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## ORIGINAL CONTRIBUTIONS

# Bone Mass, Lean Mass, and Fat Mass: Same Genes or Same Environments?

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The contributions of genetic and environmental factors to the association among bone mineral density (BMD), lean mass, and fat mass were assessed in the Sydney Twin Study of Osteoporosis (Australia), 1995–1996, in 57 monozygotic and 55 dizygotic female twin pairs of Caucasian background, aged 52.8 (standard deviation, 13) years. In multiple regression analysis, lean mass was a significant determinant of areal BMD; however, fat mass was a principal determinant of volumetric BMD. Univariate model-fitting analyses indicated that 80% and 65% of variance of lean mass and fat mass, respectively, were attributable to genetic factors. The estimated heritability of BMD for lumbar spine, femoral neck, and total body BMD was 78%, 76%, and 79%, respectively. Multivariate analyses suggested that, while the association between lean mass and fat mass was attributable mainly to environmental factors ($r_e = 0.53$, $p < 0.01$), the association among the three BMD sites was attributable to both genetic and environmental factors ($r_g = 0.64$–$0.75$, $p < 0.001$; $r_e = 0.57$–$0.70$, $p < 0.001$). Furthermore, genetic factors that affect lean mass or fat mass have minor effects on BMD. It is concluded that lean mass and fat mass, as well as bone density, are under strong genetic regulation. However, the associations between BMD and fat mass or between lean mass and fat mass appear to be mediated mainly via environmental influences. *Am J Epidemiol* 1998;147:3–16.

body composition; bone density; models, genetic; osteoporosis; twin studies

Osteoporosis is a disease characterized by an inadequate amount and/or structure of bone, which increases the susceptibility to fracture with minimal trauma. Bone mass and bone loss are strongly associated with body weight (1–4), subjects with higher body weight having higher bone density and less bone loss than their counterparts with lower weight in the same age. However, body weight is made up of two components: lean mass (LM) and fat mass (FM), and which of the components is related to bone mass is the subject of much contention. Cross-sectional analyses of data from post- and premenopausal women (5–7) suggest that bone mineral is related to fat mass, but not lean mass. In other cross-sectional studies (3, 8, 9), both lean and fat mass are related to bone mass. Thus, the relation may be dependent on the way in which bone density is expressed (10). This distinction is of clinical relevance since, if bone density is related to LM, an increase in physical activity may translate directly into protection against osteoporosis. On the other hand, if bone mineral density (BMD) is related to FM, then modification of dietary habits could play a similar role.

These discrepancies may be related to statistical and genetic confounding problems. Since lean mass and fat mass are related, when they are treated as independent factors in a multiple linear regression model, it is difficult to separate the specific effect of each factor. As a result, it will lead to the imprecise estimation of the individual parameters and functions of the parameters (11) and, hence, give inconsistent apparent relations from sample to sample. This collinearity problem cannot be resolved by statistical models but can be addressed partially by considering study design. Part of the variability of body composition and BMD is related to age, which may be viewed as an index of environments, changing significantly over a lifetime. On the other hand, there is evidence suggesting that genetic factors play a determining role in the variation of bone mass (12–14). Thus, a design that controls for these two factors could be useful. In a cotwin model, the intrapair difference reflects environmental effects (for monozygotic (MZ) pairs) and genetic and environmental effects (for dizygotic (DZ) pairs). There-

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Abbreviations: BMD, bone mineral density; DZ, dizygotic; FM, fat mass; LM, lean mass; MZ, monozygotic; rDZ, dizygotic pairs' correlation; rMZ, monozygotic pairs' correlation.

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fore, by analyzing the intrapair differences in BMD as a linear function of intrapair differences in LM and FM, researchers can control for part of the genetic factors. This approach has been shown to reduce the magnitude of colinearity (15).

Both bone mass and lean mass are under strong genetic regulation, with up to 80 percent of BMD and lean mass variances attributable to genetic factors (12–16). However, the mode of inheritance is not known, and there have been no formal analyses on the contribution of genes and environment to the covariance of body composition and bone mass. Erroneous inferences about the relation among LM, FM, and BMD could be made if the covariates (LM and FM) and BMD were mediated by the same genetic and environmental factors. Twin studies can potentially resolve this issue by analyzing the covariances among BMD, LM, and FM in MZ and DZ twins to address the question of whether heritability of BMD might reflect specific genetic effects or might be mediated by shared genetic influences on LM and FM.

This study sought to address three issues: 1) the relation between body composition and BMD, controlled for genetic factors; 2) the relative contribution of genetic and environmental factors to the intersubject variation in LM and FM; and 3) the extent to which shared genetic and environmental factors contribute to the covariation between body composition and BMD.

## MATERIALS AND METHODS

### Subjects

Twins were recruited from a media campaign and through the Australian National Health and Medical Research Council's Twin Registry, for a study into the genetics of osteoporosis. The twins were females of Caucasian background who were between 20 and 83 years, residing in the city of Sydney (Australia). The study was approved by the Ethics Committee of St. Vincent's Hospital, and written informed consent was obtained from all participants.

Baseline data including demographic characteristics and clinical history were obtained by direct interview using a structured questionnaire. Twin zygosity was determined by self-report. The probability of misclassification using this method compared with serologic markers has been estimated to be below 5 percent (17). Body height and weight were measured by a wall-mounted stadiometer and balance scale by a trained research nurse or doctor.

### Measurements of BMD and body composition

Areal BMD (in g/cm$^2$) of the lumbar spine, femoral neck, and total body and body composition (lean mass and fat mass) were measured by dual energy X-ray absorptiometry (DEXA; Lunar Corp., Madison, Wisconsin) (18). The coefficient of reliability of BMD measurements at our institution was 0.98, 0.95, and 0.96 at the lumbar spine, the femoral neck, and total body, respectively (19).

Areal BMD is derived as the ratio of bone mineral content to the projected area of a skeletal region. The quantity has the dimensions of mass/area, not a true measure of density, which would have a unit of mass/volume. To obtain an index of volumetric density (mass/volume), BMD was divided by body height (20).

As the projected area of the femoral neck is based on a constant length along the axes of the neck of 1.5 cm, it is possible to estimate the volumetric BMD at this site by noting that the femoral neck volume (FNVOL) can be expressed as a function of femoral neck bone mineral content (FNBMC) and femoral neck bone mineral density (FNBMD). After some algebra, it can be shown that $\text{FNVOL} = \pi/6(\text{FNBMC}/\text{FNBMD})^2$. The estimated femoral neck volumetric BMD is then derived as the ratio of FNBMC to FNVOL with the unit of g/cm$^3$, i.e., $[6(\text{FNBMD})^2]/[\pi(\text{FNBMC})]$. The estimated volumetric BMD at the lumbar spine was derived from Lunar's densitometry software (21).

### Data analyses

To assess the association among lean mass, fat mass, and BMD, we performed linear regression analysis in both matched and unmatched pairs. In the matched pair analysis, intrapair differences in bone density, LM, and FM (denoted by $\Delta$BMD, $\Delta$LM, and $\Delta$FM, respectively) were obtained by subtracting the value of one twin from that of the other. $\Delta$BMD was then expressed as a linear function of $\Delta$LM and $\Delta$FM; i.e., $\Delta\text{BMD} = \alpha + \beta(\Delta\text{LM}) + \gamma(\Delta\text{FM}) + \epsilon$, where $\alpha$, $\beta$, and $\gamma$ are intercept and regression coefficients associated with $\Delta$LM and $\Delta$FM, respectively, and $\epsilon$ is residual error terms. An iteratively reweighted least-squares method (22) was used to estimate the model parameters. In the unmatched analysis, each twin within a pair was treated as an individual. The BMD of the twin was then modeled as a linear function of her LM and FM in a multiple regression analysis. As the twins are not independent, the estimated error terms of regression parameters, although unbiased, tend to be correlated within pairs, which leads to underestimation of standard errors and hence overstated statistical significance (23). To avoid this problem, the generalized least-square method (22) was used with iterative adjustment for the correlation of errors within pairs. In each analysis, the backward elimination algorithm was used to select the most parsimonious equation. Assess-

ment of model adequacy was based on residual analysis.

Twin resemblance for a variable trait was assessed for MZ and DZ twin pairs separately by the intraclass correlation coefficient. 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 (24).

To estimate the heritability (proportion of variance of a trait attributable to genetic factors), we analyzed the data according to the classical twin model (25). 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 reared in the same family ($C$) and to the nonshared 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 within loci. The classical twin model assumes that additive genetic factors and dominant genetic factors are perfectly correlated in MZ pairs, while DZ pairs, 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 $\alpha$, $\delta$, $\chi$, and $\epsilon$, 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 $\alpha$, $\delta$, $\chi$, and $\epsilon$, for each variable trait, the data were summarized into 2 × 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 test. The index of heritability was obtained as the

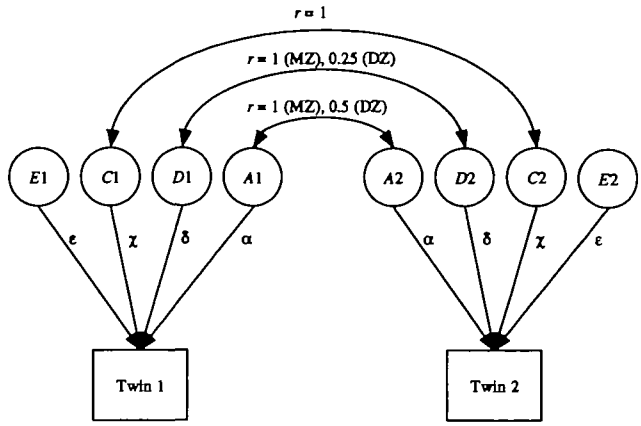


**FIGURE 1.** Classic twin model. Path diagrams illustrate the univariate twin model. Latent variables are in circles; observed variables are depicted by squares. $A$, additive genetic factors; $D$, dominant genetic factors; $C$, shared environmental factors; and $E$, nonshared environmental factors (including measurement error). The numbers associated with each factor denote twin 1 and twin 2. The correlation between $A1$ and $A2$ is 1 for monozygotic (MZ) pairs and 0.5 for dizygotic (DZ) pairs; between $D1$ and $D2$: 1 for MZ and 0.25 for DZ pairs. The correlation between shared environmental factors ($C1$ and $C2$) is assumed to be unity for both zygosities.

square of parameter $\alpha$ from the most parsimonious model.

To test the hypothesis that the same set of genes is involved in the determination of LM, FM, and BMD, the contribution of genetic environmental factors to the covariances of LM, FM, and BMD was estimated in a multivariate genetic model (figure 2). In this model, the proportion of the total variance of BMD caused by genetic effects is the sum of the squared standardized path coefficients. For example, the heritability of total body BMD is the sum of genetic factors in common with LM ($g_{e1}^2$), FM ($g_{e2}^2$), lumbar spine and femoral neck BMD ($g_{e3}^2$ and $g_{e4}^2$), and genetic factors that are specific to total body BMD ($g_{e5}^2$); i.e., $H_{\text{TBBMD}}^2 = \sum_{i=1}^{5} g_{ei}^2$. Similarly, the proportion of total variance caused by environmental factors is decomposed as $E_{\text{TBBMD}}^2 = \sum_{i=1}^{5} e_{ei}^2$.

Under this multivariate model, it is possible to estimate the genetic and environmental correlations between any two variable traits. The genetic correlation between any two variable traits $i$ and $j$ can be shown to be equal to $r_{g(i,j)} = g_{ij}/\sqrt{g_{ii} \times g_{jj}}$, where $g_{ij}$ is the genetic covariance between variables $i$ and $j$, and $g_{ii}$ and $g_{jj}$ are genetic variances of the variables $i$ and $j$, respectively. Similarly, the environmental correlation between the two traits is obtained by $r_{e(i,j)} = e_{ij}/\sqrt{e_{ii} \times e_{jj}}$, where $e_{ij}$ is the environmental covariance between variables $i$ and $j$, and $e_{ii}$ and $e_{jj}$ are

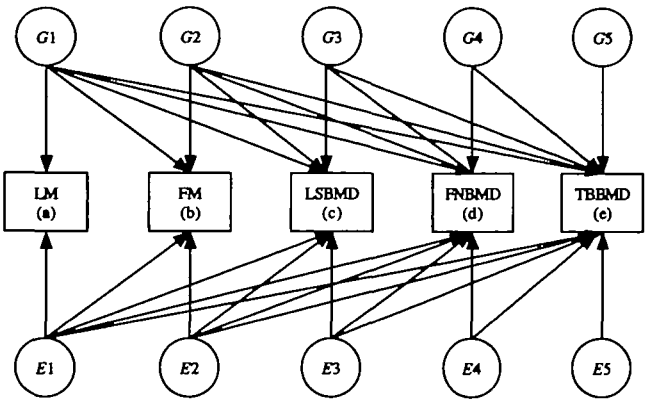


**FIGURE 2.** Cholesky factor model for the multivariables. Path diagrams depict the common and unique factors for genetic and environmental sources of variance and covariance for lean mass (LM), fat mass (FM), and bone mineral density (BMD) at the lumbar spine (LS), femoral neck (FN), and total body (TB). There are five genetic factors (*G1*, *G2*, *G3*, *G4*, and *G5*) and five nonshared environmental factors (*E1*, *E2*, *E3*, *E4*, and *E5*). The paths from *G1* to lean mass, fat mass, lumbar spine BMD, femoral neck BMD, and total body BMD are denoted by $g_{a1}$, $g_{b1}$, $g_{c1}$, $g_{d1}$, and $g_{e1}$, respectively. The paths from *G2* to fat mass, lumbar spine BMD, femoral neck BMD, and total body BMD are denoted by $g_{b2}$, $g_{c2}$, $g_{d2}$, and $g_{e2}$, respectively. The paths from *G3* to lumbar spine BMD, femoral neck BMD, and total body BMD are denoted by $g_{c3}$, $g_{d3}$, and $g_{e3}$, respectively. The paths from *G4* to femoral neck BMD and total body BMD are denoted by $g_{d4}$ and $g_{e4}$, respectively. The path from *G5* to total body BMD is denoted by $g_{e5}$. The paths of environmental factors, $e_{a1}$, $e_{b1}$, $e_{c1}$, . . ., $e_{e5}$ are denoted similarly. The figure illustrates only one twin in a pair.

environmental variances of the variables $i$ and $j$, respectively. The genetic/environmental correlation measures the extent to which the two phenotypes share genetic/environmental effects. For example, if two traits share the same genetic factors, it is expected that the genetic correlation is approximately equal to 1; on the other hand, if the two traits are genetically independent, it is expected that the genetic correlation approximates 0.

As BMD, fat mass, and lean mass are each related to age, all analyses were adjusted for age by expressing BMD and body composition measures as a linear function of age in the regression model. Standardized residuals were used as the adjusted values for genetic analyses. Preliminary univariate analyses suggested that a model with $A$ and $E$ factors fit the data adequately. Therefore, a Cholesky model (26) of decomposition including additive genetic effects ($A$) and nonshared environmental effects ($E$) was fitted to the variance-covariance matrices. All model parameters were estimated by using the maximum likelihood method via the LISREL programs, version 7 (27).

## RESULTS

This study comprised 112 female twin pairs, including 57 MZ and 55 DZ pairs, with an average age of 52.8 (standard deviation, 13) years. The two zygosities were comparable in terms of mean and variance of age, weight, height, lean mass, fat mass, and bone density (table 1). Of these twins, 155 individuals were postmenopausal (age range, 46–83 years), and 68 were premenopausal. One subject's menopausal status was unknown.

Compared with the premenopausal group, postmenopausal women had 15 percent higher fat mass (27.7 (standard deviation, 9.4) vs. 24.1 (standard deviation, 7.9) kg; $p = 0.004$) but similar lean mass (37.4 (standard deviation, 4.2) vs. 38.4 (standard deviation, 4.6) kg; $p = 0.15$). In both cross-sectional and within-pair differences analyses, fat mass was highly correlated with weight and body mass index ($r = 0.92$ and 0.94), while lean mass was moderately correlated with body mass index ($r = 0.47$) or weight ($r = 0.69$). Moreover, lean mass was linearly associated with body height via the equation LM (kg) = 0.43 × height

**TABLE 1. Characteristics of the study sample, Sydney Twin Study of Osteoporosis (Australia), 1995–1996**

| | No. of pairs | Age | | Weight (kg) | | Height (cm) | | Lean body mass (kg) | | Fat mass (kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD* | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Monozygotic twins† | 57 | 53 | 14 | 65 | 10 | 161 | 7 | 37 | 3.9 | 26 | 8.9 |
| Dizygotic twins | 55 | 52 | 13 | 67 | 12 | 161 | 7 | 38 | 4.9 | 27 | 9.3 |

| | Bone mineral density | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lumbar spine (g/cm²) | | Volumetric lumbar spine (g/cm³) | | Femoral neck (g/cm²) | | Volumetric femoral neck (g/cm³) | | Total body (g/cm²) | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Monozygotic twins | 1.10 | 0.16 | 0.12 | 0.03 | 0.89 | 0.11 | 0.36 | 0.06 | 1.11 | 0.10 |
| Dizygotic twins | 1.16 | 0.17 | 0.13 | 0.04 | 0.92 | 0.14 | 0.36 | 0.06 | 1.14 | 0.10 |

* SD, standard deviation.

† No significant difference between the two zygosities was observed in any variable.

**TABLE 2. Relations among lean mass, fat mass, and bone mineral density in monozygotic twin pairs, Sydney Twin Study of Osteoporosis (Australia), 1995–1996†**

| | Lean mass (kg) | Fat mass (kg) | Bone mineral density | | |
|---|---|---|---|---|---|
| | | | Lumbar spine (g/cm²) | Femoral neck (g/cm²) | Total body (g/cm²) |
| Higher LM‡ twin | 38.0 | 27.7 | 1.12 | 0.90 | 1.12 |
| Lower LM twin | 27.0 | 25.0 | 1.08 | 0.88 | 1.10 |
| Intrapair difference (mean ± SE‡) | 1.0 ± 0 .22* | 2.7 ± 0.99* | 0.04 ± 0.01* | 0.02 ± 0.007* | 0.02 ± 0.007* |
| | Fat mass (kg) | Lean mass (kg) | Bone mineral density | | |
| | | | Lumbar spine (g/cm²) | Femoral neck (g/cm²) | Total body (g/cm²) |
| Higher FM‡ twin | 28.9 | 37.9 | 1.11 | 0.90 | 1.12 |
| Lower FM twin | 23.8 | 37.1 | 1.08 | 0.88 | 1.10 |
| Intrapair difference (mean ± SE) | 5.1 ± 0.78* | 0.8 ± 0.23* | 0.03 ± 0.01* | 0.02 ± 0.05* | 0.02 ± 0.007* |

* Significantly different from 0 at $p < 0.001$ level.
† Intrapair differences are shown for bone density, lean mass, and fat mass between twins on the basis of lean mass (top) or fat mass (bottom).
‡ LM, lean mass; SE, standard error; FM, fat mass.

(cm) −31.14, which accounts for 48 percent of the variance of lean mass.

There were 25 women who reported estrogen use; of these, 22 pairs were discordant for estrogen usage; i.e., one twin used, but the other did not. Analysis of intrapair differences suggested that estrogen use was associated with a 0.05 ± 0.02 g/cm$^2$ (mean ± standard error, $p = 0.03$) increase in femoral neck BMD. The effect of estrogen in femoral neck BMD was taken into account in subsequent analyses. However, there was no such effect observed in lumbar spine BMD (0.03 ± 0.04 g/cm$^2$, $p = 0.5$), total body BMD, fat mass (0.62 ± 2.2 kg, $p = 0.78$), and lean mass (0.84 ± 0.73 kg, $p = 0.26$).

### Association between lean mass, fat mass, and BMD

Within MZ pairs, the twin with greater lean mass had significantly higher BMD at all sites measured. Similar trends were also observed for the twin with greater fat mass (table 2). Within twin pairs, the twin with a higher lean mass also had a higher fat mass ($r = 0.43$, $p < 0.0001$). In univariate analysis on both MZ and DZ data, both lean mass and fat mass were correlated with BMD at all sites. Intrapair differences in lean mass were positively significantly correlated with intrapair differences in lumbar spine BMD ($r = 0.38$), femoral neck BMD ($r = 0.27$), and total body BMD ($r = 0.32$; figure 3). The correlation between intrapair differences in fat mass and BMD at these sites was also statistically significant (figure 3; correlation coefficients of 0.37, 0.26, and 0.59, respectively).

In backward and stepwise multiple regression analyses based on cross-sectional (unmatched) data, BMD at all sites was negatively associated with age. Lean mass was a significant determinant of both lumbar spine and femoral neck BMD, while fat mass was an independent additional predictor of total body BMD. However, analysis on intrapair differences (matched) data revealed that lean mass was the only significant determinant of femoral neck BMD and that fat mass was only significantly associated with total body BMD, while both lean and fat mass were independent determinants of lumbar spine BMD.

Since lean mass was related to height ($r = 0.69$), an additional analysis was performed, in which lean mass was adjusted for height in the linear regression model; the adjusted lean mass was then subject to multiple regression analysis. In unmatched analysis, it was found that both fat mass and adjusted lean mass were significant determinants of lumbar spine BMD; however, only adjusted lean mass and fat mass were each significantly associated with femoral neck BMD and total body BMD, respectively. In contrast, in matched analysis, adjusted lean mass was a significant determinant of both lumbar spine and total body BMD, while fat mass was an independent additional predictor of total body BMD.

When lumbar spine and femoral neck BMD were expressed in the estimated volumetric dimension (g/cm$^3$), only fat mass was found to be a significant predictor of both lumbar spine and femoral neck BMD (in matched analysis), but only lean mass was a significant determinant of lumbar spine BMD (in unmatched analysis). However, BMD/height at all sites

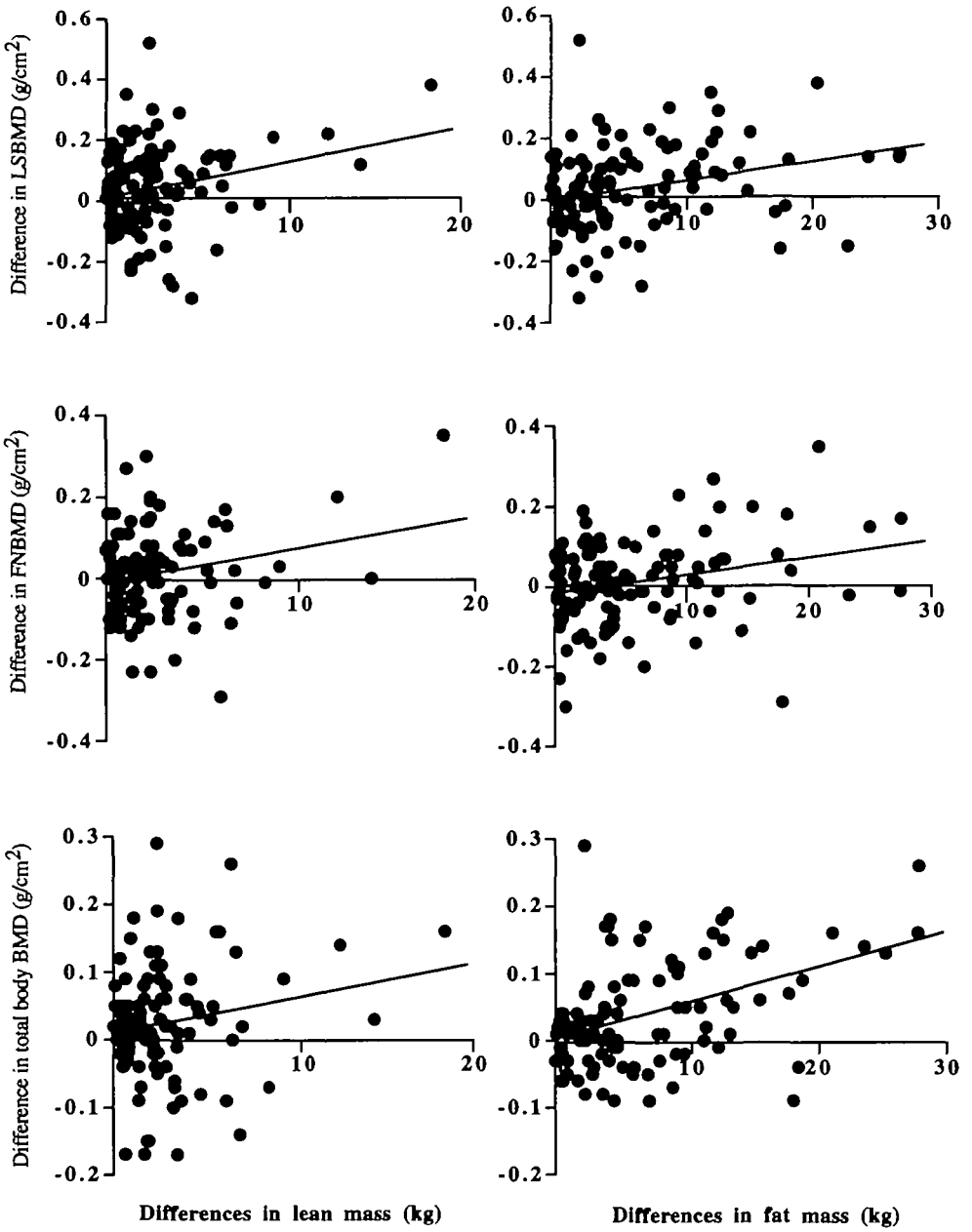


**FIGURE 3.** Relation between lean mass, fat mass, and areal bone mineral density, Sydney Twin Study of Osteoporosis (Australia), 1995–1996. Correlations between intrapair differences in lean mass and fat mass and lumbar spine (LS) bone mineral density (BMD), femoral neck (FN) BMD, and total body BMD. The differences in lean mass and fat mass were constrained to be positive (twin with lower value was subtracted from twin with higher value). In each case, within a twin pair, the twin with a higher lean or a higher fat mass was associated with higher BMD.

was related to only fat mass in either matched or unmatched analysis (table 3).

In all analyses, the maximum proportion of variation in BMD, the estimated volumetric BMD, or the BMD/height ratio attributable to the variation in lean or fat mass or both was 39 percent (total body BMD in unmatched analysis), and the minimum was 6 percent (femoral neck BMD in matched analysis).

### Univariate genetic analysis

Intraclass correlations for MZ pairs (rMZ) were significantly ($p$ = 0.03) greater than for DZ pairs (rDZ) in unadjusted lean and fat mass (figure 4). MZ pairs were also more alike than DZ pairs in BMD. The intraclass correlation ± standard error in BMD at the lumbar spine was 0.73 ± 0.08 for MZ pairs and

**TABLE 3. Relations between lean mass, fat mass, and bone mineral density, Sydney Twin Study of Osteoporosis (Australia), 1995–1996**

| Variable | Design | Regression coefficient ± SE* for a unit change† in | | | $R^2$ |
|---|---|---|---|---|---|
| | | Age (years) | Lean mass (kg) | Fat mass (kg) | |
| LSBMD* | Unmatched | −0.005 ± 0.001 | 0.009 ± 0.002 | | 0.27 |
| FNBMD* | Unmatched | −0.004 ± 0.001 | 0.007 ± 0.002 | | 0.27 |
| TBBMD* | Unmatched | −0.003 ± 0.0004 | 0.003 ± 0.001 | 0.004 ± 0.001 | 0.39 |
| LSBMD | Matched | NA* | 0.009 ± 0.003 | 0.004 ± 0.001 | 0.20 |
| FNBMD | Matched | NA | 0.008 ± 0.003 | | 0.07 |
| TBBMD | Matched | NA | | 0.005 ± 0.001 | 0.35 |
| LSBMD | Unmatched | −0.006 ± 0.001 | 0.033 ± 0.010‡ | | 0.26 |
| FNBMD | Unmatched | −0.004 ± 0.001 | 0.022 ± 0.008‡ | | 0.24 |
| TBBMD | Unmatched | −0.003 ± 0.0004 | 0.015 ± 0.006‡ | 0.004 ± 0.001 | 0.39 |
| LSBMD | Matched | NA | 0.030 ± 0.012§ | 0.004 ± 0.001 | 0.19 |
| FNBMD | Matched | NA | | 0.003 ± 0.001 | 0.08 |
| TBBMD | Matched | NA | 0.012 ± 0.007§ | 0.005 ± 0.001 | 0.37 |
| LSBMD/Ht* | Unmatched | −0.003 ± 0.0003 | | 0.002 ± 0.001 | 0.17 |
| FNBMD/Ht | Unmatched | −0.002 ± 0.0003 | | 0.001 ± 0.001 | 0.15 |
| TBBMD/Ht | Unmatched | −0.002 ± 0.0003 | | 0.003 ± 0.0004 | 0.30 |
| LSBMD/Ht | Matched | NA | | 0.003 ± 0.001 | 0.13 |
| FNBMD/Ht | Matched | NA | | 0.006 ± 0.001 | 0.06 |
| TBBMD/Ht | Matched | NA | | 0.003 ± 0.001 | 0.26 |
| Vol.* LSBMD | Unmatched | −0.001 ± 0.0001 | 0.001 ± 0.0004 | | 0.23 |
| Vol. FNBMD | Unmatched | −0.002 ± 0.0002 | | | 0.20 |
| Vol. LSBMD | Matched | NA | | 0.001 ± 0.0003 | 0.06 |
| Vol. FNBMD | Matched | NA | | 0.001 ± 0.0005 | 0.04 |

* SE, standard error; LSBMD, lumbar spine bone mineral density; FNBMD, femoral neck bone mineral density; TBBMD, total body bone mineral density; NA, not applicable; Ht, height; Vol., volumetric.

† Values are regression coefficients ± SE for a 1-kg change in lean or fat mass or a 1-year change in age. Only those coefficients statistically significantly different from 0 at the $p < 0.01$ level are shown.

‡ Based on height-adjusted lean mass (with a mean of 0 and standard deviation of 1; range, −2.2 to 5.5.

§ Based on within-pair differences in height-adjusted lean mass.

0.39 ± 0.12 for DZ pairs ($p = 0.008$); at the femoral neck (rMZ = 0.69 ± 0.07 vs. rDZ = 0.44 ± 0.11, $p = 0.05$); for total body BMD (rMZ = 0.73 ± 0.05 vs. rDZ = 0.39 ± 0.12, $p = 0.008$). After adjusting fat mass and BMD for age and lean mass for height, the differences between rMZ and rDZ remained statistically significant (table 4).

Univariate model-fitting analyses of adjusted lean mass, fat mass, and bone density indicated that the models with common and specific environmental factors (*C* and *E*) fitted the data inadequately (table 5). There was no significant effect of common environmental factors or dominant genetic factors on any trait as the goodness-of-fit of models with *ACE* and *ADE* was not significantly better than that of models with *AE*. Therefore, estimation of heritability is based on the model with additive genetic and nonshared environmental factors. It is estimated from this model that approximately 84 percent and 65 percent of variances of lean mass and fat mass, respectively, were attributable to genetic factors. Estimated heritability values of lumbar spine, femoral neck, and total body BMD were 78 percent, 76 percent, and 79 percent, respectively.

### Multivariate genetic analysis

To assess whether the observed relations between BMD at various sites and body composition were attributable to genetic or environmental factors, we performed multivariate genetic model-fitting analyses (as described in figure 2). Squared standardized path coefficients (table 6) can be interpreted as estimates of heritability of specific and decomposed values in terms of the portion in common with and independent of other genetic factors. Off-diagonal elements of this analysis were small relative to diagonal elements, which fact indicates that the majority of heritability of each variable trait is due to specific genetic factors.

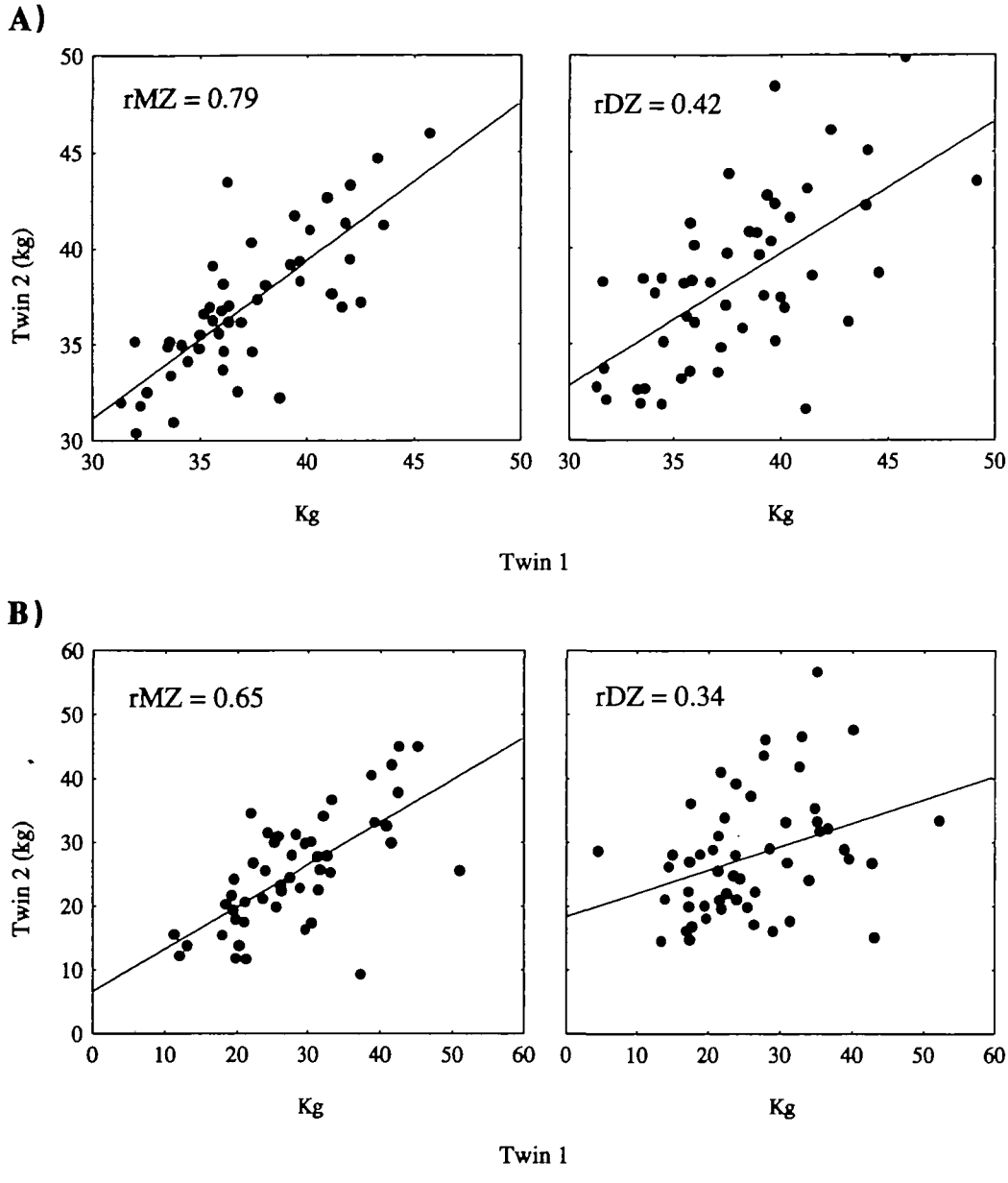


**FIGURE 4.** Familial resemblance in lean mass and fat mass, showing intrapair correlation in monozygotic (rMZ) and dizygotic (rDZ) twins for unadjusted lean mass (A) and fat mass (B), Sydney Twin Study of Osteoporosis (Australia), 1995–1996.

The heritability of fat mass in this sample was 0.65, and the portion of this due to shared genetic factors with lean mass was 0.02, whereas approximately one third of the environmental variance of FM was due to shared environment with lean mass. This is consistent with the nonsignificant genetic correlation between lean mass and fat mass ($r = 0.16$, $p = 0.24$) and the significant environmental correlation ($r = 0.51$, $p < 0.001$; table 7).

Genetic correlations among the three BMD measurements ranged from 0.58 (between femoral neck and total body BMD) to 0.75 (between lumbar spine and total body BMD). A substantial part of the genetic influence on femoral neck BMD (heritability of $H^2 = 0.78$) was due to shared genetic effects on lumbar spine BMD ($H^2 = 0.26$). Genetic factors that affect the lumbar spine BMD also had a greater influence ($H^2 = 0.43$) than did the specific genetic effect ($H^2 = 0.21$) on total body BMD ($H^2 = 0.79$). Environmental correlations between BMD measurements were all statistically different from zero, however, to a lesser extent, relative to the genetic correlations (range, $r = 0.60–0.70$).

Genetic factors that affect lean mass had a nonsignificant influence on the heritability of BMD,

**TABLE 4. Intraclass correlation in lean mass age-adjusted fat mass, lean mass, and bone density among monozygotic (MZ) and dizygotic (DZ) twins, Sydney Twin Study of Osteoporosis (Australia), 1995–1996**

| Variable | Intraclass correlation coefficient ± SE* | | p value |
|---|---|---|---|
| | rMZ* ($n$ = 57) | rDZ* ($n$ = 55) | |
| Lean mass | 0.72 ± 0.06 | 0.32 ± 0.12 | 0.003 |
| Fat mass | 0.62 ± 0.08 | 0.30 ± 0.12 | 0.032 |
| Lumbar spine BMD* | 0.74 ± 0.06 | 0.48 ± 0.10 | 0.028 |
| Femoral neck BMD | 0.73 ± 0.06 | 0.47 ± 0.11 | 0.031 |
| Total body BMD | 0.80 ± 0.05 | 0.48 ± 0.10 | 0.003 |

* SE, asymptotic standard error; rMZ, monozygotic pairs' correlation; rDZ, dizygotic pairs' correlation; BMD, bone mineral density.

accounting for less than 15 percent of the genetic component of BMD. The genetic correlation between lean mass and BMD was statistically nonsignificant. Significant genetic correlation between fat mass and BMD was observed only for the total body ($r = 0.31$, $p < 0.01$) but not for the lumbar spine and femoral neck. In contrast, environmental correlations between fat mass and BMD were slightly higher than those between lean mass and BMD ($r$ = 0.36–0.70 vs. $r$ = 0.23–0.51).

## DISCUSSION

There has been little doubt that BMD measured at various sites is one of the best measurable determinants of fracture risk (28–30). BMD is, in turn, regu-

**TABLE 5. Contribution of genetic and environmental factors in age-adjusted lean mass, fat mass, and bone mass, a summary of univariate model-fitting analysis, Sydney Twin Study of Osteoporosis (Australia), 1995–1996**

| Variable/model | Squared standardized coefficients* | | | | $\chi^2$ | p value |
|---|---|---|---|---|---|---|
| | A | D | C | E | | |
| Lean mass | | | | | | |
| *E*† | | | | 1.000 | 38.05 | <0.001 |
| *CE* | | | 0.563 | 0.437 | 14.40 | 0.006 |
| *AE* | 0.835 | | | 0.165 | 2.81 | 0.59 |
| *ACE* | 0.835 | | 0.000 | 0.165 | 2.81 | 0.42 |
| *ADE* | 0.756 | 0.079 | | 0.165 | 2.80 | 0.42 |
| Fat mass | | | | | | |
| *E* | | | | 1.000 | 373.83 | <0.0001 |
| *CE* | | | 0.506 | 0.494 | 8.26 | 0.08 |
| *AE* | 0.648 | | | 0.352 | 0.34 | 0.99 |
| *ACE* | 0.648 | | 0.020 | 0.332 | 0.34 | 0.95 |
| *ADE* | 0.624 | 0.024 | | 0.332 | 0.34 | 0.95 |
| Lumbar spine BMD‡ | | | | | | |
| *E* | | | | 1.000 | 67.93 | <0.0001 |
| *CE* | | | 0.636 | 0.364 | 14.04 | 0.007 |
| *AE* | 0.778 | | | 0.222 | 7.65 | 0.11 |
| *ACE* | 0.621 | | 0.157 | 0.222 | 7.65 | 0.11 |
| *ADE* | 0.778 | 0.000 | | 0.222 | 7.65 | 0.11 |
| Femoral neck BMD | | | | | | |
| *E* | | | | 1.000 | 59.20 | <0.0001 |
| *CE* | | | 0.629 | 0.371 | 14.91 | 0.005 |
| *AE* | 0.764 | | | 0.236 | 6.00 | 0.20 |
| *ACE* | 0.656 | | 0.108 | 0.236 | 2.75 | 0.43 |
| *ADE* | 0.764 | 0.000 | | 0.236 | 6.00 | 0.11 |
| Total body BMD | | | | | | |
| *E* | | | | 1.000 | 70.02 | <0.0001 |
| *CE* | | | 0.657 | 0.353 | 13.60 | 0.009 |
| *AE* | 0.786 | | | 0.214 | 5.19 | 0.27 |
| *ACE* | 0.625 | | 0.165 | 0.210 | 4.10 | 0.25 |
| *ADE* | 0.786 | 0.000 | | 0.214 | 5.19 | 0.16 |

* *A*, additive genetic factors; *D*, dominant genetic factors; *C*, shared environmental factors; *E*, nonshared environmental factors.
† Models *E*, *CE*, *AE*, *ACE*, and *ADE* have 5, 4, 4, 3, and 3 df, respectively.
‡ BMD, bone mineral density.

TABLE 6. Contribution of genetic and environmental factors to the variance of age-adjusted lean mass, fat mass, and bone mineral density, Sydney Twin Study of Osteoporosis (Australia), 1995–1996

| | Lean mass | Fat mass | Bone mineral density | | |
|---|---|---|---|---|---|
| | | | Lumbar spine | Femoral neck | Total body |
| Genetic factors | | | | | |
| *G*1 | 0.835 | 0.017 | 0.077 | 0.105 | 0.061 |
| *G*2 | | 0.630 | 0.000 | 0.009 | 0.063 |
| *G*3 | | | 0.701 | 0.260 | 0.428 |
| *G*4 | | | | 0.391 | 0.025 |
| *G*5 | | | | | 0.210 |
| $H^2$* | **0.835** | **0.648** | **0.778** | **0.764** | **0.786** |
| Environmental factors | | | | | |
| *E*1 | 0.164 | 0.097 | 0.034 | 0.014 | 0.061 |
| *E*2 | | 0.255 | 0.011 | 0.015 | 0.054 |
| *E*3 | | | 0.177 | 0.052 | 0.036 |
| *E*4 | | | | 0.154 | 0.007 |
| *E*5 | | | | | 0.056 |
| $E^2$* | **0.165** | **0.352** | **0.222** | **0.236** | **0.214** |

* $H^2$, index of heritability; $E^2$, environmental contribution.

TABLE 7. Genetic and environmental correlation ± standard error in age-adjusted lean mass, fat mass, and bone mineral density (BMD), Sydney Twin Study of Osteoporosis (Australia), 1995–1996†

| | Lean mass | Fat mass | Bone mineral density | | |
|---|---|---|---|---|---|
| | | | Lumbar spine | Femoral neck | Total body |
| Lean mass | | 0.52** ± 0.08 | 0.39* ± 0.08 | 0.23 ± 0.15 | 0.51** ± 0.09 |
| Fat mass | 0.16 ± 0.12 | | 0.41* ± 0.08 | 0.36* ± 0.09 | 0.70** ± 0.07 |
| Lumbar spine BMD | 0.08 ± 0.13 | 0.02 ± 0.19 | | 0.57** ± 0.08 | 0.70** ± 0.07 |
| Femoral neck BMD | 0.16 ± 0.13 | 0.05 ± 0.18 | 0.64** ± 0.09 | | 0.61** ± 0.06 |
| Total body BMD | 0.09 ± 0.14 | 0.31* ± 0.09 | 0.75** ± 0.06 | 0.58** ± 0.10 | |

* $p < 0.05$; ** $0.0001 < p < 0.01$.
† Values in the upper diagonals are environmental correlations, and values in the lower diagonals are genetic correlations.

lated by genetic, hormonal, dietary, and mechanical factors. The present study addressed a small part of this complex system by using the classic twin design. It was found that 1) both lean mass and fat mass were associated with areal BMD; however, fat mass alone appeared to have an independent effect on BMD/height ratios and volumetric BMD; 2) both lean mass and fat mass as well as BMD were under strong genetic influence; and 3) the association between fat mass (and lean mass) and BMD was mediated mainly through environmental influences.

The positive association between body weight and bone density has been well documented in several epidemiologic studies (1–3, 9). It has been shown in this study that BMD is associated with either lean or fat mass or both, depending on the skeletal site. This finding is in agreement with previous studies in twins (14–16) and cross-sectional studies (3, 8, 9, 31–34), which showed that lean mass was a better determinant of areal BMD. However, BMD ($g/cm^2$) is an areal measure, which excludes the anterior-posterior diameter; thus, an approximation of volumetric density has been suggested as a surrogate measure of true volumetric density. In this study, two estimates of volumetric BMD have been analyzed. One was derived directly from the BMD scans, and another was obtained as the BMD/height ratio (20). In both analyses, fat mass was consistently associated with volumetric BMD in matched analysis. In unmatched analysis, fat mass was the only determinant of the BMD/height ratio. These findings are in agreement with previous cross-sectional studies (5–7, 20).

It is thus clear that the relation between body composition and BMD is conditional on the unit of measurement in which BMD is expressed. As lean mass (but not fat mass) is related to height, a correction of BMD for height will reduce or eliminate the apparent effect of lean mass and increase the significance of fat

mass on BMD. There was evidence suggesting that the effect of lean mass on BMD was independent of body height, as it was noted that height-adjusted lean mass was a significant predictor of areal BMD. Statistically, because lean mass and fat mass are correlated, it is difficult to separate their *independent* effects in statistical analysis. In this study, in contrast to a previous report (15), the correlation between regression coefficients for fat mass and lean mass in matched analysis ($r = 0.43$) was higher than that in unmatched analysis ($r = 0.31$). Thus, analysis based on intrapair differences does not appear to reduce the problem of collinearity. The consequence of collinearity is that sampling estimates of the strength of the relation between body composition and BMD will vary from sample to sample. The fact that analyses based on matched and unmatched samples yielded different sets of predictor variables is a reflection of this statistical problem. It can also be argued that the conflicting findings from previous studies are also due, in part, to the sampling variation of the correlation between lean mass and fat mass.

While inevitably there is sampling variation in the relation, what appears to be consistent is that the strength of association between lean mass and areal BMD is more pronounced than between fat mass and areal BMD. For example, an increase of 10 kg in lean mass and 10 kg in fat mass was estimated to be associated with an increase of 10 percent (or approximately 1 standard deviation) and of 4 percent, respectively, of lumbar spine BMD. Is the association between bone mass and lean or fat mass due to an underlying biologic or artefactual mechanism? A study on phantom results (35) suggested that the addition of fat tissue has no significant effect on the measurement of BMD. Similarly, in vivo studies also demonstrated a nonsignificant effect of fat on lumbar spine BMD (36) and total body BMD (37). We also tested the artefactual hypothesis by analyzing data from 20 postmenopausal women in a weight-reduction interventional trial and found that, over a period of 28 days, while lumbar spine and total body BMD did not change, decreases of $2.6 \pm 0.41$ kg and $3.7 \pm 0.43$ kg (mean $\pm$ SE) in lean mass and fat mass, respectively, were observed (unpublished data). Thus, it appears that the association is biologic. Indeed, physical fitness, body weight, and muscle strength have independent effects on bone mass in samples of young and elderly men and women (1, 12, 32), with independent effects of weight and quadriceps strength on femoral neck BMD. The relation between fat mass and BMD has not been well characterized. However, an attractive proposition is that estrogen production in adipocytes influences bone mass. Nevertheless, in premenopausal women this hypothesis would not appear to be relevant (38).

Is the intersubject variation in lean mass and fat mass attributable to genetic or environmental factors? In the present study, 84 percent and 65 percent of intersubject variances of lean mass and fat mass, respectively, were attributable to genetic factors, consistent with previous estimates (16). The estimated heritability for body mass index in this sample was 72 percent, within the range of 5–90 percent reported in the literature (39–42). Taking together, there is evidence suggesting that genetic factors have a greater influence on lean mass than on fat mass. The data also indicated that shared familial environmental factors had a minimal and nonsignificant effect on these traits. Furthermore, model-fitting analysis indicated that the mode of inheritance was additive rather than dominant, as dominant genetic effects were found to be statistically nonsignificant. The cross-sectional correlation between lean mass and fat mass in this sample was moderate ($r = 0.33$, $p < 0.001$), and in bivariate analysis a significant part of this relation appeared to be due to common environmental rather than genetic factors. In fact, of the total heritability of fat mass of 65 percent, only 1.7 percent was attributable to shared genetic factors with lean mass. On the other hand, the environmental correlation between the two variable traits was 0.53 compared with 0.16 for genetic factors.

This study confirms the familial influence on bone density with estimates of heritability for the lumbar spine, femoral neck, and total body BMD of 78 percent, 76 percent, and 79 percent, respectively, which are comparable with previous estimates (12–16). However, the present study also indicates that a common source of genetic and environmental variances underlies the clustering of BMD at various skeletal areas. For example, the genetic correlation between lumbar spine and total body BMD was 0.75 compared with the environmental correlation of 0.70. Similarly, the genetic correlations between the lumbar spine and femoral neck were 0.64 compared with 0.57 for environmental factors. This indicates that genes affecting the lumbar spine BMD are more *likely* to affect total body BMD than femoral neck BMD; likewise, environmental factors affecting the lumbar spine BMD are also likely to express effects on femoral neck and total body BMD. Indeed, estimates of heritability from the bivariate twin model suggest that over half of the genetic influence on total body BMD is mediated through the genetic influence on the lumbar spine (and only a third of the genetic influence on femoral neck BMD). Somewhat lower environmental correlations between BMD sites observed in this study may relate, in part, to the differential effects of environmental

factors, such as smoking and dietary calcium intakes, on various skeletal sites.

The fact that lifestyle and dietary habits as well as body structure aggregate within families suggests that shared genetic, in addition to shared environmental, factors play a role in determining the phenotypic similarity of individuals in the same family. While the mechanism of association between either lean mass or fat mass and bone density is not known, the present study sought to determine whether the association is controlled by genetic factors. The multivariate analysis suggested that lean mass, fat mass, and bone density are more likely regulated by different genetic factors and that the association between fat mass and BMD is regulated mainly by environmental factors. In fact, less than 15 percent of the estimated heritability of bone density is due to genetic factors common with those for lean mass. Indeed, none of the genetic correlations between lean mass and bone density was significant. In contrast, the environmental correlations between fat mass (or lean mass) and BMD were all significant. This has been supported further by data in MZ twins, demonstrating that intrapair differences in BMD were related significantly to intrapair differences in lean mass and fat mass (correlation coefficients ranged from $r = 0.30$ to $r = 0.63$). Thus, significant associations exist between BMD and fat (or lean) mass entirely in the apparent absence of genetic influences.

Increased emphasis has been placed recently on the search for specific genes that influence BMD. Among others, the *vitamin D receptor* (*VDR*) gene has been reported to be associated with BMD (43). Although several studies have confirmed the relation, others have not (44, 45). The continuous, unimodal distribution of BMD is probably due to multiple genetic (and environmental) factors, each with small but additive effects. It is, thus, expected that there are several other genes yet to be found, which have effects on BMD. The present study indicates that some of the genes may have small but pleiotropic effects on both cortical and trabecular BMD. The moderate genetic correlations between BMD sites may explain the differential effects of the *VDR* gene alleles, which were observed mostly at the lumbar spine and do not necessarily translate into effects at the femoral neck, or vice versa.

The present findings must be interpreted in the context of a number of potential limitations. The data were obtained from a Caucasian population in Australia, among whom cultural backgrounds and environmental living conditions are generally homogeneous. Also, the present results were obtained in women only; thus, care should be taken when extrapolating these results to other populations and to men. However, a recent cross-sectional study of 139 healthy men (46) has found that lean mass was related to BMD (consistent with the present study's findings) and BMD/height ratios (not consistent with the present results). Hormone-related factors and environmental interaction may contribute to the divergent results for men and women.

Importantly, these data were obtained from twins, which are arguably not representative of the general unrelated population. However, the variances of bone density, lean mass, and fat mass in this twin sample are very comparable with those observed in unrelated populations. Furthermore, the finding that the strength of association between lean (or fat) mass and BMD was similar between unmatched and matched analyses suggests that the results can be generalized to nontwin populations. The use of intrapair differences in the analysis eliminates the effects of differential age and other confounding environmental factors present in most previous cross-sectional studies.

It has been argued that MZ twins are more likely to share more similar environments than DZ pairs, possibly related to their phenotypic similarity (47). This could result in an overestimate of the genetic influence derived from twin studies. However, in all the traits analyzed here, the model incorporating the effects of additive genetic and specific environmental factors (*AE* model) fit the data as well as the model with the addition of shared environmental factors (*ACE* model). This may relate to the inadequacy of power to detect a small effect in this and smaller studies (48).

Ratio variables, such as BMD/height, have been suggested to generate spurious correlations (49). However, there was no significant correlation between the BMD/height ratio and height, and the intercept term of the regression equation of BMD against height was not significantly different from zero. These findings, taking together, suggest that the adjustment of BMD for body height is valid.

In conclusion, these data indicate that the clinically relevant association between volumetric BMD and body composition is mediated only through fat mass. Furthermore, lean mass and fat mass, as with bone density, are under strong genetic regulation. While it is possible that the same genes modulate bone mass and density at different skeletal BMD sites, the association between BMD and fat mass or lean mass appears to be mediated principally via common environmental influences. These data also suggest that modulation of environmental factors could translate to clinically relevant changes in BMD and presumably fracture risk. The impact of such changes requires careful consideration.

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