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Architecture of Cortical Bone Determines in Part its Remodelling and Structural Decay

Running title: Bone architecture determines remodelling

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Abstract

Bone remodelling accelerates and becomes unbalanced after menopause; less bone is deposited than resorbed from the surface of canals traversing the cortex. The canals enlarge so the intracortical surface area enlarges. We hypothesized that cortical bone with a larger internal surface area, due to more or larger canals, is more liable to being remodelled, further enlarging the internal surface area and facilitating more remodelling and structural deterioration. For 95 monozygotic twin pairs aged 40-61 years, we measured internal cortical surface areas and structure of the distal tibia using high resolution peripheral computed tomography, and three circulating bone remodelling markers. Using principal components (PC) analyses, we identified one summary measure of intracortical and endocortical bone surface areas, cortical porosity and volumetric bone mineral density (structure PC), and one summary measure of bone remodelling markers (remodelling PC). We applied a twin regression analysis (Inference on Causation by Examination of Familial Confounding; ICE FALCON) to assess consistency with a causal component in the association between a predictor (X) and an outcome (Y) by testing if the regression coefficient for the X value of the co-twin decreases after adjusting for the X value of the twin herself. With Y = remodelling PC, the regression coefficient for structure PC in the co-twin was 0.29 ($p < 0.001$) before, and 0.18 ($p = 0.03$) after, adjusting for her own structure PC (40% lower; $p = 0.06$). With Y = structure PC, the regression coefficient for remodelling PC in the co-twin was 0.17 ($p = 0.01$) before, and 0.20 ($p < 0.001$) after, adjusting for her own remodelling PC (22% higher; $p = 0.7$). The structure of bone, its surface area to bone matrix volume configuration, contributes in part to its own remodelling and deterioration, but not vice versa.

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Introduction

The cellular machinery of bone modelling and remodelling is the final common pathway expressing all genetic and environmental factors influencing bone structure [1]. During young adulthood, remodelling is balanced; a volume of old or damaged mineralised bone matrix is removed and replaced by an equal volume of new bone matrix and no permanent structural decay occurs. After menopause, remodelling accelerates; there are more basic multicellular units (BMUs) initiated upon each of the three (intracortical, endocortical and trabecular) components of bone's inner (endosteal) surface. In addition, remodelling by each of the BMUs becomes unbalanced; each time a volume of mineralised bone matrix is removed by teams of osteoclasts, less bone matrix is deposited by the teams of osteoblasts of a BMU. This produces focal structural decay characterized by increased intracortical porosity, cortical thinning, trabecular thinning and loss of complete trabecular plates [2].

As remodelling is surface dependent, variation in remodelling intensity might be partly explained by differences in the surface area available to facilitate remodelling [3,4]. Bone fashioned with greater surface area per unit mineralised bone matrix volume could be more accessible to being remodelled and therefore decayed. For example, from studying female twin pairs [5] we found positive associations between the intracortical and endocortical surface area and bone remodelling markers and suggested that this was consistent with the notion that a larger internal surface area facilitates higher remodelling of cortical bone.

We recognised that the reverse is also plausible; higher remodelling upon cortical surfaces might result in larger surface because remodelling upon Haversian canals enlarges their cross-sectional diameter focally and so the perimeter and surface area increases at that location. Similarly, resorption upon the endocortical surface lining the medullary canal produces a concavity which might also enlarge the endocortical surface area focally. Therefore, remodelling might be self-perpetuating; a larger surface area might provide more locations for the receipt of signals from bone matrix in need of remodelling. If more remodelling further increases the surface area, a viscous cycle of accelerated deterioration of cortical bone would proceed.

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Because our previous study was cross-sectional, it was not possible to test these alternative pathways using conventional analyses. However, because it was a twin study, and because there are correlations between bone structure and remodelling between bone structure in one twin and bone remodelling markers in the co-twin, we can use a recently developed twin regression analysis for studying evidence consistent with causation to test these hypotheses which we call Inference on Causation from Examination of FAmiliaL CONfounding (ICE FALCON) [6-8]. This approach has been applied to predictors of mammographic density, a risk factor for breast cancer [6,7] and to eczema in infancy as a predictor of childhood asthma and hay fever [8].

To see how the argument works, consider sister pairs and *BRCA1* germline mutation status (X) as a predictor of breast cancer (Y); see Figure 1. The sister of a mutation carrier is at increased risk of the disease because she has a 50% chance of having also inherited the mutation. Therefore, there is a 'cross-trait cross-pair' regression coefficient for the association of a woman's breast cancer status (e.g. Y_1) with her sister's mutation status (X_2). However, it is confounded by the mutation status of the woman herself (X_1), because once a woman's mutation status is known, then in terms of that woman's breast cancer risk the mutation status of her sister becomes irrelevant. If Y_1 is regressed against both X_1 and X_2, the cross-trait cross-pair regression coefficient becomes zero.

There is an analogy between this new twin regression analysis and the classic twin model, in that both methods address issues of causation by framing a one-tailed hypothesis test. The classic twin model makes inference about the existence of unmeasured genetic causes on a single measured trait by considering the null hypothesis of no difference in correlation coefficient between monozygotic pairs and dizygotic pairs versus the alternate hypothesis of a reduced correlation in dizygotic pairs. ICE FALCON makes inference about one measured familial trait having a causal effect on another measured trait by testing the null hypothesis of no change in a regression coefficient versus the alternate hypothesis of a reduction in the regression coefficient. Neither method 'proves' causation; both seek evidence consistent with causation.

Here we have applied the twin regression analysis, first to the situation where the Y variable is a measure of

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bone remodelling for one twin and the X variables are the bone structure measures of one or both of the twins. We fitted a series of models in which first only one of the X variables was included, and then both X variables were included. We then examined whether there was any reduction in the cross-trait cross-pair regression coefficient after adjusting for the X variable of the self. Second, we tested the reverse, that bone remodelling determines bone structure, by letting the Y variable be a measure of bone structure and the bone remodelling measures of the twins be the X variables. To simplify matters, we conducted a principal components analysis to see if we could identify one or more major dimensions to each of the bone structure and bone remodelling data.

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Material and Methods

Subjects

From 2008 to 2009, through the Australian Twin Registry we recruited 113 monozygotic (MZ) female twin pairs aged 40-61 years living in Melbourne, Australia [5]. Using a questionnaire, we assessed zygosity (concordance with zygosity determined by molecular testing is about 97% [7]) and excluded twin pairs in which one or both had a hysterectomy before menopause, had an illness or used drug therapies that affect bone, or were using hormone replacement therapy, leaving 95 pairs. All subjects gave written informed consent and the study was approved by the Austin Health Ethics Committee.

High-resolution peripheral quantitative computed tomography (HR-pQCT) (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) with a isotropic resolution of 82 μm was used to quantify cortical volumetric bone mineral density (vBMD) and porosity [9]. Measurements were made at the non-dominant distal tibia. The *in vivo* precision was 0.7% to 4.4%. Daily quality control was carried out by scanning a reference phantom containing rods of hydroxyapatite (QRM Moehrendorf, Germany). Radiation exposure was ~5 μSv per measurement.

Intracortical and endocortical bone surface (BS) areas at the distal tibia were measured using marching cubes that create triangular models of the surfaces from 3D data [10]. Surface measures were validated *in vitro* using 20-micron scans of excised trabecular cubes of the radius. Bone surface area/bone volume (BS/BV) by XtremeCT correlated with BS/BV by microCT-40 ($r = 0.98$) but the absolute values were overestimated as segmentation overestimates trabecular thickness (BS/BV = 17.4 vs. 11.3 1/mm by microCT-40), mean BS = 2201 vs. 1920 mm^2 by microCT-40. The intracortical and endocortical surfaces were expressed per unit cortical tissue volume (CorticalTV; cortical bone including its pores). CorticalTV (mm^3) was expressed as the cortical CSA (mm^2) times the length of each scan (104 slices x 0.082 mm thickness).

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Fasting blood collected between 8 am and 10 am was assayed for serum osteocalcin, β- carboxyterminal cross-linking telopeptides of type I bone collagen (CTX) and procollagen type 1 amino-terminal propeptide (PINP) by electrochemiluminescence immunoassay (Elecsys 1010 Analytics, Roche Diagnostics, Germany, intra- and inter-assay CV 3-8%).

Statistical Methods

We log transformed the remodelling markers so their distributions were approximately normal. Missing data were imputed using the Random Forest method missForest in the statistical package R [11,12]. All variables were standardised to have zero mean and unit variance.

There were three bone remodelling markers and four bone structure measures (endocortical and intracortical bone surface area/mineralized bone matrix volume, porosity and cortical vBMD) so twelve analyses would have been required to assess relationships between the remodelling and structure measures. This would have led to multiple testing issues that are not necessarily resolved by Bonferroni adjustment or False Discovery Rate methods because there are multiple correlated outcomes and predictors.

We therefore used principal components (PC) analysis to see if it was justifiable to combine multiple and correlated measured variables into one or a few composite scores. The initial set of correlated variables was linearly transformed into a set of new, but uncorrelated, variables called PCs, each a linear weighted combination of the initial variables. The PCs were ordered so that the first PC explains the largest possible variation in the original data, the second PC was uncorrelated with the first component and explained additional but less variation than the first PC, and so on.

We used a twin regression analysis that allows inference on causation from examination of familial confounding by allowing a test of whether there is evidence consistent with a causal relationship underlying

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the association between the two measures [6-8]. Technical details on the statistical modelling are given in the Appendix.

Consider the situation where bone structure is a predictor (X) of bone remodelling (the outcome, Y). Given that, for both bone structure and bone remodelling, there is a correlation within twin pairs, there must be familial (genetic or environmental) factors that determine bone structure, and familial (genetic or environmental) factors that determine bone remodelling markers (see S_X and S_Y of Fig 1). If there is also a 'cross-trait cross-pair' correlation, such that the bone structure of one twin is associated with the bone remodelling markers of the co-twin, then the conventional way of analysing the data (the multivariate classic twin model) assumes that the familial factors determining bone structure and those determining bone remodelling are themselves correlated (i.e. S_X and S_Y are correlated), and this is the only reason for the cross-trait-cross-pair correlation [13-15]. If this is true, then the correlation between the bone structure of one twin and the bone remodelling of the co-twin will be independent of the twin's own remodelling or structure. On the other hand, if there is any direct causal effect of the bone structure on the bone remodelling, in that a change to the bone structure initiates a change to the bone remodelling, then in theory this independence will no longer exist.

Therefore, if the cross-trait cross-pair regression coefficient of Y_1 on X_2 does not change after adjusting for a twin's own predictor (X_1), there is no evidence for the existence of a direct causal relationship; i.e. the relationship must be attributed to genetic or environmental familial factors shared by both traits. If instead the cross-trait cross-pair regression coefficient reduces after adjusting for the twin's own predictor, the data are consistent with the existence of, at least in part, a causal component. Note that if the cross-trait cross-pair regression coefficient is not eliminated by adjusting for the predictor of the woman, this is consistent with the existence of familial factors that influence both the predictor and the outcome. That is, there can be both a direct casual pathway and common familial factors. Of course, the role of chance has to be taken into account by the statistical modelling.

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We fitted three models: the first estimates within-person cross-trait association alone; the second estimates the cross-trait cross-pair association alone; and the third estimates the cross-pair and within-self associations together. To test the null hypothesis that there is no causal effect of the predictor on the outcome against the alternate hypothesis that there is at least some causal effect, the following conditions need to be satisfied: (i) the predictor or outcome is correlated within twin pairs (whether this is due to shared genetic and/or environmental factors is irrelevant at this stage); (ii) there is an association between the predictor and the outcome within a twin; (iii) for (i) and (ii) to be strong enough that there is a detectable and statistically significant cross-trait cross-twin association; and (iv) a reduction in the magnitude of the cross-trait cross-twin regression coefficient when it is adjusted for the within-twin association.

The twin regression analyses, and the within-pair correlation for twin pairs, adjusted for body mass index (BMI) and menopausal status, were performed using the program FISHER [16,17] assuming a bivariate normal distribution and the pairs were independent of one another. Estimation of parameters and statistical inference were derived under maximum likelihood theory [16,17]. All associations were adjusted for body mass index (BMI) and menopausal status.

We assessed the statistical significance of the change in regression coefficients using nonparametric bootstrap methods. This involved randomly sampling twin pairs with replacement to obtain the same sample size as the original dataset, then fitting the models to this new data set to get a new set of estimated parameters. We then repeated the process 1,000 times to estimate the sampling distribution of the parameter estimates from which a standard error was estimated by computing the standard deviation. For bootstrap method, we used generalised estimating equations and wrote our own program in R (http://www.R-project.org/) because this was not feasible using FISHER. We used one-tailed p-values for testing of the alternative hypothesis that the cross-trait cross-pair regression coefficient decreased after adjustment.

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Results

Summary statistics for age, physical measurements, remodelling markers and structure variables at the distal tibia were given in the Table 1. PC analysis showed that the cortical structure and remodelling measures could each be reduced to one major component. For the cortical structure, the first PC was the mean of the standardised measures with tibia cortical vBMD having a similar weight but in the opposite direction; weights were -0.53, 0.52, 0.52 and 0.42 for cortical vBMD, porosity, Intracortical BS/Cortical TV, and Endocortical BS/Cortical TV, respectively. This PC explained 80% of the variance. For remodelling markers, the first PC was the mean of the standardised measures; weights were 0.58, 0.57 and 0.58 for osteocalcin, CTX and P1NP, respectively. This PC explained 87% of the variance. Therefore, the standardised cortical structure measures and the standardized remodelling measures are captured by their respective means. We refer to these as the 'structure PC' and the 'remodelling PC' respectively.

The within-pair correlation for the structure PC, adjusted for BMI and menopausal status, was 0.79 (95% CI: 0.71-0.87). The within-pair correlation for remodelling PC was 0.60 (95% CI: 0.46-0.73). Cross-trait cross-pair correlation across the structure PC in one twin and the remodelling PC in the co-twin, adjusted for BMI and menopausal status, was 0.38 (95% CI: 0.23-0.52).

The upper rows in Table 2 show the results of the twin regression analysis testing the hypothesis that bone structure determines bone remodelling. Model I shows that the PC structure was associated with the remodelling PC (regression coefficient ± standard error = 0.336 ± 0.071; $p < 0.001$). Model II shows that the PC structure of a twin was also associated with the remodelling PC of the co-twin (0.292 ± 0.079; $p < 0.001$). Model III shows that, when fitted together, the structure PC of a twin was still associated with her own remodelling PC (0.249 ± 0.075; $p = 0.001$), but the cross-twin association with the structure PC of the co-twin was reduced (to 0.175 ± 0.080; $p = 0.03$) by 40% (one-tailed $p = 0.06$).

The lower rows in Table 2 test the hypothesis that bone remodelling determines bone structure. Model I shows that the remodelling PC was associated with the structure PC (0.235 ± 0.059; $p < 0.001$). Model II

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shows that the remodelling PC of a twin was also associated with the structure PC of the co-twin (0.165 ± 0.065; p = 0.01). Model III shows that, when fitted together, the remodelling PC of a twin was still associated with her own structure PC (0.265 ± 0.058; p < 0.001), but there was a small increase in the co-twin association (to 0.202 ± 0.057; p < 0.001) of 22% that was not significant (one-tailed p = 0.7).

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Discussion

Remodelling depends on the area of the surface and the volume of mineralised bone matrix enveloped by it. The recent development of a regression analysis approach, ICE FALCON, to the study of causation using data for twin pairs meant that we could now examine whether, for cortical bone, the larger the intracortical surface area the higher the remodelling, or in reverse, the higher the remodelling the more the surface area created by excavation of large pores which creates more surface area for remodelling to occur upon [6-8]. We found that the structure PC of the co-twin was associated with the remodelling PC of a twin before, but 40% less so after taking into account her own structure PC. This was consistent with bone structure causing, in part, bone remodelling. The reverse hypothesis, that bone remodelling causes bone structure, was not supported by the data.

We cannot exclude the possibility that bone remodelling produces more surface area by excavating local concavities. This is unlikely to be substantial enough *relative* to the starting surface area to produce a detectably higher remodelling intensity in the age range of the participants. In later life, however, when chronically increased intracortical remodelling has produced giant coalescent pores, the relative contributions of peak surface area and new surface area may alter. For older women, a bi-directional causal pathway could create an ever accelerating and self-perpetuating cycle of bone loss; a larger surface is permissive for remodelling, and remodelling enlarges pores for even more remodelling to occur upon. Mineralised bone matrix is removed from an ever-decreasing volume of mineralised bone matrix. Indeed, most bone loss in old age is cortical in origin, not trabecular [18].

Our analyses suggest that peak surface area achieved at the completion of growth (rather than new surface excavated by remodelling during ageing) determines, in part, remodelling for women aged 40 to 61 years. The total surface area provides points of connection for canaliculi sending signals from damaged bone matrix in need of repair to the surface, which, at a given point, initiates remodelling of the surrounding matrix and damage repair.

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Greater endocortical surface area is formed by greater resorptive excavation of the medullary (marrow) cavity during growth. Likewise, greater numbers of osteons, each with their central Haversian canal, are formed by greater intracortical remodelling during growth. Together, the endocortical and intracortical surface form the total surface area enveloping the mineralized cortical bone matrix [19,20].

Figure 2 illustrates that, for a tubular bone of given diameter, the larger the central medullary canal and the greater the number of Haversian canals, the larger total void volume and so the reciprocally smaller mineralised bone matrix volume. The bone has a lower apparent volumetric density – there is less mineralized bone matrix volume within the periosteal envelope because there is proportionally more void volume. This creates a double hazard; after menopause, when remodelling intensity increases, there is more surface area available to allow expression of that remodelling. Each remodelling event removes more bone than it deposits, and removes it from a smaller mineralised bone matrix volume, so structural decay accelerates disproportionately in bones assembled with a high surface/volume architectural configuration. Bones assembled with relatively less mineralised bone matrix volume and more void volume, and therefore more surface area, lose their mineralized bone volume disproportionally faster. Thus, the three dimensional configuration of bone, specifically its internal surface area to mineralised bone matrix volume, plays a central role in determining the rate of bone loss and the nature of the structural decay produced during ageing. The configuration of bone itself contributes to its own decay.

This study has several limitations. A larger sample size would have given us more power to examine the hypotheses and provided more precision on the amount of cross-pair cross-trait correlations that could be explained by a causal relationship between cortical bone structure and markers of remodelling. The measurement of intracortical and endocortical surfaces was derived using the marching cube method and validation of the accuracy of this method in determining the true internal surface area is not available. The within-pair correlation for the remodelling PC was 0.60 (95% CI: 0.46-0.73), a little less than the 0.79 (95% CI: 0.71-0.87) for the within-pair correlation for bone structure PC. While measurement error in the X variable(s) can attenuate regression estimates to the null, the same is true for measurement error in the Y variable. Therefore it is unlikely that the small difference in measurement error of the two PC measures

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would explain the different findings when the X and Y variables were swapped. Our approach is novel, so that as well as replication studies using a twin or sister-pair design and ICE FALCON analyses, prospective evaluation of the relationship between surface, bone loss and structural decay will be important in trying to validate our findings and approach.

In conclusion, non-invasive assessment of bone morphology is feasible and increasingly widely able. Use of this methodology to study the skeletons of twin pairs permits insights into the pathophysiology of bone fragility. Here we have found that the data are consistent with the architecture of cortical bone, namely its surface area to mineralised bone matrix volume configuration, contributing at least in part to its own remodelling and therefore to its own decay.

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Table 1. Characteristics of the participants.

	Mean	SD
Age (years)	49.3	5.5
Height (cm)	162.6	6.3
Weight (kg)	68.8	13.5
BMI (kg/m²)	26.0	4.9
Bone remodelling markers		
Osteocalcin (ng/ml)	20.6	8.4
CTX (ng/ml)	0.361	0.174
P1NP (ng/ml)	46.7	21.0
Tibia		
Cortical vBMD (mg/cm³)	890	50.3
Cortical Porosity (%)	4.55	1.94
Intracortical BS (mm²) / Cortical TV (mm³)	0.450	0.217
Endocortical boneBS (mm²) / CorticalTV (mm³)	0.875	0.221

BMI, body mass index; CTX, β-carboxyterminal cross-linking telopeptides of type I bone collagen (β-CTX); P1NP, procollagen type 1 amino-terminal propeptide; vBMD volumetric bone mineral density TV, tissue volume; BS, bone surface.

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Table 2: Regression estimate, standard error (SE), and p-value from regression models of monozygotic twin pair data for first principal component (PC) of bone remodelling and PC of bone structure, testing probability for both direction of causation.

		Model I			Model II			Model III		
		Estimate	SE	p	Estimate	SE	p	Estimate	SE	p
		PC remodelling (Y_1)								
PC structure (X_1)	Self	0.336	0.071	< 0.001				0.249	0.075	0.001
PC structure (X_2)	Co-twin				0.292	0.079	< 0.001	0.175	0.080	0.03
		PC structure (Y_1)								
PC remodelling (X_1)	Self	0.235	0.059	< 0.001				0.265	0.058	< 0.001
PC remodelling (X_2)	Co-twin				0.165	0.065	0.01	0.202	0.057	< 0.001

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Legends to Figures

Figure 1

A path diagram representing the casual association between *BRCA1* mutation status and breast cancer for pairs of sisters. Squares represent measured variables, circles represent unmeasured variables, and the arrows indicate the direction of causation between variables. Let Y_1 and Y_2 represent the breast cancer status of sisters 1 and 2, respectively, within the same pair, and X_1 and X_2 represent their corresponding mutation status for the breast cancer susceptibility gene, *BRCA1*. S_Y represents the risk factors for breast cancer, other than *BRCA1* mutation status, that are shared by sisters; i.e. the causes of Y shared by the sisters. S_X represents the cause of X shared by the sisters, namely their shared parenthood. Causal pathways, and therefore correlations or associations, between two variables are established by proceeding backwards along causal arrow(s), then forwards along causal arrow(s). Consider going from Y_1 to X_2. There is a causal pathway from Y_1 to X_1 to S_X to X_2. Note, however, that if X_2 is known, this pathway from Y_1 to X_2 is 'blocked'. While there is a causal pathway from Y_1 to S_Y to Y_2, it stops there (one cannot reverse direction more than once) so there is no connection between Y_1 and X_2 through this route. Therefore, given knowledge of X_1, there is no association between Y_1 and X_2; see text.

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Legends to Figures (cont.)

Figure 2

For two tubular bone cross sections, A and B, of the same total diameter, the bone assembled with greater excavation of the larger medullary (marrow) cavity, B, has a relatively thinner cortex and a larger endocortical surface area. If the cortex is also assembled with higher numbers of osteons, each having their central haversian canal, the total internal surface area (intracortical plus endocortical) is larger. This bone has less mineralized bone matrix volume and more void volume within its periosteal envelope. The lesser mineralized bone matrix volume and the greater total internal surface area create a double hazard (see text) because remodeling in surface dependent.

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Figure 1:

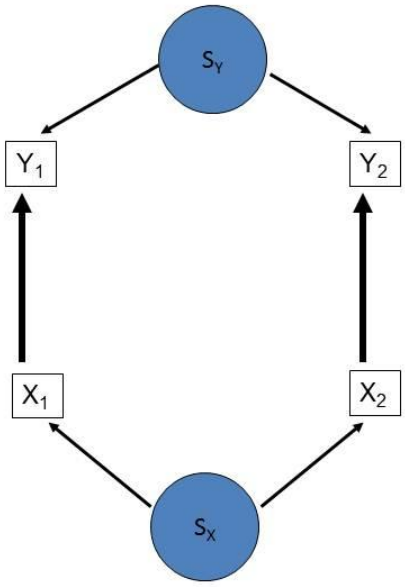

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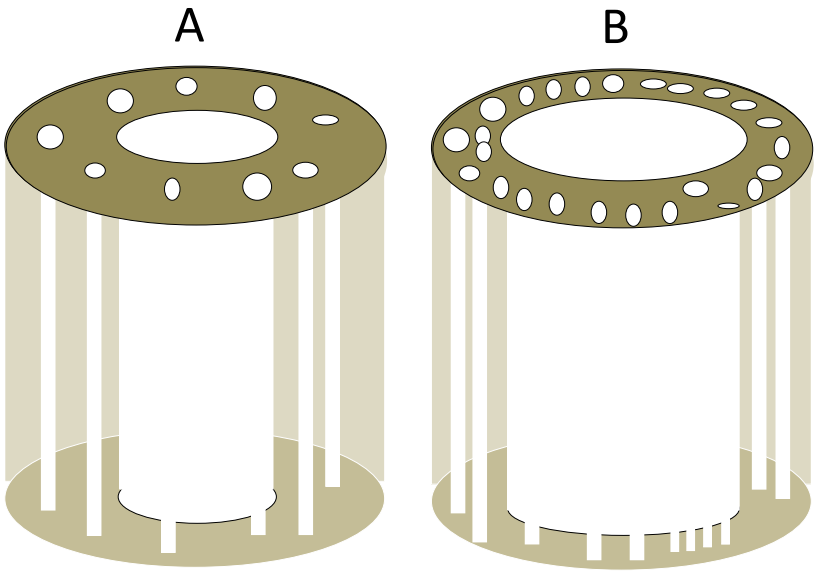
Figure 2:



More haversian canals and larger medullary canal

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Appendix

Let Y_{ij} denote a outcome of interest, with j = 1, 2 (twin 1 and twin 2, respectively) and i = 1,…, m, where m is the number of twin pairs. For each outcome, Y_{ij}, let X_{ij} denote a corresponding predictor. For simplicity, let $Y_{i,self} = Y_{i1}$ and $Y_{i,\cot win} = Y_{i2}$, and similarly define for predictors X_{ij}. Note that the choice of $Y_{i,self}$ and $Y_{i,\cot win}$ is arbitrary – data from both possibilities are used in the analysis; see below.

The first model expresses the relationship between the expected value (E) of an outcome variable and its own predictor by:

$$E(Y_{i,self}) = \alpha + \beta_{self} X_{i,self} \qquad \text{Model I}$$

$$E(Y_{i,\cot win}) = \alpha + \beta_{self} X_{i,\cot win}$$

where α is the intercept and β_{self} is the regression coefficient representing the cross-trait association.

The second model expresses the relationship between the expected value of Y_{ij} and its co-twin predictor by:

$$E(Y_{i,self}) = \alpha + \beta_{\cot win} X_{i,\cot win} \qquad \text{Model II}$$

$$E(Y_{i,\cot win}) = \alpha + \beta_{\cot win} X_{i,self}$$

where $\beta_{\cot win}$ is the regression coefficient representing the cross-trait cross-pair association.

The third model expresses the relationship using both predictors by:

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$$E(Y_{i,self}) = \alpha + \beta^{a}_{self} X_{i,self} + \beta^{a}_{\cot win} X_{i,\cot win} \qquad \text{Model III}$$

$$E(Y_{i,\cot win}) = \alpha + \beta^{a}_{self} X_{\cot win} + \beta^{a}_{\cot win} X_{i,self}$$

where $\beta^{a}_{\cot win}$ is the regression coefficient representing the cross-trait cross-pair association adjusted for its own predictor.

Estimation of the regression coefficients is conducted under a bivariate normal model which also estimated the correlation between $E(Y_{i,self})$ and $E(Y_{i,\cot win})$. The models above can be easily extended to allow for inclusion of multiple predictors, such as BMI and menopausal status, and to allow the cross-trait cross-pair associations to depend on zygosity and other pair-specific variables.

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Highlights

- We applied a twin regression analysis to assess consistency with causation
- The method permits insights into association between bone remodelling and structure
- Cortical bone surface area to bone matrix volume, contributes to its own remodelling
- Bone structure contributes at least partly to bone remodelling, but not vice versa

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