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Original Article

Hylonome

Analysis of the independent power of age-related, anthropometric and mechanical factors as determinants of the structure of radius and tibia in normal adults. A pQCT study

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Abstract

To compare the independent influence of mechanical and non-mechanical factors on bone features, multiple regression analyses were performed between pQCT indicators of radius and tibia bone mass, mineralization, design and strength as determined variables, and age or time since menopause (TMP), body mass, bone length and regional muscles' areas as selected determinant factors, in Caucasian, physically active, untrained healthy men and pre- and post-menopausal women. In men and pre-menopausal women, the strongest influences were exerted by muscle area on radial features and by both muscle area and bone length on the tibia. Only for women, was body mass a significant factor for tibia traits. In men and pre-menopausal women, mass/design/strength indicators depended more strongly on the selected determinants than the cortical vBMD did ($p<0.01$-0.001 vs n.s.), regardless of age. However, TMP was an additional factor for both bones ($p<0.01$-0.001). The selected mechanical factors (muscle size, bone lengths) were more relevant than age/TMP or body weight to the development of allometrically-related bone properties (mass/design/strength), yet not to bone tissue "quality" (cortical vBMD), suggesting a determinant, rather than determined role for cortical stiffness. While the mechanical impacts of muscles and bone levers on bone structure were comparable in men and pre-menopausal women, TMP exerted a stronger impact than allometric or mechanical factors on bone properties, including cortical vBMD.

Keywords: Muscle-Bone Interactions, Bone Biomechanics, Bone Tomography, QCT, pQCT, Osteoporosis

Introduction

Three current concepts in Osteology concern: **1.** the primary role of mechanical factors in the determination of bones as support structures, with regional muscle contractions playing a dominant role; **2.** the servo-regulated bone adaptations to mechanical usage through the re-distribution of the available mineralized tissue as a function of bone strains by bone mechanostat, and **3.** the modulation of bones' mechanical adaptation by non-mechanical factors (chiefly, the endocrine-metabolic system). The well-known genetic and endocrine-metabolic relationships between bone and muscle growth and development[1] fail to explain the regional adaptations of bone to mechanical usage[2]. This study aims to further disentangle the role of some selected mechanical variables as independent factors relevant to the development of the structural efficiency of human long bones, over the known influence of other, anthropometric and age-related confounders.

Such investigation should show some functional influences of muscle strength (mass) and bone levers[3] on one or both of the two natural components of bone structural stiffness and strength, namely, the mechanical quality and the spatial distribution of the mineralized tissue[4]. These relationships are usually blunted by *anthropometric* associations between bone, muscle and fat *masses*[5,6], as well as affected by genetic and endocrine-metabolic factors[1,7,8] which can bias the "true" biomechanical interactions

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between muscle and bone *strength*, especially when bone "strength" is taken as a correlate of the DXA-assessed bone "mass"[6]. Nevertheless, increasing evidence supports a direct mechanical influence of regional muscles' strength on the structural determination of the affected bones, rather concerning bone geometry than bone mass[3,9-27], including studies in long-time bed resting and quadriplegic individuals[28,29].

pQCT is a suitable technology for evaluation of bone volumetric density, mass, design, and strength[30], as well as muscle mass. Several pQCT-assessed indicators of those properties are alometrically associated. However, the analysis of their relationships with other variables related to the mechanical environment of the skeleton such as the mass or strength of the regional muscles and the length of long-bone levers can reveal the independent influence of these factors on bone structure and strength over any genetic or allometric association[3,7-9,12]. Thus, some additional influences of age and anthropometric factors like body weight and height that would *modulate* the biomechanical determination of bone features[2,6] could be duly evaluated and eventually ruled out by multi-factorial analyses.

In this study, we performed multiple regression analyses[31] of the influences of some representative mechanical, age-related and anthropometric determinants (age or time since menopause (TMP), body mass, length of the studied bone, and size of the regional musculature) on suitable pQCT indicators of long-bone trabecular and cortical mass, cortical tissue "quality"(vBMD), diaphyseal design, and structural stiffness as dependent variables, in the radii and tibiae of healthy men and pre- and post-MP women. The study aimed to: **1.** Compare the relationships between the assessed bone properties and correlates of their mechanical determinant factors (regional muscles' "strength" -cross-sectional area-, bone levers' length) with those related to their obvious anthropometric relationships with the whole (portable) body mass. **2.** Assess the age-dependence of the relationships in men vs women and in pre- vs post-MP women. **3.** Test the differences between the relationships evaluated for allometrically-related bone variables (mass-, design- or strength-related indicators) and those found for the bone tissue "quality" indicator, cortical vBMD. **4.** Compare the studied relationships in the tibia and radius, as body-weight bearing and non-bearing bones. **5.** Evaluate the possible interference of gender and women's reproductive status as natural "non-mechanical" factors on the above relationships, regardless of the nature or of any further dependence of the involved variables.

Materials and methods

The sample

Forty-seven men aged 25-82 years, 70 pre-MP women of 25-50 years, and 122 post-MP women of 50-82 years were recruited for study as healthy volunteers. None of them had a history of drinking or smoking habits, fractures, bone diseases, or treatments with bone-seeking drugs, or was following any systematic plan of physical activity. A brief description of the characteristics of this sample is given in Table 1. Every participant gave his/her written informed consent before being included in the study. The study was approved by the *Bioethics Committee, Faculty of Medicine, National University of Rosario, Argentina*.

Tomographic determinations

Standard pQCT scans (*XCT-2000, Stratec, Germany*) were obtained from the dominant forearm (at 4% and 66% of the ulna length from its distal end; R-4 and R-66 sites) and legs (at 4%, 14%, 38%, and 66% of the tibia length from its distal end; T-4, T-14, T-38 and T-66 sites). The R-4 and T-4 sites allowed studying chiefly the trabecular tissue. The R-66, T-14 and T-38 sites were apt to analyze cortical bone. The T-14 site presents the minimal values of both cortical mass and cross-sectional moments of inertia (CSMI's) as a typical diaphyseal design to stand uniaxial compression stress[32]. The T-38 site has larger CSMI values than the former as an adaptation to stand both bending and torsion stresses[32]. In R-66 and T-66 sites the CSA of the regional musculature is maximal. Of these two sites, only R-66 was selected to study (radial) cortical bone.

The X-ray beam of the scanner has a thickness of 2.5 mm, and the pixel edge size was set to 0.5 mm for 4%, 14% and 38% sites, and at 0.8-mm for the 66% sites. All image analyses were performed with the integrated XCT software in its version 5.50. For all sectional images we applied the parameters *contmode* 2, *peelmode* 2, and *cortmode* 1. Threshold values for total and cortical bone were selected at 398.5 and 700.0 mg/cm^3, respectively. The following pQCT indicators were determined as allowed in each site studied[30]:

1. **Trabecular, cortical and total bone mass indicators** (assessed at R-4 and T-4 for total and trabecular bone, and at R-66, T-14 and T-38 for cortical bone):
   - *Total, trabecular or cortical BMC*, in mg/mm of slice thickness.
   - *Cross-sectional area of total, cortical, and trabecular bone* (total area, cortical area, trabecular area), in mm^2. The trabecular area determined by the standard procedure of concentrically peel off the image until only the central 45% of its area is left for analysis.
   - *Total vBMD*= total BMC/(total area * slice thickness), and trabecular vBMD= trabecular BMC/(trabecular area * slice thickness), in mg/cm^3.
2. **Indicator of the mechanical "quality" (intrinsic stiffness, elastic modulus) of the mineralized tissue** (assessed in the "cortical" sites, R-66, T-14 and T-38):
   - *Cortical vBMD*= cortical BMC / cortical area * slice thickness), expressed in mg/cm3.
3. **Bone perimeters and cortical thickness** (assessed in the "cortical" sites, R-66, T-14 and T-38):
   - *Endo-cortical perimeter*= internal perimeter of the cortical area, assessed automatically as the length of the regularized circumference corresponding to the internal side of the cortical bone section ("Endo-C", ring model; the ring model was used to avoid erratic results derived from small periosteal discontinuities, which are especially frequent in the post-MP women), in mm.

	Men (n =47)	Pre-MP women (n=70)	Post-MP women (n=122)
Age, yr (range, mean±SD)	15-77, 31.4±13.2	18-63, 33±11	39-87, 58.9±8.9
Time since MP, yr (range, mean±SD)	—	—	1-46, 12.3±9.0
Body weight (mean±SD)	76.4±11.3	59.0±8.6	69.9±11.5
Body height (mean±SD)	176±7	163±7	158±7
Radius length, mm (mean±SD)	288±18	261±18	255±26
Tibia length, mm (mean±SD)	400±20	366±22	362±26

Table 1a. Age and anthropometric characteristics of the studied sample.

		Men (n=47)		Pre-MP women (n=70)		Post-MP women (n=122)	
	Site %	Radius	Tibia	Radius	Tibia	Radius	Tibia
Cortical vBMD (g/cm^3)	14	—	1098±34	—	1116±34	—	1067±51
Cortical vBMD (g/cm^3)	38	—	1121±29	—	1152±30	—	1112±448
Total BMC (g/cm)	4	1.52±0.25	4.67±0.87	1.06±0.29	3.06±0.51	0.98±0.26	2.81±0.51
Total vBMD (g/cm^3)	4	410±64	278±33	359±62	255±43	324±72	344±72
Trabecular vBMD (g/cm^3)	4	226±45	264±36	190±49	222±31	155±42	202±39
Peri-C (mm)	14	—	87±6.5	—	77±6.5	—	82±18
Peri-C (mm)	38	—	86±5.3	—	74±1.3	—	73±4.7
Peri-C (mm)	66	68±4.7	—	60±11.2	—	61±7.6	—
Endo-C (mm)	14	—	72±7.9	—	65±7.4	—	68±7.1
Endo-C (mm)	38	—	45±5.7	—	39±5.0	—	43±6.7
Endo-C (mm)	66	63±5.9	—	56±11.2	—	58±8.3	—
Cortical thickness (mm)	14	—	4.82±0.95	—	3.79±0.66	—	3.39±0.79
Cortical thickness (mm)	38	—	13.10±1.88	—	10.47±1.38	—	9.80±1.58
Cortical thickness (mm)	66	1.86±0.68	—	1.31±0.69	—	1.10±0.69	—
Cortical BMC (g/cm)	14	—	2.31±0.35	—	1.68±0.23	—	1.49±0.27
Cortical BMC (g/cm)	38	—	3.95±0.56	—	2.87±0.37	—	2.69±0.35
Cortical BMC (g/cm)	66	0.56±0.22	—	0.34±0.19	—	0.29±0.19	—
Cortical area (mm^2)	14	—	210±30	—	148±21	—	139±20
Cortical area (mm^2)	38	—	353±51	—	246±38	—	241±29
Cortical area (mm^2)	66	59.9±20.4	—	38.0±19.2	—	32.1±19.0	—
xMI (mm^4)	14	—	13967±3670	—	7924±2162	—	7797±1678
xMI (mm^4)	38	—	21414±5233	—	11290±3455	—	11278±2878
xMI (mm^4)	66	2059±728	—	1072±561	—	890±565	—
pMI (mm^4)	14	—	30023±7366	—	17094±4510	—	16730±3510
pMI (mm^4)	38	—	37686±8848	—	19434±5149	—	19905±4377
pMI (mm^4)	66	5498±2279	—	2692±1611	—	2182±1466	—
Stress-Strength Index (mm^3)	14	—	1524±289	—	992±196	—	910±171
Stress-Strength Index (mm^3)	38	—	2071±396	—	1292±254	—	1249±206
Stress-Strength Index (mm^3)	66	537±118	—	350±173	—	322±86	—
Cross-sectional muscle area (mm^2)	66	6002±2129	7382±1037	4174±1881	5640±815	4103±2071	5721±930

Table 1b. Tomographic indicators relevant to the study (means±SD).

- *Periosteal perimeter*= external perimeter of the cortical area, assessed automatically as "Peri-C" following a similar procedure to that applied to calculate "Endo-C" (ring model), in mm.
- *Averaged cortical thickness*, calculated as [Peri-C - Endo-C] / 2π. It expresses the mean absolute cortical thickness, independently of the architectural design of the diaphyseal section.

4. Indicators of the mechanical efficiency of diaphyseal design to resist failure in bending and torsion:

- *Second cross-sectional moments of inertia of cortical area (MI's*, integral sums of the products of the area of every "cortical" pixel and its squared distance to the reference, bending or torsion axis). All the reference axes (*x* for anterior-posterior bending, *y* for lateral bending, *z* for torsion) were determined automatically by the software as

passing through the center of mass of the image, calculated in two different ways, namely, concerning resistance to deformation in anterior-posterior bending (*x-axial MI, xMI*), and concerning resistance to deformation in torsion (*polar MI, pMI*).

5. Indicator of bone torsional structural stiffness/strength:
- "*Stress-strain index*"= (cortical vBMD / cortical vBM_{Max}) * (pMI / R_{Max}), being cortical $vBMD_{Max}$ (maximum physiological value for vCtD) equal to a proposed, 1.2 mg/cm^3 value, and R_{Max} the maximal radius of the image.

6. Indicator of muscle strength (mass):
- *Cross-sectional muscle area* = area of the region resulting as the difference between the total and the [fat + bone] area of the image obtained using a 0.8-mm pixel size without image filtering.

Statistical methods

Stepwise-type, multiple correlation/regression analyses were applied[31] to evaluate the independent influence of some selected determinants on the above pQCT indicators. To optimize the results according to study's aims and minimizing errors by omission, excess or unsuitability of the independent variables, the number of these confounders was reduced to only the following, age-related, anthropometric or mechanical factors:

1. Age (for men and pre-MP women) or time since menopause (TMP).
2. Body mass.
3. Length of the studied radius or tibia, determined by standard anthropometric measurements.
4. Maximal muscle mass of the studied forearm or leg (muscle cross-sectional area at R-66 and T-66).

Inclusion of TMP instead of age in post-MP women, as well as definition of post-MP stage in women by age >50, were decided to avoid both the inhomogeneity of age at menopause and the well-known, larger influence of TMP than age *per se* on bone features in aged women. We excluded other potential determinants that either do not show a priori any significant interrelationship; or, on the contrary, are so closely inter-related (high *co-linearity*) that the risk that the algorithm exclude one of them because of the mere presence of the other grows too high. These comprised body height (highly co-linear with bone length), body fat mass (highly co-linear with body weight), and the *body-mass index* (mechanically irrelevant, or a possible agonist)[33,34], as well as other classic confounders (smoking/drinking habits, bone-affecting treatments, genetic factors, fracture history, etc.) as detected by a careful anamnesis.

The applied tests[31] provided the values and statistical significances of: **1.** *the partial regression coefficients (β) for each indicator* with respect to each significant determinant in every instance of analysis, and **2.** *the squared global correlation coefficients (R^2)* of every analysis performed for the whole group of significant determinants selected in every instance. The β values indicated the magnitude of the variation of the analyzed indicator, expressed in SD units (i.e. as Z-scores), per each SD unit of variation of the selected determinant (standardized effect size), keeping all other included determinants constant. The statistical significance of β coefficients expressed the particular suitability of the analyzed variable as an independent determinant factor (independent "determinant power"). R^2 values and significances indicated the fitness of the selected analytical model in every instance tested. Independent variables found non-significant were disregarded in each analysis. The significance level was established at $p<0.05$.

Results

Table 1-b describes the means and SD's of the most relevant tomographic indicators determined as allowed in all bone sites scanned in the three groups studied.

Table 2 shows the β and R^2 coefficients calculated from the multiple regression analyses performed in the forearms (Table 2-a) and legs (Table 2-b) of the three groups, comprising the pQCT indicators shown in Table 1. The indicators are classified into:

1. *Bone tissue "quality" estimator* (cortical vBMD, directly associated with the intrinsic stiffness or elastic modulus[35], assessed at R-66, T-14 and T-38;
2. *Total and trabecular mass indicators* assessed at R-4 and T-4 (total BMC, total vBMD, trabecular vBMD) and cortical mass indicators (cortical BMC, cortical area) assessed at R-66, T-14 and T-38;
3. *Bone perimeter-related indicators* (not biologically regulated), diaphyseal perimeters ("PeriC", "EndoC"), and cortical thickness, assessed at R-66, T-14 and T-38;
4. *Indicators of the cross-sectional design of the diaphyses* concerning resistance to bending and torsion (biologically regulated) (xMI, pMI), assessed at R-66, T-14 and T-38; and
5. *Diaphyseal structural stiffness indicator*, stress-strength index, assessed at R-66, T-14 and T-38.

In Table 2, the data obtained in the radii (**a**) and tibiae (**b**) were arranged as they were employed for analysis according to the aims of the study and the current understanding of mechanical and systemic influences on bone structure[2,4,6,7,9,30,33,36-42], from the following four different points of view: ***1.*** *Concerning the dependent (determinant) variables ($X_{1,2,3,4}$, upper rows).* ***2.*** *Concerning the dependent (determined) variables (Yi, left columns).* ***3.*** *Concerning the different groups studied (men, pre- and post-MP women; left, central and right groups of columns).* ***4.*** *Concerning the mass-bearing nature of the studied bones (tibia, radius).* A detailed description of the behavior of the independent and dependent variables studied (points 1 & 2, above) follows.

Partial influence of the independent (determinant) variables

Concerning the age-related factors, in the men and pre-MP women, age exerted a significant, negative partial influence on some trabecular and cortical mass indicators (trabecular and total vBMD, cortical area, $p<0.05$ to $p<0.01$) only in the tibiae, with no effect on the other indicators in both bones. However,

TABLE 2-a. (FOREARM).

	MEN					PRE-MP WOMEN					POST-MP WOMEN				
	AGE (yr)	BODY MASS (kg)	RADIUS LENGTH (cm)	MUSCLE AREA (mm^2)	R^2	AGE (yr)	BODY MASS (kg)	RADIUS LENGTH (cm)	MUSCLE AREA (mm^2)	R^2	TMP (yr)	BODY MASS (kg)	RADIUS LENGTH (cm)	MUSCLE AREA (mm^2)	R^2
Tissue stiffness estimator (R-66)															
Cortical vBMD, g/cm^3											-0.35***				*0.12****
Trabecular mass indicators (R-4)															
Trabecular vBMD, g/cm^3				0.36**	*0.24***						-0.28***				*0.08***
Total BMC, g/cm				0.58***	*0.33****				0.52***	*0.27****	-0.23**			0.19*	*0.11****
Total vBMD, g/cm^3			0.32*		*0.10**						-0.36***				*0.13****
Bone perimeters & thickness (R-66)															
Periosteal perimeter, mm				0.63***	*0.39****				0.58***	*0.34****	0.27***			0.37***	*0.12****
Endocortical perimeter, mm				0.36**	*0.13***				0.33**	*0.11***				0.34**	*0.11****
Averaged cortical thickness, mm				0.33*	*0.11**				0.22*	*0.05**					
Cortical mass indicators (R-66)															
Cortical BMC, g/cm				0.61***	*0.37****				0.52***	*0.27****	-0.37***				*0.13****
Cortical area, mm^2		0.23*		0.55***	*0.46****				0.54***	*0.29****	-0.30***			0.20*	*0.15****
Design & strength indicators (R-66)															
Moment of inertia for bending, mm^4				0.547***	*0.29****				0.62***	*0.38****				0.43***	*0.19****
Moment of inertia for torsion, mm^4				0.64***	*0.41****				0.69***	*0.47****				0.53***	*0.28****
Stress-strain index, mm^3				0.58***	*0.44****				0.31***	*0.18****				0.44***	*0.28****

TABLE 2-b. (LEG).

	MEN					PRE-MP WOMEN					POST-MP WOMEN				
	AGE (yr)	BODY MASS (kg)	TIBIA LENGTH (cm)	MUSCLE AREA (mm^2)	R^2	AGE (yr)	BODY MASS (kg)	TIBIA LENGTH (cm)	MUSCLE AREA (mm^2)	R^2	TMP (yr)	BODY MASS (kg)	TIBIA LENGTH (cm)	MUSCLE AREA (mm^2)	R^2
Tissue stiffness estimator (T-14, T-38)															
Cortical vBMD, g/cm^3 (T-14)											-0.49***				*0.24****
Cortical vBMD, g/cm^3 (T-38)											-0.23*				*0.05**
Trabecular mass indicators (T-4)															
Trabecular vBMD, g/cm^3	-0.34*				*0.11**	-0.35**			0.26**	*0.15****	-0.29***	0.36***		0.23**	*0.30****
Total BMC, g/cm				0.34*	*0.11***		0.36**		0.30*	*0.32****					
Total vBMD, g/cm^3				0.36**	*0.13***		-0.32**		0.26*	*0.14****	-0.27**	0.18**			*0.10***
Bone perimeters & thickness (T-14, T-38)															
Periosteal perimeter, mm (T-14)			0.46***	0.28*	*0.36****		0.49***		0.24*	*0.41****	0.23**		0.48***	0.17*	*0.06**
Periosteal perimeter, mm (T-38)			0.62***	0.29**	*0.57****							0.23**			*0.38****
Endocortical perimeter, mm (T-14)			0.39**		*0.15***		0.52***			*0.26****		0.31***	0.32***		*0.10****
Endocortical perimeter, mm (T-38)			0.33*		*0.11***			0.43***		*0.19****	0.25**		0.17*		*0.17****
Averaged cortical thickness, mm (T-14)									0.37**		-0.31***				*0.12****
Averaged cortical thickness, mm (T-38)				0.41**	*0.17***					*0.14****	-0.32***				*0.10****
Cortical mass indicators (T-14, T-38)															
Cortical BMC, g/cm (T-14)			0.40**	0.40**	*0.41***		0.20*	0.35**	0.35**	*0.43****	-0.24**	0.24**	0.18*	0.23**	*0.30****
Cortical BMC, g/cm (T-38)			0.42**	0.40**	*0.17***		0.34**	0.30**	0.27*	*0.45****	-0.34***		0.37***		*0.24****
Cortical area, mm^2 (T-14)	-0.30**	-0.44*			*0.27****		0.38**	0.31**		*0.33****	-0.39***		0.31***	0.20*	*0.31****
Cortical area, mm^2 (T-38)			0.46***	0.39**	*0.46****		0.47***	0.31***		*0.43****	-0.26**		0.41***	0.21*	*0.29****
Design & strength indicators (T-14, T-38)															
Mom. of inertia, bending, mm^4 (T-14)			0.50***	0.33*	*0.47****		0.19***	0.33***	0.27**	*0.59***	-0.22**	0.27**	0.38***	0.17*	*0.37****
Mom. of inertia, bending, mm^4 (T-38)			0.48***	0.37**	*0.42****		0.42***	0.41***		*0.48****			0.51***	0.27***	*0.33****
Mom. of inertia, torsion, mm^4 (T-14)			0.45***	0.36**	*0.56****		0.41***	0.29***	0.29**	*0.56****	-0.19*	0.32***	0.28**	0.19*	*0.32****
Mom. of inertia, torsion, mm^4 (T-38)			0.57***	0.35**	*0.32***		0.47***	0.38***		*0.51****		0.18*	0.49***	0.23**	*0.39****
Stress-strain index, mm^3 (T-14)			0.36**	0.39**	*0.44****		0.36***	0.35***	0.31**	*0.58****	-0.39***	0.25**	0.34***	0.28***	*0.35****
Stress-strain index, mm^3 (T-38)			0.43***	0.43***			0.33***	0.44***	0.25*	*0.59****	-0.19*	0.17*	0.47***	0.26***	*0.37****

Table 2. Partial regression (β) and squared global correlation (R^2) coefficients of the stepwise-type multiple regressions calculated between the studied bone indicators of the stiffness of the mineralized tissue (cortical vBMD); the trabecular, total or cortical mineralized mass (trabecular vBMD, total BMC, total vBMD, cortical area, cortical BMC), and the diaphyseal cross-sectional geometry (non-regulated variables: Endo-C, Peri-C, cortical thickness; regulated variables, xMI, pMI), and structural stiffness/strength (stress-strength index) of the radii (**Table 2-a**) and tibiae (**Table 2-b**) of the studied men and pre- and post-MP women, as dependent variables (Y_i, **left columns**), and their selected, possible biological determinant variables, age or TMP, body weight, bone length and maximal cross-sectional area of the regional muscles (muscle area), as independent variables (X_i, **upper rows**). Only the significant β coefficients are shown. Asterisks (*,**,***) indicate their $p<0.05$, $p<0.01$, and $p<0.001$ levels of statistical significance, respectively. All the R^2 coefficients are indicated in the corresponding column when the analytical power of the assayed model was significant.

in the post-MP women, TMP exerted the largest impact on the variation of almost all indicators in the tibiae (mostly $p<0.01$ to $p<0.001$) and on all trabecular and cortical mass indicators in the radii (mostly $p<0.001$). Importantly, TMP was the only significant independent factor concerning the variation of the mineralized tissue "quality" (cortical vBMD), both in radius and tibia ($p<0.05$ to $p<0.001$ for both β and R^2 coefficients).

Concerning body mass, as expected, it affected radius and tibia differently.

In the radii, body mass was unrelated to any indicator in the women. In the men, its influence was relevant only to the radial cortical mass indicator, cortical area, with a low statistical significance ($p<0.05$).

In the tibiae, however, body mass was a significant factor of the variation of many bone features, albeit with important inter-group differences. *In the men*, body mass contributed only to cortical area in T-14 ($p<0.05$). *In the women*, body mass was significant for trabecular mass (total BMC in pre- and post-MP women; total vBMD only in post-MP women; $p<0.01$ to $p<0.001$); cortical mass (BMC, area; $p<0.05$ to $p<0.001$); bone perimeters (in pre-MP women, Endo-C and Peri-C in T-14, $p<0.001$; in post-MP women, Endo-C in T-14 and Peri-C in T-38, $p<0.001$ and $p<0.01$) and, particularly, the diaphyseal design (MI's) and structural stiffness/strength (stress-strength index) indicators (pre->MP women, always $p<0.001$; post-MP women, p n.s. to $p<0.001$). These features were more evident in pre-MP than in post-MP women, especially concerning cortical bone.

No influence of body mass was detected on bone tissue "quality" (cortical vBMD).

Concerning bone length, its influence differed in weight-bearing and non-bearing bones.

Radial length had virtually no influence on any indicator, except for total vBMD in R-4 in the men ($p<0.05$).

Tibia length was unrelated to trabecular mass (trabecular vBMD, total BMC and vBMD) and cortical tissue "quality" (cortical vBMD). However, it was relevant to cortical mass (BMC, area; $p<0.05$ to $p<0.001$) and, particularly, to diaphyseal design (MI's) and stiffness/strength (stress-strength index) (mostly $p<0.001$), generally more evidently in T-38 than in T-14 in all groups. In general terms, tibia length was a relevant factor to the variation of most tibia indicators of cortical bone features in all groups. In the post-MP women, the influence of TMP was comparable to that of the tibia length on cortical bone mass and was the only relevant factor to cortical bone "quality" (cortical vBMD) and cortical thickness. However, the influence of tibia length on bone design and strength (MI's, stress-strength index) superseded that of TMP (virtually always $p<0.001$ vs erratic p values from n.s. to $p<0.001$).

No influences of bone length on cortical vBMD were detected in any bone or group.

Concerning the regional musculature, muscle mass (area) was always a relevant factor to bone traits (excepting only the cortical vBMD) for both radii and tibiae, in all groups, with highly significant R^2 values (mostly $p<0.01$ to $p<0.001$). Nevertheless, results showed both inter-limb and inter-group differences.

In the forearms of the men and pre-MP women, muscle mass was virtually the only significant factor that affected trabecular BMC ($p<0.001$) and all cortical bone features ($p<0.05$ to $p<0.001$), except only for the radial cortical vBMD (p n.s.). Forearm muscles contributed also significantly to radial trabecular vBMD variation in males ($p<0.01$). In the post-MP women, the impact of the forearm muscle area on trabecular and cortical mass and diameters was generally less evident (p n.s. to $p<0.001$), although it was still highly significant for the diaphyseal design and strength (always $p<0.001$ for both β and R^2 values).

In the legs, the influence of muscles on tibia traits was also evident in all groups, and generally stronger and more conspicuous in men than in women. However, it was generally less significant here than in forearms. Contrary to forearms, muscle influences on the tibiae were compounded with those exerted by the other three studied determinants: bone length (in all groups), body mass (in pre- and post-MP women), and TMP, with generally high R^2 values ($p<0.01$ to $p<0.001$). The impact of regional muscles on MIs and stress-strength index was generally larger than that exerted on cortical perimeters and thickness in men (mostly $p<0.001$ vs p n.s. to $p<0.01$) and post-MP women ($p<0.05$ to $p<0.001$ vs p n.s. to $p<0.05$). In the pre-MP women that difference was less evident. In general terms, the influence of calf muscles on bone indicators tended to be less significant than that of tibia length in all groups.

No influence of regional muscles on bone tissue "quality" (cortical vBMD) was observed.

Influences exerted on the dependent (determined) variables

Age affected negatively trabecular mass (trabecular and/or total vBMD) in men and pre-MP women slightly and only in the tibiae (p n.s. to $p<0.01$), but TMP exerted a significant, negative influence on trabecular bone in both radii and tibiae ($p<0.01$ to $p<0.001$). Body mass was a significant determinant of trabecular mass indicators only in the tibiae of pre- and post-MP women (p n.s. to $p<0.001$). Bone length was a significant independent factor of only the total vBMD variation, and exclusively in the males' radius ($p<0.05$). *In both radii and tibiae*, at least one of the trabecular mass indicators (trabecular and total vBMD, total BMC) depended on muscle area in all groups and bones, with variable significance ($p<0.05$ to $p<0.001$). Both indicators comprising combined amounts of trabecular and cortical tissues (total BMC and vBMD) reflected some influence of the musculature in the legs (only) of all groups ($p<0.05$ to $p<0.01$).

The influences of the four independent factors studied on cortical bone indicators were quite different in radii and tibiae.

In the radii, all diaphyseal perimeters/thickness ($p<0.05$ to $p<0.001$) and especially mass, design and stiffness indicators (always $p<0.001$) depended critically on muscle mass in all instances of comparison in men and pre-MP women (with R^2 values reaching $p<0.01$ to $p<0.001$ levels of significance), and generally more significantly and conspicuously than the trabecular indicators did. In the pre-MP women, muscle mass was the only relevant factor to the variation of these indicators,

with high R^2 coefficients (mostly $p<001$). Men's bones showed similar influences, but cortex size (cortical area, not cortical BMC) was also significantly dependent on body mass ($p<0.05$), this analysis providing the largest R^2 coefficient for the group (0.46, $p<0.001$). In the post-MP women, muscles were the most relevant factors to the variation of cortical diameters (Endo-C, Peri-C) and design and strength indicators (MI's, stress-strength index) (β coefficients almost always $p<0.001$, R^2 coefficients always $p<0.001$). However, the influence of muscles on cortical mass (BMC, area) was little or no evident (only for area, $p<0.05$), and was compounded with a significant (negative) influence of TMP (β and R^2 values always $p<0.001$). Nevertheless, TMP did not affect diaphyseal design or stiffness (MI's, stress-strength index), and its influence of the diaphyseal Peri-C was significantly positive (β and R^2 values, $p<0.001$). In addition, TMP was the only significant (negative) factor of cortical vBMD variation (β and R^2 values, $p<0.001$).

In the tibiae, in general terms, the independent influence of the musculature on cortical bone was somewhat stronger and more conspicuous in men than in women, and less significant than it was in the radii, in all groups, where the $p<0.001$ level of significance was reached only in a few instances, and almost only in the post-MP women. Contrasting with the radii, within groups, the tibia length was generally more strongly associated with diaphyseal mass, design and stiffness indicators (mostly $p<0.001$) than the other studied factors, with generally highly significant R^2 values in every analytical instance (mostly $p<0.001$). The additional influence of body mass and age-related factors on cortical bone indicators varied widely between groups. In the men, virtually no influence of body mass on cortical mass was observed, and that of age (negative) was very humble (only on cortical area, $p<0.01$). In the pre-MP women, body mass was relevant to the variation of diaphyseal mass, design and stiffness, even more than bone length or muscle mass, in almost every instance, while age showed only a weak influence the R^2 values were highly significant ($p<0.001$) in all instances. In T-14, body mass' influence was even evident on diameters ($p<0.001$), regardless of bone length ($p<0.001$ for both R^2 values). In the post-MP women, the independent influence of body mass on cortical features (mostly p n.s. to $p<0.01$) was generally weaker than it was in the pre-MP ones (mostly $p<0.001$). Coincidently, the TMP was the most relevant factor to the variation of all cortical indicators (with positive influences on Peri-C), excepting only the design and stiffness indicators in T-38. The R^2 values for all these analytical instances in post-MP women reached a $p<0.001$ level of statistical significance, with the only exception of that for Peri-C at T-14 ($p<0.05$).

In summary, bone length exerted the strongest influence on tibia cortical features in males, and the second one in females. The mass and design indicators were all more dependent on the mechanical determinants than Peri-C and cortical thickness were. In the males, both Peri-C and cortical thickness were dependent on bone length and musculature, in this order, regardless of age and body mass. In the post-MP women, they depended on the TMP, bone length and body mass, in this order, with little or no influence of the musculature. Importantly, as observed in the radii, the only significant factor of cortical vBMD variation was the TMP.

The generally high significances of the R^2 coefficients of all the above relationships (mostly $p<0.001$), with the only exception of cortical vBMD in men and pre-MP women, would reflect the correspondingly high statistical power of the selected models for every instance of analysis. Of note, almost all the R^2 coefficients were significant (mostly $p<0.001$) when only post-MP women were studied, and when the studied relationships involved pQCT indicators of bone design or strength of all groups, in both limbs (always $p<0.001$).

Discussion

In general terms, results show that:

1. in men and pre-MP women, the most relevant factors to the development of trabecular or cortical bone features were only the muscle area for the radius and both muscle area and bone length (this latter only for cortical bone) for the tibia;
2. only for women, was body mass a significant factor for the variation of tibia (not radius) traits;
3. in men and pre-MP women, the relationships between cortical or trabecular bone mass or, particularly, the cortical cross-sectional design indicators and their selected determinant factors, were the most significant and conspicuous in the study, and were also much closer than those found for the bone tissue "quality" (intrinsic stiffness) indicator, cortical vBMD;
4. the regional specificity of some of the above relationships along the tibia could be related to differences in the type of predominant stress in the studied bone sites;
5. all the above relationships were independent of age, but, in post-MP women, TMP was an additional contributor to the variation of both radius and tibia features.

Within the model-related limitations, those findings are discussed below according to the proposed influence of mechanical and non-mechanical factors in the development of bone structure and strength.

Concerning the mechanical environment

In agreement with earlier observations[42], results show that, in men and pre-MP women, the independent variables which have some dynamic correlate (bone length, musculature) were more relevant to the development of bone mass, structure and strength than the age-related and anthropometric factors (age, TMP, body mass) which would have no mechanical correlate, or just exert a static (non-dynamic) influence on the skeleton. In addition, many studies have afforded evidence that physical activity induces changes in bone size/geometry rather than on other bone features, and that the directionality of the induced stresses could orient the induced responses of the corresponding bone modeling drifts[3,12,16-20,22-24,26,27,32,33,38-40,43-46], as observed here.

The passive influence of body-mass bearing on the mechanical adaptation of bones is not clearly established. Obese children have lower muscle-to-fat and bone-to-muscle mass ratios in the forearms than in the legs[47]. Women have substantially larger fat mass than men have. This fat mass addition does not generally scale with muscle bulk. Unfortunately, fat mass could not be determined in this study, but it could have expressed itself via the observed, independent influence of body mass upon tibia traits. Combining the observed effects of body mass in men (absence) and in women (presence), the present study provides further evidence against the assumption of weight-bearing as being important to bones' mechano-adaption. In this connection, the correlation shown between the whole-body fat mass and pQCT indicators of cortical tibial-femoral bone mass, geometry and strength in healthy girls was significantly attenuated after adjustment for muscle area[48]. Interestingly, some pQCT studies in trained people suggest that muscles usage, in addition to muscle mass, is a relevant factor to bone structure development[45,49].

The indicators of bones' cross-sectional design and structural stiffness (thought to be feedback-controlled as a function of the mechanical usage of the skeleton)[2] were generally more significantly correlated with the mechanical factors studied (bone length, muscle mass) than the indicators of bone mass or diameter-related features or the "quality" indicator, cortical vBMD (which would not be feedback-controlled) in all groups studied. Accordingly, the R^2 coefficients of the corresponding analytical instances were quite higher and more significant for the former than for the latter. These findings are in consonance with the current concepts concerning the biomechanical regulation of bone mass distribution as a function of directional mechanical influences as predicted by the *mechanostat* theory[2,4,6,7,9,33,34,36-43,50].

The particular influences of torsion stress on tibia shaft geometry deserve a separate comment. Some observations made in tennis players and throwers, in whom torsion has been suggested to be the likely side-different loading pattern in the humerus[43,44], can be interpreted in the light of these findings. In fact, pronation and supination are strong torque generators in the forearm, and even other forearm muscles could generate torsion, given their eccentricity of origin and insertion in relation to the neutral axis of either of the bones. Of note, the torsional moment will depend on the muscular force and the distance from the center of rotation (i.e. the neutral axis), but not on the radius length. Thus, if torsion were also the prevailing driver in mechano-adaption of the radius in that cohort, then this would elegantly explain why radius length was unrelated to radius traits. In any case, the strong association of muscle area to radius traits underlines the importance of the local musculature. The generally more significant dependence of bone design indicators on bone length as assessed at T-38 than at T-14 sites in this study would support this view. Results from the MUST study, in which *in vivo* bone deformation was assessed in humans with a novel optical tracking approach[51-53], demonstrate that torsion is a prevailing deformation mode in the human tibia during locomotion[11,51]. The presence of torsional deformation had not been considered in most, if not all past studies[54], which is probably due to the technical difficulties arising from strain gauge measurements[55]. The most recent results from the MUST study now demonstrate[56] that regional calf muscle contractions specifically induce torsional deformation of the tibia, whilst anterior-posterior bending is the prevailing deformation mode from heel touch down to mid-distance in walking, running and stair climbing. Importantly, the line-of-action of the body's center of mass is passing behind the tibia during heel touch down, and the center of mass is lifted from heel touch down to mid-distance. It seems logical, therefore, that both muscle area (likely through torsional deformation), as well as tibia length (likely through anterior-posterior bending) have been revealed as important determinants of tibia traits in this study. Anatomically speaking, the torsion is most likely to arise from the soleus muscle's origin of both the tibia and the fibula, although part of it could also be caused by rotatory acceleration of the body's center of mass around the stance leg[56]. However, our model calculation suggests that the latter contribution is probably small. In addition, the MUST study also suggests that anterior-posterior bending results from momentum (= mass * velocity) gained from non-regional muscle contractions.

Concerning the non-mechanical (systemic, endocrine-metabolic) environment

The gender-related differences between the above relationships have reflected the known interference of *"non-mechanical" factors*, regardless of the qualitative or quantitative nature and the age-related, anthropometric or mechanical dependence of the involved variables, as could be shown for sex hormones in earlier human studies by DXA[5,57] and in OX rats[58]. Nevertheless, the "areal" character of the DXA determinations limited their interpretation to the anthropometric field, with no clear biomechanical correlate.

Strikingly, this study, in which the cortical BMD was measured volumetrically by pQCT, shows that the cortical vBMD was fully independent of any influence from the anthropometric (body mass) or "mechanical" (regional muscle mass, bone lengths) factors selected. This contrasts with the significant dependence of the other bone indicators studied upon the selected determinant variables in many instances. The relative independence of cortical vBMD from the mechanical environment, which has been evident also by testing the bone-muscle relationships in rapidly-growing, pre-pubertal children[48,59] and in young and older trained and untrained individuals[19,60-63], could be hypothetically attributed to the relatively low natural variability of that property[38,45,64]. Some strong biological reasons seem to explain that relative invariance. In bones with similar functions, there is a fairly stable relationship between the mineral content of bone matrix or "solid" tissue and its intrinsic stiffness (elastic modulus)[65], which is always evident if the latter is measured regarding the optimal orientation of the matrix collagen fibers[66]. Furthermore, this relatively little variability of the mineral concentration of bone matrix seems to have resulted from a "trade-off" between bone tissue stiffness and toughness through Evolution[67]. These relationships can only

be altered naturally by enhancing the micro-porosity of the "solid" bone[35,68,69], a phenomenon that happens naturally in post-MP women and in elderly persons because of well-established causes[70], but always as a direct effect, i.e. by no means suggesting the participation of any feedback regulation mechanism. This poses the question, whether the cortical vBMD could or not act as a *determinant*, rather than a *determined* variable, within the scope of the mechanostat theory[2]. Currey had already risen this interesting question[71]. Reasonably, the stiffness of the mineralized tissue (a mechanical correlate of its volumetric mineral density, disregarding porosity and fiber directionality) could be an independent determinant factor of the tissue ability to transduce the strains derived from mechanical usage into detectable signals to osteocytes. Thus, bone tissue "distribution" (concerning the efficiency to support the usual types of stress) might adapt to bone tissue "stiffness", perhaps at every point of the moving skeleton. We have described what we coined "distribution/quality" (d/q) relationships in long bones of rodents of different strains or species[72-75], rats treated with bisphosphonates, glucocorticoids, PTH or rhGH[76-79], and healhty, sedentary or trained men and women[32]. Those d/q curves (easily determinable by pQCT) showed always negative, hyperbole-shaped relationships between the cross-sectional MIs ("distribution" indicators, y) and the cortical vBMD (tissue "stiffness" indicator, x) of the same bone sections throughout the human tibia. There is some evidence that departing from this natural relationship could lead to bone fragility[77], even in normal persons[80].

At any rate, the relative insensitivity of the cortical vBMD (a "qualitative" indicator) to anthropometric and mechanical factors (all "quantitative" variables) observed in this study suggests that these factors should not influence independently the intracortical remodeling during the habitual mechanical usage of the skeleton in the assayed conditions. However, the cortical vBMD did show independent, significant relationships with TMP, in agreement with the above comments. This points out the relevance of the endocrine environment to the development of some bone's traits in some instances. Nevertheless, in healthy individuals, this matter would tend to assume some clinical relevance only in post-MP women. To note, in others' studies in aged men[81] and women[42], the age- or body-mass-adjusted, pQCT-assessed values of both radial and tibia cortical vBMD (not trabecular vBMD) were found significantly correlated with muscle strength/power indicators. It was also shown that post-MP women express different, sex-hormone-dependent cortical vBMD responses than pre-MP women to the same muscle strength and to the same level of high-impact exercise[46,82].

Limitations of the study

In addition to all traditional limitations imposed by sample size and technical matters on any biological investigation, the interpretation of this study is obviously restricted to the scope of the analytical method employed. In this concern, the selection of just muscle mass and bone levers as "mechanical factors" could be regarded either as its strongest feature or as a severe limiting condition, depending on the spirit of the observer. We think that, from the positive side, this selection restricts the ambit of mechanical factors to just the two main "strain-inducers" to the bones, namely, the source of the strength of regional muscles' contractions, and the multiplication of that strength by the length of bone levers. The mechanical influence of body weight should have been neutralized by studying simultaneously weight-bearing and -nonbearing bones. An analogous observation could be made about the selection of just age (or TMP) and body weight as "confounders" within the set of independent (determinant) factors, yet in this regard there exist a number of criteria to take into account for selection, which have been duly discussed.

The determination of muscle area disregarded the muscle fat content. In normal adults, this method should not affect the accurateness of the measurement; however, in the post-MP women studied this could have over-estimated the real values. This fact could have affected the comparison between groups as a descriptive fashion, i.e. as shown in Table 1. However, it could be reasonably assumed that this source of error might not have distorted significantly the relationships of the other variables with muscle area as assessed by the β-coefficients in any of the groups studied. Nevertheless, muscle area could be regarded as a good correlate of muscle mass, rather than strength. Therefore, all regression analyses performed between any other indicator and muscle area should be taken as a (reasonable) approximation. This inconvenience may affect somehow the inter-group comparisons with the post-MP women, though not those calculated within groups.

Conclusions

1. Concerning the *mechanical* influences on the skeleton, the selected mechanical factors (maximal cross-sectional area of the regional muscles, bone lever lengths) were more relevant than the selected age-related or anthropometric determinants or confounders to the development of a number of allometrically-associated bone properties (mass, design, strength). The influence of musculature on bone traits seems to be independent from the weight-bearing or -nonbearing nature of the bones; however, both muscles and bone-lever influences could somewhat depend on the predominant stress pattern induced by customary usage at each bone site, especially concerning bending and torsion. Nevertheless, the mechanical environment of the skeleton would not be that critical to the biological determination of bone tissue "quality", at least concerning the mineralization-related mechanical properties of the hard tissue.
2. Concerning the *endocrine-metabolic* influences (restricted to only those of sex hormones in this study), the mechanical impacts of muscles and bone levers on bone structure seem to be comparable in men and pre-MP women in qualitative terms. However, in the post-MP women, the TMP could exert a stronger (negative) impact than other, allometric or mechanical factors did on any kind of bone property, including the "tissue quality" (cortical vBMD), with the probable exception of the diaphyseal design.

3. Results suggest that the cortical vBMD might be a determinant, rather than a determined variable within the analyzed model (geometric properties changing as adaptive manifestations to changes in the mechanical environment and in bone tissue stiffness), perhaps with a most relevant role in the feedback mechanism configuring the bone *mechanostat*.

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