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Original Article

Differences of bone mineral mass, volumetric bone mineral density, geometrical and structural parameters and derived strength of the tibia between premenopausal and postmenopausal women of different age groups: a peripheral Quantitative Computed Tomography (pQCT) study

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Abstract

Menopause constitutes a significant cause of bone loss, and it is currently debated whether bone mass is preserved or begins to decline substantially before that time in women. We used pQCT of the tibia to estimate differences of bone mineral mass, bone geometry and derived strength between premenopausal and postmenopausal Caucasian women of different age-groups per decade of age (20-79y). For each individual, we assessed total, trabecular and cortical bone mineral content (BMC, mg) and volumetric bone mineral density (BMD, mg/cm^3); total and cortical cross-sectional areas (CSA, mm^2); periosteal circumference (PERI_C, mm); endosteal circumference (ENDO_C, mm); mean cortical thickness (CRT_THK, mm); and Stress-Strain Index (SSI) . Comparisons were made both between premenopausal (N=84) and postmenopausal (N=231) women as distinct groups, and among women of the different age-groups. Our results indicated that premenopausal women had significantly higher trabecular and cortical BMC and vBMD, with higher cortical CSA, CRT_THK and SSI than postmenopausal women. Moreover, significant differences of trabecular but not cortical BMC, vBMD or SSI were found between women of the younger (<48y) age-groups. PERI_C, ENDO_C displayed lower values in the 20-29y group and higher values in the 70-79y group, denoting significant differences of bone geometry with aging.

Keywords: Aging, Menopause, Bone mass, peripheral Quantitative Computed Tomography, Bone Geometry

Introduction

From birth until the completion of skeletal growth, bones grow in length and width, increasing their size and accumulating mass, while also developing their individual shape and structure[1-2]. Aging, on the other hand, is associated with decreases of bone mass and deterioration of micro-architectural properties of trabecular and cortical bone that may lead to skeletal fragility[3-4]. However, it is currently debated whether substantial bone loss starts at early adulthood or later in life.

In women, menopause has been shown to constitute a significant cause of bone loss[5-6]. Although bones constantly undergo changes throughout life to meet mechanical and metabolic needs, a common-held belief in the scientific community still remains that before menopause practically no net bone loss occurs in healthy eumennorhic individuals[7-8]. This notion has been supported by a number of previously published studies with dual energy X-ray absorptiometry (DXA) suggesting that, from early adulthood and until the peri-menopause, bone mineral density (BMD) of the lumbar spine, distal forearm and proximal femur remains stable or decreases minimally, although site-dependent differences concerning the extent of bone loss have also been report-

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ed[9-13]. Despite its utility in the clinical setting, DXA is a two-dimensional technique that provides measurements of areal (mg/cm^2) rather than volumetric (mg/cm^3) BMD, being thus largely affected by bone size. As a result, larger bones present higher values of areal BMD than smaller bones, while their volumetric density may be identical[14]. Moreover, DXA does not possess the discerning ability to differentiate between trabecular and cortical bone, so that independent changes in each compartment cannot be assessed. Finally, parameters of microarchitecture are not captured by DXA, so that age-related differences or changes of bone structure remain unnoticed.

In recent years, quantitative computed tomography (QCT), performed both at central and peripheral sites has been employed to provide a more thorough understanding of the changes that bones undergo through out life. QCT presents numerous advantages as compared to DXA, since it provides measurements of bone mineral mass and volumetric BMD of the trabecular and cortical compartment separately[15]. Moreover, peripheral QCT enables the study of long bones such as the radius and tibia by capturing geometrical and structural properties that are subject to change in time such as bone cross-sectional areas (CSA), bone perimeters at the periosteal and endosteal surface and mean cortical thickness. Although significantly fewer studies of this nature have been published until present time, their results[16-20] clearly suggest that, at multiple skeletal sites, significant bone loss is evident from young adulthood in healthy women, contrary to what the DXA studies have previously reported. To assess age-related differences or changes, most researchers study age-groups of individuals usually per decade, and menstrual status in women is usually presented as percentage of the total study population being pre- or postmenopausal; however, timing of menopause with regard to age is often not reported[16-17,20]. As a result, it is not clear in such studies whether (and how many) women especially in the 40-50y and 50-60y groups were pre or post menopause.

In a previously published study, we assessed age-related differences of bone mass, geometry and derived strength in treatment-naive postmenopausal women aged 48-80y using pQCT of the tibia[21]. Our aim in the present study was to examine premenopausal and postmenopausal women of different age-groups per decade in order to assess differences of total, trabecular and cortical BMC and vBMD, and also of geometrical and micro-architectural properties of bone, as well as Stress-Strain indexes (SSI) between them, using pQCT of the tibia. To be certain that only women of the same menstrual status were included in each age group, all postmenopausal women included in the study were required to have natural menopause after the age of 48y. Our interest was also to examine whether premenopausal women of different age-groups presented with significant differences of measured bone variables between them despite being eumennhoric.

Materials and methods

Subjects

Participants in the study were Caucasian ambulatory women of the general population between 20 and 79 years of age who visited our department and were assessed for bone metabolic diseases. To be selected, postmenopausal women were required to have natural menopause after the age of 48 years. We excluded individuals with: 1) Previous or current use of medication for osteoporosis including hormone replacement therapy; 2) bone metabolic diseases (Paget's disease, Osteogenesis Imperfecta, Hypoparathyroidism, Hyperparathyroidism, Osteomalacia, Fibrous Dysplasia); 3) fragility fractures of the axial and/or appendicular skeleton excluding fractures of the fingers and toes; 4) systemic chronic diseases known to affect bone (autoimmune diseases, endocrine, haematological, neurological or renal disorders, malignancies); 4) previous or current use of glucocorticoids *per os*; 5) history of immobilisation, amenorrhea, or participation in sport more than once a week. All women were assessed in 2 different groups based on their menstrual status (premenopausal women aged 20-48y versus postmenopausal 49-79y) and 6 different age-groups per decade of age: 20-29y, 30-39y, 40-48y, 49-59y, 60-69y, 70-79y. The study protocol was approved by the Scientific and Ethics committee of our institution and all participants provided informed consent.

Anthropometry and menstrual status

Standing height (cm) was measured by a wall-mounted stadiometer and body weight (kg) using a digital scale. The length of the tibia (cm) was assessed *in vivo* in all individuals, as the distance between the most prominent part of the medial malleolus and the medial tibial condyle. Menstrual status and menstrual cycle characteristics were self-reported, and menopause was defined as the absence of menstruation for at least 12 months.

pQCT

The XCT-2000 scanner (Stratec Medizintechnic, Pforzheim, Germany) was used to scan the non-dominant tibia of each participant. According to the manufacturer, the X-ray beam generated by this scanner has a thickness of 2.5 mm and the pixel edge size was set to 0.5 mm. All scans were performed by one trained technician and according to the manufacturer's protocol, and scan speed was set at 10 mm/s. In brief, after measuring the length of the tibia, a scout view over the tibiotalar joint was obtained in order to locate the tibial plafond. Using the tibial plafond as a reference line, 3 radiological slices were obtained for each individual at the 4%, 14% and 38% of tibia length. The 4% site is adjacent to the tibial plafond, and mainly composed of trabecular bone. The 14% site represents the distal tibial epiphysis and provides estimates of subcortical and cortical bone, and the 38% site represents the distal diaphysis and is composed mainly of cortical bone. All Image analyses were performed with the in-

tegrated XCT Stratec software, version 5.50. For the 4% site, Contour Mode 1 was selected to separate the soft tissue from the outer edge of the bone with a threshold of 180 mg/cm^3 (pixels >180 mg/cm^3 were recognised as bone). Peel Mode 1 (Area A% 45.0) was selected to separate between trabecular and cortical bone and leave an inner region of the total bone CSA of 45% of purely trabecular bone. For the 14% and 38% sites, Cortical Mode 1 with a threshold of 710 mg/cm^3 to define cortical bone was used. To determine SSI at the 14% and 38% sites, Cortical Mode 1 with a threshold of 480 mg/cm^3 was used, according to the manufacturer's protocol. For each individual we assessed: total BMC (TOT_CNT) and trabecular BMC (TRB_CNT) in mg/mm of slice thickness), total volumetric BMD (TOT_DEN) and trabecular vBMD (TRB_DEN) in mg/cm^3 and total bone area of the cross-section (TOT_A, mm^2) at the 4% site; cortical BMC (CRT_CNT, mg/mm) and vBMD (CRT_DEN, mg/cm^3) at the 14% and 38% sites; total CSA (TOT_A, mm^2) and cortical CSA (CORT_A, mm^2) at the 14% and 38% sites; periosteal circumference (PERI_C, mm), corresponding to the external perimeter of the tibial cross-section, and endosteal circumference (ENDO_C, mm) corresponding to the internal perimeter of cortical bone (14% & 38% sites); mean cortical thickness (CRT_THK, mm) at the 14% and 38% sites; and SSI (mm^3) at the 14% and 38% sites. SSI provides an estimate of bending and torsional bone strength and is calculated according to the following formula: SSI= Σ (r^2 a CD/ND/Rmax) where a is the area of a voxel, r is the distance of the voxel from the centre of the cross-section, CD is the measured cortical density for each individual at the specific measurement site, ND is the normal (maximal) density of cortical bone adjusted for porosity on the light microscopic level which is set at 1200mg/cm^3, and Rmax is the maximum distance from the centre of the cross-section[22-23].

The short-term *in vivo* precision (CV) in our laboratory for all the variables used herein has been assessed in a separate study and has been estimated between 0,5% and 2%.

Statistical analysis

Data are expressed as mean±standard deviation or median for quantitative data and as percentages for categorical data. The Kolmogorov-Smirnov test was used for normality analysis of the parameters. The comparison of pQCT variables among the age groups was performed using the 1-way analysis of variance model (One way ANOVA). Pairwise comparisons were performed using the Bonferroni test. Kruskal-Wallis test and Mann-Whitney test were used in case of violation of normality. All tests are 2-sided, statistical significance was set at p<0.05. All analyses were carried out using the statistical package SPSS version 17.00 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL).

Results

315 women met the inclusion and exclusion criteria and were included in the study; of them 84 were premenopausal (age 20-48y) and 231 were postmenopausal (age 49-79y). Participants' demographic and anthropometric data per group are shown in Table 1. Differences concerning pQCT variables of trabecular and cortical bone between premenopausal and postmenopausal women are shown in Table 2. As expected, premenopausal women had significantly higher values of both trabecular and cortical BMC and vBMD than post-menopausal women (p<0.0005). Moreover, they exhibited significantly larger cross-sectional areas of cortical bone both at the 14% and 38% sites (p<0.0005), with lower periosteal and endosteal circumference and higher cortical thickness than postmenopausal women (Table 2). Premenopausal women displayed also significantly higher values of SSI than postmenopausal women both at the 14% and 38% sites (p<0.0005) (Table 2).

Differences of pQCT variables between the six different age-groups at the 4% site are shown in Table 3. Again as expected, the highest values of Total and Trabecular BMC and vBMD were found in women aged 20-29y and the lowest in women 70-79y. Moreover, women 20-29y were found to have significantly higher total and trabecular BMC and vBMD than all other age-groups. No differences concerning these variables were found between women 30-39y and 40-48y. TRB_DEN was found significantly higher in the 49-59y group than in the 60-69y (p=0.039) and 70-79y (p<0.005) age-groups, and so was TOT_DEN (p<0.05 and p<0.005 respectively).

Differences of cortical BMC (CRT_CNT) and vBMD (CRT_DEN) between age-groups at the 14% and 38% sites are shown in Table 4. Highest values for both variables were observed in the 30-39y group, although differences between the 20-29y and 30-39y groups were not statistically significant. All women in the younger age groups had significantly higher CRT_DEN than women 49-59y and older, and lowest values of CRT_CNT and CRT_DEN at both sites were found in women 70-79y.

Differences of geometrical and architectural variables as well as SSI between the different age-groups at the 14% and 38% sites are shown in Table 5. Total CSA was shown to be higher at the 14% site compared to the 38% site; as a result, periosteal (PERI_C) and endosteal (ENDO_C) circumference presented greater values in all age-groups at the 14% than the 38% site. Values of SSI were found to be lower at the 14% site than at the 38% site in all age-groups, and the same observation was made for cortical BMC and vBMD, cortical CSA and cortical thickness, depicting that the 38% site is a much richer cortical site than the 14% site . Total CSA, PERI_C and ENDO_C were found to have lowest values in the 20-29y group and highest values in the 70-79y group at both sites; however, differences between the youngest (20-29y) and the oldest (70-79y) age-groups were statistically significant only at the 14% site. Cortical thickness (CRT_THK) was found to have highest values in the 20-29y group and lowest in the 70-79y group, and differences between them were statistically significant at both sites. Values of SSI were found to be higher in the younger age-groups (20-29y, 30-39y, 40-48y) without significant differences between them at both measurement sites; significant differences of

Table 1. Participants' anthropometric data (Mean±Standard Deviation). No statistically significant differences (NS) were found between the different age-groups concerning height, weight and tibia length.

Groups	N	Height (cm)	Weight (kg)	Tibia Length (mm)
Premenopausal	**84**	**165.8±6.3**	**62.9±4.3**	**357.4±16.9**
20-29y	23	166.2±5.3	62.5±5.1	357.3±20.0
30-39y	20	165.5±4.1	63.2±4.9	354.2±19.7
40-48y	41	164.9±6.4	63.7±6.4	358.8±22.5
p-value		**NS**	**NS**	**NS**
Postmenopausal	**231**	**165.0±7.2**	**62.1±8.4**	**356.8±17.2**
49-59y	80	165.1±5.8	63.6±5.8	356.9±17.7
60-69y	82	165.4±4.3	62.3±5.5	357.0±18.9
70-79y	63	164.6±4.7	61.6±6.2	356.7±19.4
p-value		**NS**	**NS**	**NS**

Table 2. Differences of pQCT variables between premenopausal and postmenopausal women (values expressed as Mean±Standard Deviation).

	PREMENOPAUSAL WOMEN (N=84)	POSTMENOPAUSAL WOMEN (N=231)	p-value
TRB_CNT (mg/mm, 4% SITE)	93.25±19.16	81.12±18.01	<0.0005
TRB_DEN (mg/cm^3, 4% SITE)	205.70±35.22	178.84±36.41	<0.0005
CRT_CNT (mg/mm, 14% SITE)	176.45±28.51	141.22±31.71	<0.0005
CRT_DEN (mg/cm^3, 14% SITE)	1152.00±34.99	1054.92±62.64	<0.0005
CRT_CNT (mg/mm, 38% SITE)	306.27±46.46	267.44±43.14	<0.0005
CRT_DEN (mg/cm^3, 38% SITE)	1204.24±44.02	1126.37±45.46	<0.0005
CRT_A (mm^2, 14% SITE)	153.02±23.33	132.78±24.86	<0.0005
CRT_A (mm^2, 38% SITE)	254.26±37.04	236.94±35.59	<0.0005
CRT_THK (mm, 14% SITE)	2.25±0.35	1.90±0.41	<0.0005
CRT_THK (mm, 38% SITE)	4.65±0.60	4.26±0.68	<0.0005
PERI_C (mm, 14% SITE)	75.40±5.90	76.78±5.23	0.048
PERI_C (mm, 38% SITE)	69.36±4.79	69.27±3.94	0.865
ENCO_C (mm, 14% SITE)	61.26±6.59	64.87±6.75	<0.0005
ENDO_C (mm, 38% SITE)	40.16±5.14	42.47±5.70	0.001
SSI (mm^3, 14% SITE)	1324.6±281.6	1142±200.7	<0.0005
SSI (mm^3, 38% SITE)	1560.9±212.3	1291.1±201.6	<0.0005

SSI between women of the younger age-groups as compared to women aged 49-59y and older were also found at both measurement sites.

Discussion

In this cross-sectional study, we compared differences of multiple bone variables between age-groups of women 20-79 years per decade of age, using pQCT of the tibia. Menstrual status was also taken into consideration so that we could determine differences of bone mass, geometry and structure between premenopausal and postmenopausal women in total, and between women of the different age-groups. Moreover, we studied 3 radiological slices for each individual, in order to monitor differences of bone variables at multiple sites. Our results indicated that, when pooled together, premenopausal women presented significantly higher values of trabecular and cortical BMC and vBMD, with larger cortical CSA and higher mean cortical thickness than postmenopausal women. Premenopausal women were also found to have significantly lower periosteal circumference (14% site) and endosteal circumference (14% and 38% sites) than postmenopausal women. In addition, premenopausal women presented significantly higher values of SSI at both measurement sites,

Table 3. Differences of Total and Trabecular BMC and vBMD between age-groups at the 4% site.

VARIABLES	Age Groups						p-value
	20-29y	30-39y	40-48y	49-59y	60-69y	70-79y	
TOT_CNT (mg/mm)	311.5±43.2	268.4±51.1$^{*\,\$\$}$	265.1±45.5$^{**\,\$\$}$	256.9±41.2$^{**\,\$\$}$	244.9±42.8$^{**\,\$\$}$	213.5±34.3**	<0.0005
TRB_CNT (mg/mm)	106.3±15.7	88.0±20.5$^{*\,\$\$}$	87.9±17.2$^{**\,\$\$}$	87.1±17.2$^{**\,\$\$}$	81.7±17.0$^{**\,\$\$}$	70.7±16.4**	<0.0005
TOT_DEN (mg/cm^3)	304.0±41.4	269.8±35.8$^{*\,\#\,\$\$}$	265.0±38.2$^{*\,\#\,\$\$}$	258.8±34.1$^{*\,\#\,\$\$}$	242.5±38.0$^{**\,\$\$}$	207.1±28.6**	<0.0005
TRAB_DEN (mg/cm^3)	230.7±34.0	196.0±33.4$^{*\,\$\$}$	197.2±30.8$^{**\,\$\$}$	194.0±32.3** $\$\$\#$	179.7±34.0$^{**\,\$\$}$	152.0±31.0**	<0.0005
*p<0.05 vs 20-29y, **p<0.005 vs 20-29y, $^{\#}$p<0.05 vs 60-69y, $^{\$}$p<0.05 vs 70-79y, $^{\$\$}$p<0.005 vs 70-79y.							

Table 4. Differences of cortical BMC and vBMD between age-groups at the 14% and 38% sites.

VARIABLES	Age Groups						p-value
	20-29y	30-39y	40-48y	49-59y	60-69y	70-79y	
CRT_CNT (mg/mm, 14% site)	180.3±25.6 $^{**\,\$\$\,\#\#}$	178.3±31.4 $^{*\$\$\,\#\#}$	173.4±29.0 $^{*\,\$\$\,\#\#}$	155.3±25.5 $^{\#\#}$	144.2±30.0 $^{\#\#}$	116.1±28.0	<0.0005
CRT_CNT (mg/mm, 38% site)	304.4±44.4 $^{\$\$\,\#\#}$	316.0±48.5 $^{*\,\$\$\,\#\#}$	302.6±47.0 $^{\$\$\#\#}$	282.6±38.6 $^{\#\#}$	270.1±41.8 $^{\#\#}$	240.7±39.6	<0.0005
CRT_DEN (mg/cm^3, 14% site)	1150.0±35.0 $^{**\,\$\$\,\#\#}$	1171.4±29.1 $^{**\,\$\$\,\#\#}$	1143.6±34.6 $^{**\,\$\$\,\#\#}$	1095.8±46.6 $^{\$\$\#\#}$	1054.0±51.5 $^{\#\#}$	996.8±51.6	<0.0005
CRT_DEN 38% (mg/cm^3)	1204.4±40.7 $^{**\,\$\$\,\#\#}$	1238.8±38.6 $^{**\,\$\$\,\#\#}$	1187.3±38.9 $^{**\,\$\$\,\#\#}$	1144.6±39.7 $^{\#\#}$	1128.0±38.6 $^{\#\#}$	1096.6±49.6	<0.0005
*p<0.05 vs 49-59y, **p<0.005 vs 49-59y, $^{\$}$p<0.05 vs 60-69y, $^{\$\$}$p<0.005 vs 60-69y, $^{\#}$p<0.05 vs 70-79y, $^{\#\#}$p<0.005 vs 70-79y.							

Table 5. Differences of geometrical and architectural variables between age-groups at the 14% and 38% sites.

VARIABLES	Age Groups						p-value
	20-29y	30-39y	40-48y	49-59y	60-69y	70-79y	
TOT_A (mm^2, 14% site)	443.9±60.0$^{\#}$	450.0±76$^{\#}$	463.9±76	457.6±62.6$^{\#\#}$	466.5±60.5$^{\#}$	498.5±64.1	0.003
TOT_A (mm^2, 38% site)	379.0±48.8	381.0±57	389.7±53	378.0±43.4	383.5±44.0	390.0±44.0	0.678
PERI_C (mm, 14% site)	74.5±5.0$^{\#}$	75.0±6.2$^{\#}$	76.1±6.2$^{\#}$	75.7±5.1$^{\#}$	76.4±5.1$^{\#}$	79.0±5.1	0.002
PERI_C (mm, 38% site)	68.9±4.5	69.0±5.1	69.8±3.9	68.8±3.9	69.3±4.0	69.9±3.9	0.682
ENDO_C (mm, 14% site)	59.8±5.5$^{\#\#}$	60.9±6.6$^{\#\#}$	62.3±7.1$^{\#\#}$	62.8±6.1$^{\#\#}$	64.1±6.5$^{\#\#}$	69.1±6.3	<0.0005
ENDO_C (mm, 38% site)	39.5±5.1$^{\#\#}$	39.5±5.3$^{\#\#}$	40.9±5.1$^{\#\#}$	40.3±4.5$^{\#\#}$	42.3±5.4$^{\#\#}$	46.0±6.1	<0.0005
CRT_A (mm^2, 14% site)	156.9±23.1 * $\$\$\,\#\#$	152.0±25$^{\$\#\#}$	151.3±23 $^{\$\$\#\#}$	141.3±20.5$^{\#\#}$	136.0±23.8$^{\#\#}$	115.5±24.3	<0.0005
CRT_A (mm^2, 38% site)	253.1±38.6$^{\#\#}$	255.0±37.6$^{\#\#}$	254.5±36.8$^{\#\#}$	246.9±32.9$^{\#\#}$	239.1±34.1$^{\#}$	218.9±31.3	<0.0005
CRT_THK (mm, 14% site)	2.3±0.3 $^{*\,\$\$\,\#\#}$	2.2±0.3$^{\$\#\#}$	2.2±0.3$^{\$\$\,\#\#}$	2.0±0.3$^{\#\#}$	1.95±0.4$^{\#\#}$	1.57±0.4	<0.0005
CRT_THK (mm, 38% site)	4.7±0.7$^{\#\#}$	4.7±0.6$^{\#\#}$	4.6±0.6$^{\#\#}$	4.5±0.6$^{\#\#}$	4.3±0.7$^{\#\#}$	3.8±0.6	<0.0005
SSI (mm^3, 14% site)	1334.0±198.1 $^{*\#\#\,\$}$	1315.2±187.1 $^{\#\#\,\$}$	1325.1±194.3 $^{\#\#\$}$	1225.6±203.8 $^{\#\#}$	1159.9±212$^{\#}$	1039.9±185.9	<0.0005
SSI (mm^3, 38% site)	1564.4±201.3 $^{**\,\$\$\,\#\#}$	1543.6±192.7 $^{**\,\$\$\#\#}$	1563.8±199.6 $^{**\,\$\$\#\#}$	1374.2±216.5 $^{\#\#}$	1339.7±229$^{\#}$	1262.1±185.8	<0.0005
*p<0.05 vs 49-59y, **p<0.005 vs 49-59y, $^{\$}$p<0.05 vs 60-69y, $^{\$\$}$p<0.005 vs 60-69y, $^{\#}$p<0.05 vs 70-79y, $^{\#\#}$p<0.005 vs 70-79y.							

and this finding suggests that they presented higher bending and torsional bone strength at these measurement sites compared to postmenopausal women. Our research of the relevant literature revealed very few previously published studies that examined differences of bone variables between premenopausal and postmenopausal women using pQCT of the tibia. Consequently, our results concerning all aforementioned variables are in full agreement to those of Reina et al[24] and Cointry et al[25] who used exactly the same pQCT scanner, scanning sites and software for image analysis that we did to assess differences at the tibia between premenopausal (age 25-50y, N=70) and postmenopausal women (50-82y, N=122), with the addition that we report also levels of statistical significance of the differences that we found. Our results are also in agreement to those of Uusi-Rasi et al[26], who used a similar scanner than we did (XCT3000, Stratec) but scanned only one site at the mid shaft of the tibia to assess prospectively age-related changes of trabecular and cortical vBMD in 2 groups of premenopausal (age 32-28, N=79) and 108 postmenopausal (age 66-72y, N=108) women.

While it could be hypothesised that differences of bone variables between premenopausal and postmenopausal women emphasise the importance of sex hormones regarding bone mass and microarchitecture, it is also true that before menopause women are of younger age. Our comparisons between different age-groups of women of the same menstrual status indicated that significant differences were found concerning trabecular BMC and vBMD between premenopausal women of different decades of age, while no such differences were found for cortical bone. As shown in Tables 3 and 4, women 20-29y presented significantly higher trabecular BMC and vBMD than women 30-39y and 40-48y, while cortical BMC and vBMD presented smaller and non-significant differences among these age-groups. Moreover, as shown in Table 5, derived bending and torsional strength as given by SSI did not present significant differences between the younger age-groups at both measurement sites. Other researchers have also reported significant differences of trabecular and not cortical vBMD or SSI between young women of similar age-groups in previously published studies. Russo et al[16] using the same scanner and 2 of the 3 scanning sites that we did, reported higher trabecular vBMD of the tibia in women 20-39y (N=55) vs 40-49y (n=47) and older, stating that their results support a pattern of decline in trabecular BMD starting in young adulthood. Their results concerning cortical vBMD (38% site) were also similar to ours, since they reported minor differences between women 20-39y vs 40-49y (1068.7±44.0 vs 1073.4±33.3 mg/cm^3). Riggs et al[18] also assessed age-related differences of trabecular and cortical bone at various skeletal sites between men and women of different age-groups. Although they used different scanners than we did, their results for all scanning sites including the proximal and distal tibia indicated that differences in trabecular but not cortical vBMD were apparent at young adulthood among the age-groups and continued throughout life, while decreases in cortical vBMD were apparent in midlife. In an extension of their study in order to include longitudinal data, Riggs et al[19] validated their previous results and concluded that substantial trabecular, but not cortical, bone loss was found to begin in young adult women at all 3 skeletal sites measured. Especially for the distal tibia, these authors presented evidence that the rate of bone loss for trabecular bone was higher in women 20-29y and 80y+ (-0.76% per year) while only -0.11% to -0.15% per year was lost in women 50-59y and 60-69y respectively. As for cortical bone, estimated rates of bone change were 0.25% per year in women 20-29y and -0.02% per year between 30-39y and reached significantly higher levels thereafter (-0.41% per year in women 50-59y). Similar results of early trabecular bone loss and declines of cortical bone after the age of 50y have also been reported from Yuen et al[27] who assessed age-related differences in Chinese women using the XCT3000 scanner. Our results concerning SSI are also in accordance to those of Sherk and Bemben[28], who reported no significant differences of SSI at various measurement sites (including the 15%, 35% and 40% of the length of the tibia) between women 20-29y (N=15), 30-39y (N=14) and 40-49y (N=15).

Popular theoretical considerations in the field of osteoporosis suggest that before menopause the amount of bone resorbed by osteoclasts in each remodelling unit is replaced by an equal amount of bone produced by osteoblasts[7-8]. However, the works of Harold Frost dating from the 1950's comprising the bone's Mechanostat theory[29] suggest that bone tissue physiologically regulates its mass and structure based primarily on mechanical stimuli that are subject to change constantly throughout one's life. Mechanical usage is principally a function of muscle activity, performed within the laws of gravity, and bones have been shown to adopt within certain limits to stress imposed to them, via thresholds, either by enhancing bone formation or by favouring bone resorption. Levels of physical activity are usually higher in younger individuals, and the bone's cross-section and mass at the distal part of the tibia have been shown to be adaptive to a compressive stress pattern resulting from kinetic activities[30]. Especially in adolescent females, it has also been shown that thresholds of bone formation are significantly influenced by sex hormones, so that trabecular vBMD is approximately equal for both sexes at the completion of skeletal growth, despite differences of bone size and muscle mass among males and females[31]. Within this context, we feel that it is possible that women in their '20's exhibit trabecular mass that is significantly higher than that of women in their '30's or '40's, and redistribution of trabecular bone as part of the completion of bone growth (as also postulated by Riggs et al) or bone loss due to lower physical activity or other events (i.e pregnancy, lactation) may be the cause of the differences we found. Trabecular bone, given its higher rate of bone remodelling, is more susceptible to change than cortical bone, and indeed neither we nor other researchers found differences regarding variables of cortical bone mass and structure between women of the younger age groups- and this observation correlates well in our view with the assumption that the distal diaphysis of the tibia retains its strength for many decades, at least until midlife. This assumption is further strengthened by our

findings concerning SSI at both cortical measurement sites that we used (14% and 38%), and our results demonstrated that there were no differences of SSI between women of the younger age groups; however, after the age of 49y differences of SSI become apparent, both versus the younger age groups, as well as between groups of older women, denoting that estimated bending and torsional bone strength diminishes with aging. Given the cross-sectional nature of our study and the significant inter-individuality of subjects assessed in all studies, we feel however that such hypotheses need to be addressed in the future with the use of longitudinal data, even though other researchers have previously reported the same results as we do based on prospective studies.

Our findings concerning differences of variables of bone geometry and mean cortical thickness between the different age groups are also in agreement with previously published work of other researchers, although such results have rarely been presented. In our study, differences for TOT_A and PERI_C were more pronounced and gained statistical significance at the 14% rather than the 38% site, possibly due to the larger cross-sectional area that it exhibits, as also shown in other studies[30,32]. Nonetheless, significant between groups differences for ENDO_C, CRT_A and mean CRT_THK (as well as SSI) were depicted both at the 14% and 38% site, probably given that the 38% site represents better the cortical bone of the distal diaphysis. TOT_A, PERI_C and ENDO_C were higher in the 70-79y group than all other age groups, with Δ TOT_A 70-79y vs 20-29y ranging from +2.92% to +12.3% at the 38% and 14% sites respectively, ΔPERI_C ranging from +1.49% -to +5.98%, and ΔENDO_C ranging from +15.5% to +16.5%. These results are in unison with those of Russo et al[20] and Lauretani et al[17] who reported ΔTOT_A of women 20-29 vs 90y of +5.7% at the 38% site and expansion of the medullary cavity with large increases of medullary bone area. These authors also concluded that periosteal apposition is a function produced by aging in order for the bone to compensate for losses produced by endocortical absorption and increased cortical porosity that are also prominent in older age. Our results of SSI further demonstrate that with aging, such a mechanism may not be adequate in order to preserve bone strength at previous levels, since values of SSI were found to be significantly lower in women 70-79y than all other age groups. Our results are also in accordance to those of Riggs et al[18] who reported increases of total area of 2-7% as measured at the distal tibia and of 4-10% at the proximal tibia between women 20-90y depending on whether adjustments to height are made. Cortical CSA and cortical thickness in our study were found significantly lower in the 70-79y group at both measuring sites, with differences ranging for ΔCRT_A 70-79y vs 20-29y ranging from -13.5% to - 26.3% and ΔCRT_THK from -18.6% to - 32.9% at the 38% and 14% sites respectively. Lauretani et al[17] similarly reported differences of CRT_A of -13.4% between women 20-90y at the 38% site, while Riggs et al[18] reported differences of CRT_A between women 20-90y ranging from -9% to -15% for the distal tibia and -14% to -18% at the proximal tibia depending on whether adjustments to height are made.

Our study has also limitations: it is a cross-sectional study that can only estimate differences of measured bone variables between women of different age-groups and cannot depict age-related changes of bone quantity and bone quality of the same individuals for a given period of time. Moreover, due to its resolution, the scanner that we used does not provide information concerning trabecular micro-architecture (i.e. trabecular number, thickness, separation etc) or cortical porosity. It is our understanding that information regarding such parameters may reveal additional differences between age-groups, further elucidating how the micro-structural elements of bone change through time. Although very few studies with high-resolution pQCT have been published until present time, their results in Caucasian[33] and Chinese women[34] indicate significant negative correlations with age of trabecular bone volume per tissue volume (BV/TV), trabecular number and thickness, cortical vBMD and cortical area and significant positive correlations for total area, trabecular network inhomogeneity and cortical porosity.

Our study is one of the few to report differences of bone mass, geometry, architecture and derived strength between premenopausal and postmenopausal women of different age-groups using pQCT of the tibia. We found significant differences of trabecular BMC and vBMD between women 20-29y versus 30-39y and 40-49y, while no such differences regarding cortical bone were observed among those age groups. Cortical bone loss was apparent after midlife in our study, and all women 20-49y had significantly higher cortical BMC and vBMD than women 49-59y and older, although it is not clear to what extent these results could be attributed to menopause or result from various other causes including bone adaptation to lower physical activity. Our results also of higher values of endosteal and periosteal circumference and total CSA, and lower mean cortical thickness in women 70-79y than all other age-groups suggest that bone loss in the cortical compartment is mainly driven by endosteal absorption and possibly (though not measured in our study) increased cortical porosity within the cortical shell, and periosteal apposition could be regarded as a defence mechanism in an attempt to preserve adequate bone strength. Our results concerning SSI, however, demonstrated that such a compensatory mechanism does not suffice to preserve strength at previous levels, and bending/torsional strength is actually diminished with aging. We believe that longitudinal studies of long duration with quantitative computed tomography are further needed to validate such results, so that rates of changes of bone parameters in different age-groups and at different sites could be monitored in more detail and enable a more thorough understanding of age-related bone loss.

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