. Cross-sectional studies in twins [20-23] and families [11-16, 18] have shown that peak bone mass is largely influenced by genetic factors . A cross sectional design however cannot directly assess the magnitude of bone loss heritability. Although bone density may be affected by many genes at different skeletal sites and in different age groups, it is likely that the magnitude of individual genetic effects differs in different populations and in different environmental settings [22]. The San Antonio Family osteoporosis study on Mexican American extended families showed similar results on heritability of bone loss to ours [17]. Our study is the first large longitudinal twin genetic epidemiological study to directly quantify the genetic versus environmental components of bone loss variance in peri- and postmenopausal women. Previous twin studies that have measured longitudinal changes in BMD were much smaller studies in men or younger subjects and the findings were not consistent [31-33]. A longitudinal study of sisters did find a significant genetic influence on change in femoral neck BMD [34], but the study subjects were premenopausal (mean age 35.3 years) and it was unclear whether this effect persists across the menopause. Our findings in the total hip and femoral neck sites suggest it does not. Greater environmental effects at the hip may decrease the power to show genetic effects at that site.

The benefits of defining the genes causing bone loss and subsequent osteoporotic fractures include identification of individuals who are at greater risk and a better understanding of the disease pathophysiology, which will facilitate the finding of novel therapeutic or preventative targets. Whether genetic tests can actually predict who are at risk of developing osteoporosis is uncertain. In theory, if all of the genes that cause the disease can be identified, and their interaction with each other and with environmental factors understood, then heritability figures from twin studies and family studies suggest that this information will be useful in predicting those who are at risk. However, the depth of our knowledge currently falls far short of this goal. Genes that have been implicated, to date, in osteoporosis make only minor contributions individually to bone density and fracture risk, and are not yet of great clinical value. Because osteoporosis is a polygenic disease, predictive tests are likely to involve the typing of several genes, and tests for single genes are less likely to be of clinical significance. However our data suggest that studies aimed at identification of genes that are involved in bone loss should use lumbar spine or forearm bone loss as the preferred phenotypes, with hip bone loss being less likely to show bone loss influenced by genetic factors.

Our study has several strengths and limitations. The mean annual BMD loss in our study was lower than reported in some previous longitudinal studies [6-8, 42-44]. However these high rates of bone loss have mainly been observed in the elderly and our observed rates of loss are similar to other studies of peri- and postmenopausal women [44-46]. Moreover, rates of bone loss may be influenced by multiple factors including age, body composition and environmental factors. A large percentage of our twins had exposure of greater than 12 months to estrogen therapy but adjusting for hormone use did not affect the results. Moreover, when the analysis was confined to the twins who had never had HRT, a significant effect of heritability on bone loss was still evident in the lumbar spine. Because baseline BMD is used to calculate rate of loss, bone loss rate can never be truly independent of baseline BMD [27]. Although this phenomenon may account for the observation of faster bone loss in women with higher BMD, the effect of baseline BMD appears to be small [47]. In a re-analysis, controlling for baseline BMD, of 75 women followed for 9.5 years, it was estimated 67% of postmenopausal variation was attributable to premenopausal BMD, whereas 29% was attributable to the bone loss rate [27]. Our figure of around 40% heritability in bone loss would not be inconsistent with that analysis. Our study sample is 10

fold larger than previous longitudinal twin studies and of satisfactory duration (mean 5 years follow-up), which should diminish the effects of measurement error related to small changes in BMD over time. Inferring a genetic aetiology by contrasting MZ and DZ twins rests on the assumption that the twins share a common family environment to the same extent. This assumption may not hold for a number of environmental variables that might affect bone loss such as exercise and smoking [48]. However the effects of these covariates are likely to be modest.

In conclusion, our data suggest that although genetic effects on bone loss with ageing are less pronounced than on peak bone mass, they account for around 40% of the between-individuals variation in bone loss at the lumbar spine, forearm and whole body in pre- and post-menopausal women. These findings provide a rational basis for the identification of genes that are involved in the regulation of bone loss.

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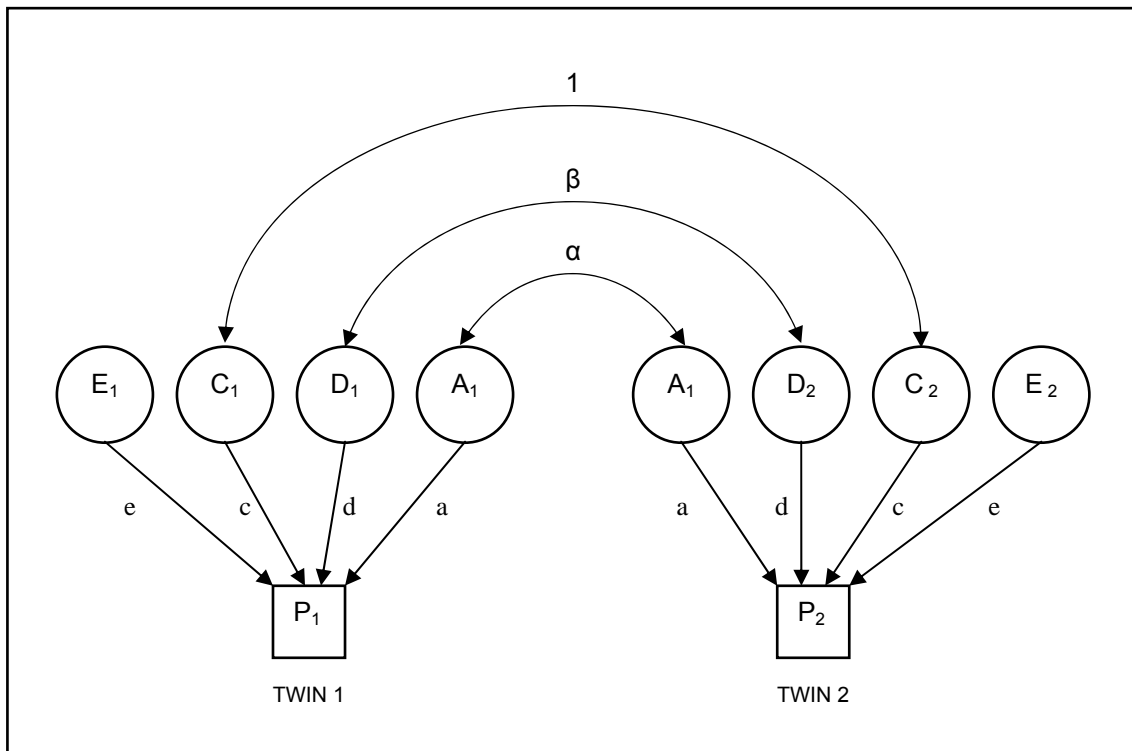
Tables and Figure

Figure 1. Path diagram for MZ and DZ twins measured on a single phenotype. Each phenotype (P1, P2) is caused by a linear combination of latent additive genetic (A), dominant genetic (D), common environmental (C) and unique environmental (E) variables. Each latent variable is standardized (i.e. has mean of zero and a variance of one) and the path coefficients of each latent variable on the observed phenotypes are estimated (i.e. a, d, c, e). From biometrical genetics theory, the additive genetic correlation between pairs (a) is 1 for MZ twins and 0.5 for DZ twins. The correlation between dominance variance components (b) is 1 for MZ twins and 0.25 for DZ twins. The correlation between common environmental effects is one for MZ and DZ twins by definition.

Table 1. Baseline anthropometric and lifestyle characteristics of twins

	N	MZ Twins Mean ± SD	N	DZ Twins Mean ± SD
Age (years)	354	56.2± 8	370	56.1 ± 8
Time between scans (years)	354	5.25 ± 2.21	370	4.57 ± 2.24*
Weight (kg)	354	64.88 ± 10.93	370	67.61 ± 12.99*
Height (cm)	354	160.09 ± 6.82	370	160.57 ± 6.25
BMI (kg/m^2)	354	25.35 ± 4.16	370	26.21 ± 4.76*
Menopausal Status:				
Peri-menopausal	53		68	
Post-menopausal	301		302	
Status changed during the study	41		45	
Hormone Replacement Therapy:				
Never taken for more than 6 months	132		133	
Ever taken for more than 6 months	222		237	

Table 2. Baseline bone mineral density measurements and rate of bone loss

	N	MZ Twins Mean ± SD	N	DZ Twins Mean ± SD
Baseline BMD (g/cm^2)				
Lumbar Spine (L1-L4)	354	0.98 ± 0.16	370	0.98 ± 0.16
Total hip	352	0.89 ± 0.12	370	0.90 ± 0.14
Femoral Neck	352	0.75 ± 0.12	370	0.76 ± 0.12
Total Forearm	354	0.53 ± 0.06	369	0.53 ± 0.06
Whole Body	352	1.07 ± 0.11	360	1.07 ± 0.12
Average overall change in BMD (%)				
Lumbar Spine (L1-L4)	354	-1.69 ± 5.11	370	-1.23 ± 4.73
Total hip	352	-1.43 ± 4.68	370	-1.19 ± 4.01
Femoral Neck	352	-1.86 ± 5.01	370	-1.38 ± 4.80
Total Forearm	347	-3.27 ± 4.65	365	-2.72 ± 3.40
Whole Body	324	-0.80 ± 3.59	295	-0.73 ± 3.04
Average annual change in BMD (% per year)				
Lumbar Spine (L1-L4)	354	-0.40 ± 1.41	370	-0.35 ± 1.44
Total hip	352	-0.21 ± 1.41	370	-0.33 ± 1.22
Femoral Neck	352	-0.38 ± 1.45	370	-0.34 ± 1.67
Total Forearm	347	-0.82 ± 2.19	365	-0.72 ± 0.91
Whole Body	324	-0.16 ± 0.80	295	-0.17 ± 0.82

* $p < 0.05$

Figure 2a. Intraclass correlations for annual change in Lumbar Spine BMD
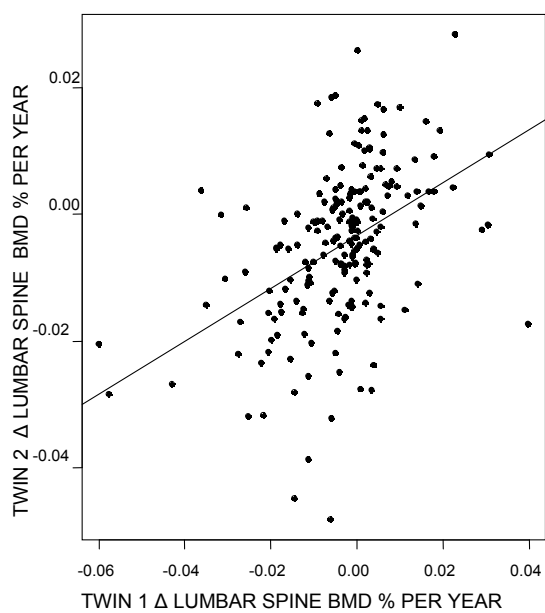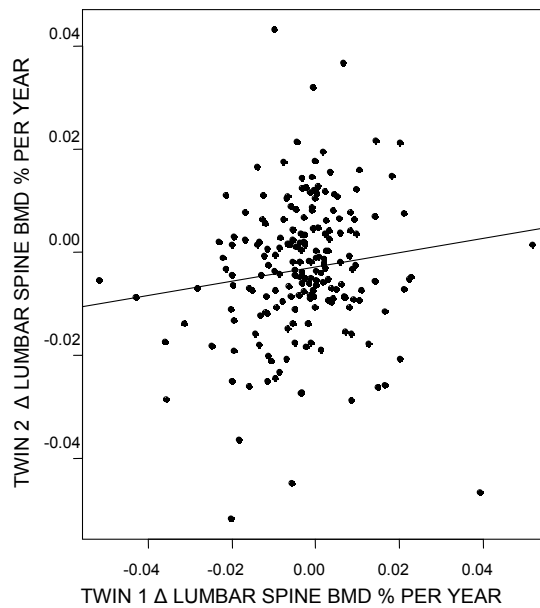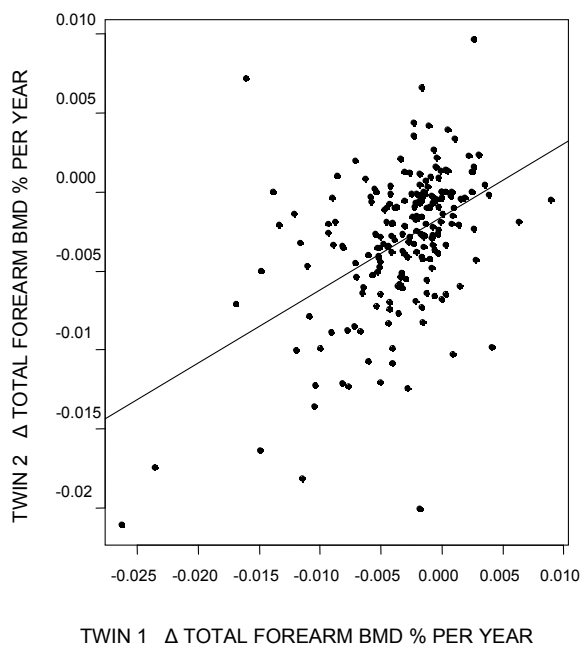

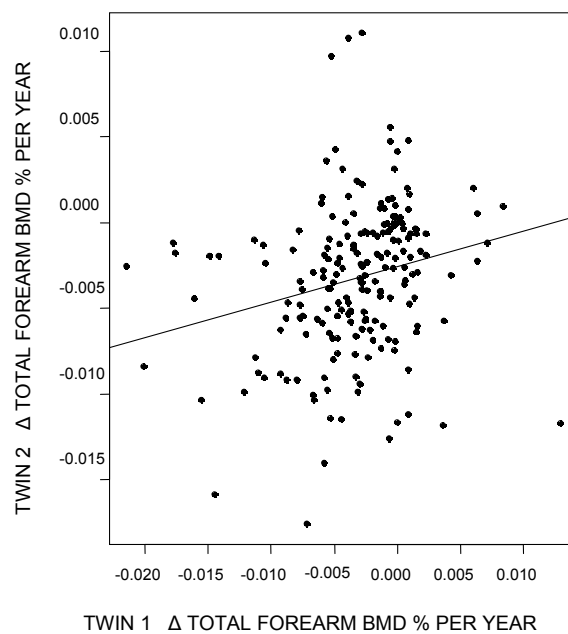MZ TWINS (r=0.44)   DZ TWINS (r=0.22)

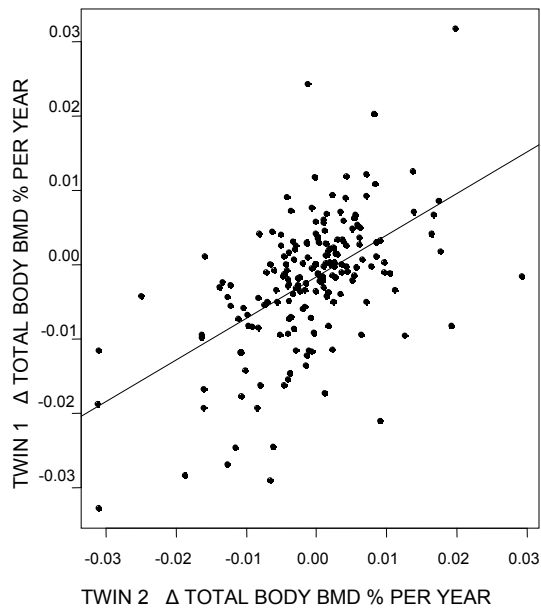Figure 2b. Intraclass correlations for annual change in Total Forearm BMD

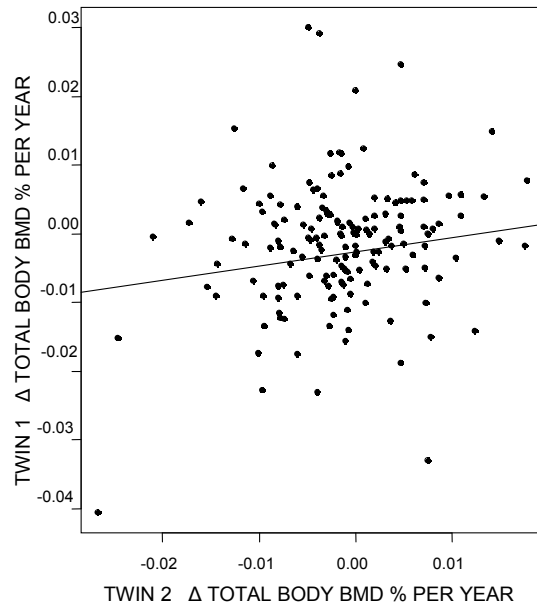

MZ TWINS (r=0.47)   DZ TWINS (r=0.20)

Figure 2c. Intraclass correlations for annual change in Whole Body BMD

MZ TWINS (r=0.47)   DZ TWINS (r=0.19)

Table 2. Standardised parameter estimates for HRT-user inclusive and exclusive models.

	Average % per year	Not adjusted for HRT		Adjusted for HRT	
		Squared standardised coefficients			
		A Estimate (95% C.I.)	E Estimate (95% C.I.)	A Estimate (95% C.I.)	E Estimate (95% C.I.)
All twins over 45 including HRT users 362 twin pairs (177 MZ pairs 185 DZ pairs)	Lumbar spine	**0.384** (0.043 - 0.551)	**0.553** (0.449 - 0.678)	**0.376** (0.036 - 0.552)	**0.551** (0.448 - 0.675)
	Total Forearm	**0.487** (0.211 - 0.584)	**0.513** (0.416 - 0.628)	**0.490** (0.188 - 0.585)	**0.510** (0.415 - 0.626)
	Whole body	**0.442** (0.171 - 0.549)	**0.559** (0.451 - 0.682)	**0.447** (0.165 - 0.553)	**0.553** (0.447 - 0.676)
No HRT users 186 twin pairs (91 MZ pairs 95 DZ pairs)	Lumbar spine	**0.501** (0.089 - 0.693)	**0.420** (0.308 - 0.576)		

HRT – Hormone Replacement Therapy
C.I. – Confidence Intervals