Author(s): Mikkola, Tuija; Sipilä, Sarianna; Rantanen, Taina; Sievänen, Harri; Suominen, Harri; Kaprio, Jaakko; Koskenvuo, Markku; Kauppinen, Markku; Heinonen, Ari

Title: Genetic and environmental influence on structural strength of weight-bearing and non-weight-bearing bone: a twin study

Year: 2008

Version:

Please cite the original version:

Mikkola, T., Sipilä, S., Rantanen, T., Sievänen, H., Suominen, H., Kaprio, J., Koskenvuo, M., Kauppinen, M., & Heinonen, A. (2008). Genetic and environmental influence on structural strength of weight-bearing and non-weight-bearing bone: a twin study. Journal of Bone and Mineral Research, 23(4), 492-8. https://doi.org/10.1359/jbmr.071205

Genetic and Environmental Influence on Structural Strength of Weight-Bearing and Non-Weight-Bearing Bone: A Twin Study

Tuija Mikkola[1,2], Sarianna Sipilä[2], Taina Rantanen[1], Harri Sievänen[3], Harri Suominen[1], Jaakko Kaprio[4,5], Markku Koskenvuo[4], Markku Kauppinen[2], Ari Heinonen[1]

[1]Department of Health Sciences and [2]Finnish Centre for Interdisciplinary Gerontology, University of Jyväskylä, Jyväskylä, Finland, [3]Bone Research Group, UKK Institute for Health Promotion Research, Tampere, Finland, [4]Department of Public Health, University of Helsinki, Helsinki, Finland, [5]Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland

Funding Sources: The Finnish Ministry of Education, Peurunka Rehabilitation and Exercise Foundation, University of Jyväskylä, Juho Vainio Foundation, Academy of Finland Center of Excellence in Complex Disease Genetics

Running title: Genes, Environment and Bone Strength

Email addresses: Tuija Mikkola: tuija.mikkola@sport.jyu.fi, Sarianna Sipilä: sipila@sport.jyu.fi, Taina Rantanen: taina.rantanen@sport.jyu.fi, Harri Sievänen: harri.sievanen@uta.fi, Harri Suominen: harri.suominen@sport.jyu.fi, Jaakko Kaprio: Jaakko.Kaprio@helsinki.fi, Markku Koskenvuo: markku.koskenvuo@helsinki.fi, Markku Kauppinen: markku.kauppinen@sport.jyu.fi, Ari Heinonen: ari.heinonen@sport.jyu.fi

Corresponding author:

Tuija Mikkola

University of Jyväskylä

Department of Health Sciences

P.O. Box 35 (Viveca)

FIN-40014 University of Jyväskylä

Finland

Tel. +358 14 260 2149

Fax +358 14 260 4600

Email: tuija.mikkola@sport.jyu.fi

Number of words in abstract: 341

Number of words in manuscript: 5940 ; Number of characters in manuscript: 32850

Number of figures: 2 black/white

CONFLICT OF INTEREST

All authors have no conflicts of interest.

A bivariate genetic analysis among 217 older female twin pairs showed that although the structural strength of tibia and radius are mainly regulated by same genetic and environmental factors, the tibia is more affected by environment.

ABSTRACT

Introduction The habitual loading environment of the bone may modulate the relative contribution of genetic and environmental factors to bone structure. The purpose of this study was to estimate the contribution of the common and site-specific genetic and environmental factors to inter-individual variation in compressive structural strength of the weight-bearing tibia and non-weight-bearing radius.

Methods Peripheral quantitative computed tomography scans were obtained from both members of 103 monozygotic (MZ) and 114 dizygotic (DZ) 63 to 76-year-old female twin pairs to estimate the compressive strength of the distal tibia and distal radius. Quantitative genetic models were used to decompose the phenotypic variance into additive genetic, shared environmental and individual environmental effects at each bone site, and to investigate whether these bone sites share genetic or environmental effects.

Results The MZ and DZ twins did not differ in mean age, height, weight or bone structural strength. The age-adjusted Cholesky model showed that additive genetic factors accounted for 83% (95% CI 77-88%) of the variance in radial strength and 61% (52-69%) of the variance in tibial strength and these were fully correlated. A shared environmental factor accounted for 15% (10-20%) of tibial strength. An individual environmental factor accounted for 17% (12-23%) of the variance in radial strength and 10%

(5-17%) of the variance in tibial strength. The relative contribution of an individual environmental factor specific to tibial strength was 14% (11-18%).

Conclusions The results suggest that in older women, the majority of the individual differences in the compressive structural strength of the forearm and leg are regulated by genetic and environmental factors that are common to both bone sites. However, the relative importance of environmental factors was greater for the weight-bearing tibia than for the non-weight-bearing radius. Thus, the heritability of bone strength seems to vary between skeletal sites according to differences in the typical loading environment.

Key words: Osteoporosis, Bone strength, Heritability, Mechanical loading, Aging

INTRODUCTION

Osteoporotic fractures, which mainly affect older people, are a major health problem world-wide. [1] For the development of preventive strategies for fractures it is important to understand factors underlying individual differences in bone fragility. Thus far, both genetic and environmental factors have been shown to influence bone properties, but the contribution of genes has usually been reported to be larger than that of the environment, varying in different studies from 40% to 99%. [2-9]

Previous studies on the heritability of bone phenotypes have predominantly focused on areal bone mineral density (aBMD).[3, 7, 8, 10-13] However, due to its planar nature aBMD is not able adequately to capture the most important aspects of bone structural strength, i.e., material properties, three-dimensional architecture and geometry of bone.[14] Indeed, changes in bone geometry through the redistribution of bone mineral may remain undetected by aBMD.[15] Genetic influences on bone geometry, such as cross-sectional area, estimated with dual-energy X-ray absorptiometry (DXA), have been studied recently.[4, 9] However, analyses of phenotypes of true bone geometry, such as those measured with peripheral quantitative computed tomography (pQCT), might provide more precise estimates of heritability of bone geometry.

Mechanical loading is one of the most important environmental factors in terms of bone strength and is considered to be a major regulator of bone properties.[16] Different bone sites have very different daily loading environments; customary daily loading on the lower limb bones is mostly of the compressive type and is caused by locomotion accompanied by body weight, whereas the upper limb bones are not typically subjected to such loads at all. Interestingly, some athlete studies have suggested different responses in upper and lower limb bones to altered loading.[17, 18] In addition, in the absence of gravity during a space flight bone mineral is lost in the lower limbs but not in the upper limbs.[19] Therefore,

different combinations and overall relative proportions of genetic and environmental effects may underlie the properties of bone in differently loaded skeletal sites. The aim of this study was to evaluate the relative contribution of genetic and environmental effects to individual differences in estimated bone compressive strength in older women at two structurally similar bone sites, i.e. the distal site of the tibia in the weight-bearing lower limb and the distal site of the radius in the non-weight-bearing upper limb. We also aimed at investigating to what extent the compressive strength of these skeletal sites share genetic and environmental factors.

METHODS

Subjects

The present study is a part of the Finnish Twin Study on Aging (FITSA), a study on the genetic and environmental influences on the disablement process in older women. The participants were recruited from the nationwide Finnish Twin Cohort which comprises all same-sex twin pairs born before 1958 and with both co-twins alive in 1975.[20, 21] In the age group of 63–76 years, there were 1,260 respondent female pairs. An invitation to participate in the study was sent, on the basis of age and zygosity, to 414 twin pairs aged 63-76 years. To be included in the study, both members in a pair had to agree to participate. Reasons for nonparticipation were refusal (106 pairs), poor health status (85 pairs), or death (6 pairs) of one or both twin sisters. The zygosity of the twin pairs was confirmed using a battery of 10 highly polymorphic gene markers in DNA extracted from a venous blood sample. Finally 103 monozygotic (MZ) and 114 dizygotic (DZ) twin pairs arrived at the laboratory where clinical examination and several tests of health and functional capacity were performed. On arrival, the participants provided a written informed consent. The study was approved by the ethics committee of the Central Finland Health Care District.

Bone assessments

Peripheral quantitative computed tomography (pQCT) (XCT 2000, Stratec Medizintechnik GmbH, Pforzheim, Germany) scans were obtained from the distal tibia and distal radius on the side of the dominant hand. The distal tibia was scanned at 5 % of the measured tibial length proximal to the distal end of the tibia and the distal radius at 4% of the measured segment length proximal to the distal end of the radius. The radius was successfully scanned and analyzed in 191 MZ and 210 DZ individuals. The reasons for missing bone measurements or analyses of the radius were: previous fracture at the scanned site (n=29), substantial movement artefacts (2), and inability of the bone analysis to separate bone from the surrounding tissues (very low volumetric bone mineral density) (2). The data on the tibia were obtained from 196 MZ and 216 DZ individuals. The reasons for missing bone measurements or analyses were: substantial movement artefacts (n=7), leg did not fit into the opening of the pQCT device (7), inaccurate positioning of the leg (3), metal in the tissues in the scanned region (2) and inability to perform the measurement (3). The analysis of the pQCT images was performed with software designed for analyzing cross-sectional CT images (Geanie 2.1, Commit; Ltd, Espoo, Finland). To separate the bone from the surrounding soft tissues, a density threshold of 130 mg/cm^3 was used for both bone sites. The main outcome was compressive bone strength index, BSIc (g^2/cm^4). To be able to calculate BSIc, total cross-sectional area (ToA, mm^2, including the bone marrow area) and total volumetric bone mineral density (ToD, mg/cm^3) were analyzed. BSIc was calculated as ToD^2 · ToA, where the first term denotes the apparent compressive strength of bone tissue (~ a material property) and the latter the load-bearing cross-sectional area.[22, 23]

Diseases, medication and physical activity

Self-reports of acute and chronic diseases and medication had been obtained earlier by a questionnaire and were confirmed by a physician during the clinical examination. Those who reported using hormone replacement therapy (HRT) currently or had used it for at least one year over the last 6 years were

considered to be HRT users. Those who reported taking systemic corticosteroid treatment currently or who had taken it for at least one year during the last six-year period were classified as corticosteroid users.

Those reporting no other physical activity but light walking no more than two times a week at the most were rated as sedentary in the classification of current physical activity. Those reporting walking or other light exercise at least three times a week, or moderate intensity exercise up to two times a week, were rated as moderately active. If a participant reported moderate or vigorous exercise at least three times per week, she was rated as active.[24]

Data analysis

The equality of the means of the continuous variables and the equality of the distributions of the categorical variables between the MZ and DZ twins were analyzed with the adjusted Wald test and the equality of variances was tested with the variance ratio test, taking into account the dependence of observations between the co-twins (Stata 8.0, Stata Corp.). The within-pair resemblance in each bone characteristic was estimated separately for the MZ and DZ groups using intra-class correlation coefficients (ICC) (SPSS 14.0, SPSS Inc.). These correlations give indicative estimates of the genetic and environmental components of the variances.[25]

Univariate genetic analyses were carried out to evaluate the genetic and environmental contributions to each bone characteristic separately (ToD, ToA, BSIc) for the radius and the tibia. In these analyses the total variance can be decomposed into additive genetic effects (A), nonadditive genetic effects (D), shared environmental effects (C) and individual environmental effects (E). A refers to the sum of the effects of the individual alleles over the loci, whereas D refers to interactions between alleles at the same or different loci. C includes factors that are shared by both twins, such as those related to their

childhood environment, and E consists of exposures that are not shared by the co-twins, such as diseases and accidents that have affected only one sibling. The genetic analyses in twin design are based on the phenotypic resemblance of MZ and DZ twins. The information that MZ co-twins share 100% of their genes whereas DZ co-twins share on average 50% of their genes is used in the analysis. Further, both in MZ and DZ pairs the within-pair correlations of C (r=1) and E (r=0) are equal while the correlation of D is different in MZ (r=1) compared to DZ (r=0.25) co-twins.[25] The possible combinations of the different effects that can be tested in the genetic models are the full models (ACE and ADE) and their submodels (AE, CE and E). The model with D but not A (DE) is biologically implausible and hence not tested, while D and C cannot be estimated in the same model (ADCE) using data that consists of twin pairs reared together.[26] The alternative univariate models obtained were compared against the full model (ACE or ADE) by Akaike's information criterion (AIC = -2 x log-likelihood - 2 x degrees of freedom), which is smaller for better fitting models, and by the p-value of the χ^2 difference between models.

The preliminary analyses of the effect of rheumatoid arthritis, cerebrovascular disease, HRT usage, corticosteroid usage, previous fractures, hip or knee osteoarthritis and smoking on radial and tibial compressive strength were performed using linear regression analysis (SPSS 14.0). Only those predictors with $\beta \geq 0.1$ were left in the model. To adjust for the effect of these predictors on the genetic and environmental proportions, the residuals of the regression model were used as input data in the univariate analyses.

Bivariate genetic analyses utilize the cross-twin cross-trait covariances within MZ and DZ pairs.[27] A bivariate Cholesky model[28] was used to evaluate to what extent common and site-specific genetic and environmental factors influence radial and tibial structural compressive strength. This structural equation model consists of the genetic and environmental effects (A_1, C_1, E_1) that are common to both

variables (radial and tibial structural compressive strength) and of the genetic and environmental effects (A_2, C_2, E_2) that are specific to the second variable (tibial structural compressive strength). The analysis was started with the hypothetical full ACE bivariate model. To obtain a more parsimonious model, the full model was modified by dropping the nonsignificant or small parameters one by one.

The univariate and multivariate genetic analyses were performed with Mx software[29] using the full information maximum likelihood method with raw data input. In all the genetic analyses age was used as a covariate.

RESULTS

The characteristics of the MZ and DZ individuals are presented as means, variances and distributions in Table 1. The zygosities differed only in the variances of age and tibial compressive strength.

The ICCs of bone characteristics were higher in MZ than DZ pairs and suggested the presence of additive genetic and shared environmental effects (Table 2). Thus, the genetic analyses were based on the ACE model. The age-adjusted univariate models are presented in Table 3. The effect of age explained 10 % of the variances in radial ToD and radial BSIc and 3% of the variances in tibial ToD and tibial BSIc. Age was not associated with ToA in either the radius or tibia.

The AE models showed good fit for all the bone variables whereas the CE and E models showed poor fit with the data. In the AE models, the proportion of variance accounted for by genetic effects was 84% and 88% for radial ToA and tibial ToA, respectively (Table 3). The contribution of genetic effects to radial ToD, tibial ToD, radial BSIc and tibial BSIc varied from 77 to 80%. However, for tibial BSIc the p-value of the χ^2 difference between the models indicated that the AE model resulted in an almost

significantly worse fit with the data than the ACE model and therefore the ACE model cannot be ignored. In the ACE model for tibial BSIc the relative contribution of genetic effects was 49% and the contribution of shared environmental effects 26%, the rest being accounted for by individual environmental factors.

In the linear regression models HRT usage, rheumatoid arthritis, previous fracture and osteoarthritis were left in the model. These predictors together explained 9.7% of the variance in radial BSIc and 8.3% of the variance in tibial BSIc. After adjusting for the effect of these predictors, the ICCs were 0.77 (r_{MZ}) and 0.45 (r_{DZ}) for radial BSIc and 0.69 (r_{MZ}) and 0.50 (r_{DZ}) for tibial BSIc. In the univariate models for radial BSIc adjusted with the predictors, the relative proportions of A, C and E were 77% (48-84%), 0 (0-26%) and 23% (16-32%), respectively. For tibial BSIc the corresponding proportions were 53% (22-78%), 19% (0-44%) and 28% (21-39%).

The bivariate analyses for radial and tibial compressive strength began from the full ACE model (-2 log-likelihood=2352.5, df=804, AIC=744.5) (Fig. 1) since the univariate models for tibial BSIc indicated the possibility of a shared environmental effect. The final model (-2LL=2354.2, df=805, AIC=744.2, p-value of the χ^2 difference between the models=0.188) (Fig. 2) consisted of additive genetic effects common to both traits, shared environmental effects specific to tibial BSIc and both common and tibia-specific individual environmental effects. Dropping C completely from the model worsened the fit significantly (-2LL=2403.8, df=806, AIC=791.8, $p<0.001$). In the final model additive genetic factors accounted for 83% (95% CI 77-88%) of the variance in radial BSIc and for 61% (52-69%) of the variance in tibial BSIc and these were fully correlated. The relative contribution to tibial BSIc of a shared environmental factor was 15% (10-20%). An individual environmental factor accounted for 17% (12-23%) of the variance in radial BSIc and 10% (5-17%) of the variance in tibial

BSIc. The relative contribution of an individual environmental factor, specific to tibial BSIc was 14% (11-18%). The correlation between the individual environmental factors was 0.64 (0.51-0.74).

DISCUSSION

The novel findings in this study were, first, that in older women, over 80% of the variance in compressive structural strength of the non-weight-bearing radius was accounted for by inter-individual genetic differences, whereas but in the weight-bearing tibia 60% of the variance was attributable to genetic factors and, second that the genetic factors were the same for both bone sites. In addition, the radius and tibia shared a large proportion of the environmental effects. Previous studies, which have mainly focused on aBMD, have suggested that in women the degree of heritability in weight-bearing hip and non-weight-bearing forearm aBMD is similar, heritability estimates varying between 66% and 88%.[2, 3, 30, 31] Our results on volumetric density are, however, in line with the previous studies since the present univariate models showed that the genetic contribution to volumetric density in both radius and tibia was similar, at around 80%.

The heritability of bone geometry has been studied less than that of aBMD. Two previous studies, a pedigree[9] and a family study,[4] have found a similar degree of heritability, approximately 60%, for femoral neck cross-sectional area estimated with DXA. In our study, the proportion of genetic effects on the actual tibial cross-sectional area was higher, at over 80%. The lower proportion of heritability in bone geometry in the previous two studies may be caused by differences in the age, sex and ethnicity of the subjects, all of which are likely to influence the heritability estimates of bone phenotypes.[2, 11, 32] Also differences in study design (family versus twin study), bone measurement method and measurement site may have contributed to the differences between the previous studies and our study. It should also be borne in mind that the planar nature of DXA compromises its ability to assess bone

size accurately[14] whereas pQCT allows direct measurement of the actual cross-sectional area. The heritability of the cross-sectional area of radius measured with pQCT has recently been estimated to be 27% and the heritability of volumetric BMD 49%.[33] Those estimates, however, are not comparable with ours, since they are residual heritabilities after adjusting for several covariates, such as height and weight.

Our results showed a total genetic overlap between radial and tibial compressive structural strength, i.e., same genes or genes that are in close linkage to each other regulate compressive structural strength in both the radius and the tibia. It has been suggested previously that bone mineral mass in the weight-bearing hip and non-weight-bearing forearm share sets of genes but are likely also to be regulated by separate genes. [3, 8] Our finding that tibial and radial compressive structural strength share their genes totally may be due to the fact that the distal radius and distal tibia which were measured in this study are both bone sites at the end of long bones with a similar bone structure composed of both trabecular and cortical bone. Further, in the early phase of human evolution, quadrupedal locomotion was common, and the tibia and radius had a similar function. It is therefore plausible that the tibia and radius share more genes than do the hip and radius. Nevertheless, owing to sample size in our study the possibility of site-specific genetic influences cannot be excluded.

According to the bivariate model, radial and tibial compressive strength are partly influenced by the same environmental factors. This is in line with previous studies that have shown that both common and site-specific environmental factors underlie bone mineral mass in the weight-bearing hip and non-weight-bearing forearm.[3, 8] The environmental factors common to both upper and lower limb bone are likely to include factors that act systemically, influencing the whole skeleton, such as nutrition, medication, smoking and some diseases.[34-38] This was supported by our finding that diseases and medication acted on radial and tibial compressive strength in a similar way.

Apart from common environmental factors, our results showed that tibial compressive strength was more susceptible to environmental factors than radial compressive strength. The reason to this may lie in different loading environments in these bones. Since the distal tibia is subjected to compressive loads generated by body weight-bearing locomotion while the radius is not typically loaded in this way, we hypothesized that the differences observed in the environmental effects on these bone sites are a result of differences in the predominant loading environments in the upper and lower limb. The central role of loading in the tibia is supported by previous observations on female athletes, whose legs are exposed to high exercise-induced mechanical loading.[17, 18] These women have been shown to have considerably higher density and more favorable geometry in the tibia than non-athlete controls. Previous findings also emphasize the importance of habitual physical activity for lower limb bone mineral mass and geometry in older women.[39] In addition, the bone mass of the lower limbs is rapidly reduced during space flights and in bed rest (i.e. periods of lack of daily loading) whereas the upper limb bones maintain their bone mass in these conditions.[19, 40] It may be that loading considerably increases the variance in bone strength in the lower limbs and therefore increases the relative proportion of environmental effects. Further, in the absence of strong effect of weight-bearing loading in the upper limbs relatively greater proportion of the variance in bone strength is consequently attributable to genetic factors.

An important strength in our study was that it was population-based. However, caution must be exercised when applying the results to other populations than Caucasian older women since heritability estimates are always population-specific. Also, heritability of properties of bone seems to decrease with aging[11] probably since rate of bone loss is less heritable than density.[41] Nevertheless, most of the previous studies have estimated the heritability of bone properties mainly in younger persons or have studied a wide age-range[3, 5, 7, 9, 10, 30, 31] whereas our study gives now estimates for older women who

are at a particularly high risk for sustaining osteoporotic fractures.[1] However, although our sample was population-based the inclusion criteria may have led to the exclusion of pairs with at least one sister with poor health. This may have reduced the variance in the bone phenotypes, increased the similarity within the pairs and thus influenced the heritability estimates. A further strength in our study was that the bone measurements were performed using pQCT, which can give precise information on the volumetric mineral density and geometric properties of given bones.[42] Also, the multivariate analysis increases the statistical power of the genetic analyses and thus improves the reliability of the results.[43]

In conclusion, in older women same genetic factors underlie bone structural strength in both the weight-bearing tibia and non-weight-bearing radius. These two bone sites are also largely influenced by the same environmental factors although the weight-bearing tibia is more affected by environment than the radius. The latter finding may be attributable to differences in the typical loading environment of these bone sites.

ACKNOWLEDGEMENTS

This study was supported by grants from the Finnish Ministry of Education, Peurunka Rehabilitation and Exercise Foundation, University of Jyväskylä, Juho Vainio Foundation, Academy of Finland Center of Excellence in Complex Disease Genetics

REFERENCES

1. Cummings SR, Melton LJ 2002 Epidemiology and outcomes of osteoporotic fractures. Lancet **359:**1761-1767.

2. Lenchik L, Hsu FC, Register TC, Lohman KK, Freedman BI, Langefeld CD, Bowden DW, Carr JJ 2004 Heritability of spinal trabecular volumetric bone mineral density measured by QCT in the Diabetes Heart Study. Calcif Tissue Int **75:**305-312.

3. Yang TL, Zhao LJ, Liu YJ, Liu JF, Recker RR, Deng HW 2006 Genetic and environmental correlations of bone mineral density at different skeletal sites in females and males. Calcif Tissue Int **78:**212-217.

4. Xu H, Long JR, Yang YJ, Deng FY, Deng HW 2006 Genetic determination and correlation of body weight and body mass index (BMI) and cross-sectional geometric parameters of the femoral neck. Osteoporos Int **17:**1602-1607.

5. Harris M, Nguyen TV, Howard GM, Kelly PJ, Eisman JA 1998 Genetic and environmental correlations between bone formation and bone mineral density: a twin study. Bone **22:**141-145.

6. Howard GM, Nguyen TV, Harris M, Kelly PJ, Eisman JA 1998 Genetic and environmental contributions to the association between quantitative ultrasound and bone mineral density measurements: a twin study. J Bone Miner Res **13:**1318-1327.

7. Nguyen TV, Howard GM, Kelly PJ, Eisman JA 1998 Bone mass, lean mass, and fat mass: same genes or same environments? Am J Epidemiol **147:**3-16.

8. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S 1987 Genetic determinants of bone mass in adults. A twin study. J Clin Invest **80:**706-710.

9. Sun X, Lei SF, Deng FY, Wu S, Papacian C, Hamilton J, Recker RR, Deng HW 2006 Genetic and environmental correlations between bone geometric parameters and body compositions. Calcif Tissue Int **79:**43-49.

10. Jian WX, Long JR, Li MX, Liu XH, Deng HW 2005 Genetic determination of variation and covariation of bone mineral density at the hip and spine in a Chinese population. J Bone Miner Metab **23:**181-185.

11. Brown LB, Streeten EA, Shapiro JR, McBride D, Shuldiner AR, Peyser PA, Mitchell BD 2005 Genetic and environmental influences on bone mineral density in pre- and post-menopausal women. Osteoporos Int **16:**1849-1856.

12. Deng FY, Lei SF, Li MX, Jiang C, Dvornyk V, Deng HW 2006 Genetic determination and correlation of body mass index and bone mineral density at the spine and hip in Chinese Han ethnicity. Osteoporos Int **17:**119-124.

13. Livshits G, Deng HW, Nguyen TV, Yakovenko K, Recker RR, Eisman JA 2004 Genetics of bone mineral density: evidence for a major pleiotropic effect from an intercontinental study. J Bone Miner Res **19:**914-923.

14. Sievänen H 2000 A physical model for dual-energy X-ray absorptiometry--derived bone mineral density. Invest Radiol **35:**325-330.

15. Adami S, Gatti D, Braga V, Bianchini D, Rossini M 1999 Site-specific effects of strength training on bone structure and geometry of ultradistal radius in postmenopausal women. J Bone Miner Res **14:**120-124.

16. Frost HM 2000 The Utah paradigm of skeletal physiology: an overview of its insights for bone, cartilage and collagenous tissue organs. J Bone Miner Metab **18:**305-316.

17. Heinonen A, Sievänen H, Kannus P, Oja P, Vuori I 2002 Site-specific skeletal response to long-term weight training seems to be attributable to principal loading modality: a pQCT study of female weightlifters. Calcif Tissue Int **70:**469-474.

18. Nikander R, Sievänen H, Uusi-Rasi K, Heinonen A, Kannus P 2006 Loading modalities and bone structures at nonweight-bearing upper extremity and weight-bearing lower extremity: a pQCT study of adult female athletes. Bone **39:**886-894.

19. Vico L, Collet P, Guignandon A, Lafage-Proust MH, Thomas T, Rehaillia M, Alexandre C 2000 Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet **355:**1607-1611.

20. Kaprio J, Koskenvuo M 2002 Genetic and environmental factors in complex diseases: the older Finnish Twin Cohort. Twin Res **5:**358-365.

21. Kaprio J, Sarna S, Koskenvuo M, Rantasalo I 1978 The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program. Prog Clin Biol Res **24 Pt B:**179-184.

22. Carter DR, Hayes WC 1976 Bone compressive strength: the influence of density and strain rate. Science **194:**1174-1176.

23. Martin RB 1991 Determinants of the mechanical properties of bones. J Biomech **24 Suppl 1:**79-88.

24. Grimby G 1986 Physical activity and muscle training in the elderly. Acta Med Scand Suppl **711:**233-237.

25. Posthuma D, Beem AL, de Geus EJ, van Baal GC, von Hjelmborg JB, Iachine I, Boomsma DI 2003 Theory and practice in quantitative genetics. Twin Res **6:**361-376.

26. Rijsdijk FV, Sham PC 2002 Analytic approaches to twin data using structural equation models. Brief Bioinform **3:**119-133.

27. Plomin R, DeFries JC, McClearn GE, McGuffin P 2001 Behavioral Genetics, 4th ed, Worth Publishers, New York, NY, USA.

28. Neale MC, Cardon LR 1992 Methodology for Genetic Studies of Twins and Families, Kluwer, Dordrecht, Netherlands.

29. Neale MC, Boker SM, Xie G, Maes HH 2003 Mx: Statistical Modeling. 6th ed, Department of Psychiatry, Virginia Commonwealth University, Richmond VA, USA.

30. Hunter DJ, de Lange M, Andrew T, Snieder H, MacGregor AJ, Spector TD 2001 Genetic variation in bone mineral density and calcaneal ultrasound: a study of the influence of menopause using female twins. Osteoporos Int **12:**406-411.

31. Knapp KM, Andrew T, MacGregor AJ, Blake GM, Fogelman I, Spector TD 2003 An investigation of unique and shared gene effects on speed of sound and bone density using axial transmission quantitative ultrasound and DXA in twins. J Bone Miner Res **18:**1525-1530.

32. Lei SF, Chen Y, Xiong DH, Li LM, Deng HW 2006 Ethnic difference in osteoporosis-related phenotypes and its potential underlying genetic determination. J Musculoskelet Neuronal Interact **6:**36-46.

33. Havill LM, Mahaney MC, L Binkley T, Specker BL 2007 Effects of genes, sex, age, and activity on BMC, bone size, and areal and volumetric BMD. J Bone Miner Res **22:**737-746.

34. Cranney A, Guyatt G, Griffith L, Wells G, Tugwell P, Rosen C, Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group 2002 Meta-analyses of therapies for postmenopausal osteoporosis. IX: Summary of meta-analyses of therapies for postmenopausal osteoporosis. Endocr Rev **23:**570-578.

35. Christiansen P, Steiniche T, Brixen K, Hessov I, Melsen F, Charles P, Mosekilde L 1997 Primary hyperparathyroidism: biochemical markers and bone mineral density at multiple skeletal sites in Danish patients. Bone **21:**93-99.

36. Matkovic V, Landoll JD, Badenhop-Stevens NE, Ha EY, Crncevic-Orlic Z, Li B, Goel P 2004 Nutrition influences skeletal development from childhood to adulthood: a study of hip, spine, and forearm in adolescent females. J Nutr **134:**701S-705S.

37. Martin JC, Munro R, Campbell MK, Reid DM 1997 Effects of disease and corticosteroids on appendicular bone mass in postmenopausal women with rheumatoid arthritis: comparison with axial measurements. Br J Rheumatol **36:**43-49.

38. Ward KD, Klesges RC 2001 A meta-analysis of the effects of cigarette smoking on bone mineral density. Calcif Tissue Int **68:**259-270.

39. Uusi-Rasi K, Sievänen H, Pasanen M, Oja P, Vuori I 2002 Associations of calcium intake and physical activity with bone density and size in premenopausal and postmenopausal women: a peripheral quantitative computed tomography study. J Bone Miner Res **17:**544-552.

40. Shackelford LC, LeBlanc AD, Driscoll TB, Evans HJ, Rianon NJ, Smith SM, Spector E, Feeback DL, Lai D 2004 Resistance exercise as a countermeasure to disuse-induced bone loss. J Appl Physiol **97:**119-129.

41. Hui SL, Koller DL, Foroud TM, Econs MJ, Johnston CC, Peacock M 2006 Heritability of changes in bone size and bone mass with age in premenopausal white sisters. J Bone Miner Res **21:**1121-1125.

42. Sievanen H, Koskue V, Rauhio A, Kannus P, Heinonen A, Vuori I 1998 Peripheral quantitative computed tomography in human long bones: evaluation of in vitro and in vivo precision. J Bone Miner Res **13:**871-882.

43. Schmitz S, Cherny SS, Fulker DW 1998 Increase in power through multivariate analyses. Behav Genet **28:**357-363.

TABLE 1 CHARACTERISTICS OF TWIN INDIVIDUALS

	Monozygotic individuals		Dizygotic individuals			
	n	Mean (SD)	n	Mean (SD)	p^a	p^b
Age (yrs)	205	68.3 (3.7)	226	68.8 (3.1)	0.251	0.004
Height (cm)	205	158.0 (6.5)	226	159.1 (5.8)	0.193	0.143
Body weight (kg)	205	69.6 (11.8)	226	70.7 (12.2)	0.449	0.654
Time since menopause (yrs)	201	18.7 (5.8)	224	19.9 (5.7)	0.085	0.781
Radius	191		210			
ToA (mm^2)		344 (45)		346 (45)	0.680	0.980
ToD (mg/cm^3)		281 (56)		276 (60)	0.452	0.308
BSIc (g^2/cm^4)		0.28 (0.10)		0.27 (0.11)	0.682	0.114
Tibia	196		216			
ToA (mm^2)		981 (119)		1009 (125)	0.068	0.498
ToD (mg/cm^3)		259 (42)		251 (47)	0.166	0.095
BSIc (g^2/cm^4)		0.66 (0.19)		0.65 (0.23)	0.591	0.028
		n (%)		n (%)		
Cerebrovascular dysfunction		15 (7.3)		14 (6.2)	0.690	
Hip or knee osteoarthritis		79 (39)		75 (33)	0.298	
Rheumatoid arthritis		5 (2.4)		11 (5.3)	0.169	
Previous fracture(s)		37 (18)		37 (16)	0.670	
HRT users		45 (22)		48 (21)	0.879	
Corticosteroid users		3 (1.5)		11 (4.9)	0.058	
Current smokers		7 (3.4)		11 (4.9)	0.506	
Physical activity					0.535	
sedentary		18 (8.8)		20 (8.8)		
moderately active		123 (60)		122 (54)		
active		64 (31)		84 (37)		

ToA, total cross-sectional area; ToD, total volumetric bone mineral density; BSIc, compressive strength; Previous fracture(s), previous fractures at age 60 or older; HRT users, hormone replacement therapy users;

[a] Adjusted Wald test

[b] Variance ratio test

TABLE 2 WITHIN-PAIR INTRA-CLASS CORRELATION COEFFICIENTS (ICC) FOR BONE CHARACTERISTICS

	MZ		DZ	
	ICC	95% CI	ICC	95% CI
Radius				
ToA	0.85	(0.79-0.90)	0.51	(0.35-0.65)
ToD	0.77	(0.67-0.84)	0.50	(0.33-0.63)
BSIc	0.80	(0.71-0.86)	0.49	(0.32-0.62)
Distal tibia				
ToA	0.88	(0.82-0.92)	0.48	(0.32-0.62)
ToD	0.76	(0.66-0.84)	0.49	(0.32-0.62)
BSIc	0.73	(0.62-0.81)	0.56	(0.41-0.68)

ToA, total cross-sectional area of bone; ToD, total volumetric bone mineral density; BSIc, compressive strength

TABLE 3 UNIVARIATE GENETIC MODELS ADJUSTED FOR AGE FOR CROSS-SECTIONAL AREA, VOLUMETRIC BONE MINERAL DENSITY AND ESTIMATED COMPRESSIVE STRENGTH IN RADIUS AND TIBIA.

		Model fit				Standardized estimates (95% CI)					
Variable	Model	-2LL	df	ΔAIC	p	A		C		E	
Radius											
ToA	ACE	360.4	396			0.62	(0.37-0.87)	0.22	(0-0.45)	0.16	(0.12-0.22)
	AE	362.3	397	-0.1	0.164	0.84	(0.78-0.88)			0.16	(0.12-0.22)
	CE	386.7	397	24.3	p<0.001			0.68	(0.60-0.75)	0.32	(0.25-0.40)
	E	501.6	398	137.2	p<0.001					1.00	(1.00-1.00)
ToD	ACE	570.0	396			0.68	(0.38-0.83)	0.09	(0.00-0.36)	0.24	(0.17-0.33)
	AE	570.3	397	-1.7	0.572	0.77	(0.68-0.83)			0.23	(0.17-0.32)
	CE	588.7	397	16.7	p<0.001			0.57	(0.47-0.66)	0.43	(0.34-0.53)
	E	664.0	398	90.0	p<0.001					1.00	(1.00-1.00)
BSIc	ACE	1051.8	396			0.79	(0.50-0.85)	0.01	(0.00-0.28)	0.20	(0.15-0.28)
	AE	1051.8	397	-2.0	0.939	0.80	(0.72-0.85)			0.20	(0.15-0.28)
	CE	1079.1	397	25.3	p<0.001			0.57	(0.46-0.65)	0.43	(0.35-0.54)
	E	1154.2	398	98.4	p<0.001					1.00	(1.00-1.00)
Distal tibia											
ToA	ACE	1170.4	407			0.85	(0.60-0.92)	0.04	(0.00-0.29)	0.12	(0.08-0.16)
	AE	1170.5	408	-1.9	0.799	0.88	(0.84-0.92)			0.12	(0.09-0.16)
	CE	1224.6	408	52.2	p<0.001			0.65	(0.57-0.72)	0.35	(0.28-0.43)
	E	1337.5	409	163.0	p<0.001					1.00	(1.00-1.00)
ToD	ACE	384.4	407			0.67	(0.39-0.84)	0.11	(0.00-0.36)	0.22	(0.16-0.31)
	AE	384.9	408	-1.5	0.459	0.78	(0.70-0.84)			0.22	(0.16-0.30)
	CE	405.6	408	19.3	p<0.001			0.59	(0.49-0.67)	0.41	(0.33-0.51)
	E	491.9	409	103.5	p<0.001					1.00	(1.00-1.00)
BSIc	ACE	1666.9	407			0.49	(0.22-0.79)	0.26	(0.00-0.49)	0.24	(0.18-0.34)
	AE	1670.3	408	1.5	0.063	0.77	(0.68-0.83)			0.23	(0.17-0.32)
	CE	1679.1	408	10.2	p<0.001			0.61	(0.52-0.69)	0.39	(0.31-0.48)
	E	1774.4	409	103.5	p<0.001					1.00	(1.00-1.00)

A, additive genetic effects; C, shared genetic effects; E, individual environmental effects
ToA, total cross-sectional area; ToD, total volumetric bone mineral density; BSIc, compressive strength
-2LL, -2 times log-likelihood; df, degrees of freedom; ΔAIC, difference in the Akaike's information criterion between the model and full model; p, p-value of the χ^2 difference between the model and full model

FIGURE LEGENDS

FIG. 1. THE FULL HYPOTHETIC ACE CHOLESKY MODEL FOR RADIAL AND TIBIAL COMPRESSIVE STRUCTURAL STRENGTH. THE PERCENTAGES (95% CONFIDENCE INTERVALS) ARE THE PROPORTIONS OF THE TOTAL VARIANCE OF THE BONE CHARACTERISTICS EXPLAINED BY EACH GENETIC AND ENVIRONMENTAL FACTOR.

FIG. 2. REDUCED CHOLESKY MODEL FOR RADIAL AND TIBIAL COMPRESSIVE STRUCTURAL STRENGTH. THE PERCENTAGES (95% CONFIDENCE INTERVALS) ARE THE PROPORTIONS OF THE TOTAL VARIANCE OF THE BONE CHARACTERISTICS EXPLAINED BY EACH GENETIC AND ENVIRONMENTAL FACTOR.