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ORIGINAL ARTICLE

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Brazilian normal static bone histomorphometry: effects of age, sex, and race

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Abstract Bone histomorphometry values for normal individuals within different populations have been well established. We studied iliac crest bone samples from 125 healthy Brazilian subjects. The effect of sex, race, and age variables on histomorphometric parameters was evaluated. Bone volume showed a trend to decrease with age in both sexes, being significantly higher in black females and Caucasian males. Interactions among sex, race, and age had no effect on trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp). However, age had a significant effect on Tb.Th and Tb.Sp, and sex had an impact on Tb.Sp. Trabecular number (Tb.N) was higher in black females than in males and was higher in Asian males than in females. Among females, Tb.N was lower in Asians than in other races and was higher in blacks than in Caucasians and or in those of mulattos. In addition, Tb.N was higher in males under 10 than in males over 50 years old, was higher in females under 10 than in females in any other age bracket, and was lower in females in the 41–50 age bracket than in younger females. Osteoid volume and osteoid surface were significantly higher in males than in females, and a significant age-related difference in osteoid thickness was observed. No significant sex-related or race-related differences were found in terms of resorption, although eroded surface decreased with age. In conclusion, sex, race, and age, as well as interactions among these three variables, were found to affect some static histomorphometric indexes in healthy Brazilian subjects.

Key words bone histomorphometry · age-related bone loss · race and sex differences · normal iliac bone

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Introduction

Bone strength and fracture susceptibility are significantly influenced by peak bone mass and age-related physiological bone loss. Bone mass progressively increases over the first two decades of life, peaking in the third or fourth [1]. Peak bone mass and bone loss are both dependent on sex, heredity, ethnicity, physical activity, and nutritional factors [2–4]. However, age-related bone loss can also be influenced by factors such as smoking, alcohol consumption, and drug use [5]. In females, bone loss is accentuated, decreasing in the late thirties and accelerating during the first 5–10 years after menopause [6].

Several methods have been used to study bone mass. Histomorphometry is the most accurate and widely used method for the analysis of bone tissue [7–9]. Histomorphometry can be used to analyze various parameters, allowing the quantitative assessment of bone remodeling and structure, thereby providing information that cannot be obtained using other methods [10]. Over the past 20 years, various authors have established their own standard values for normal bone [10]. However, normal values can vary as a result of the influence of factors such as race, geography, and diet.

The aim of this study was to establish reference values for static histomorphometric parameters used to evaluate bone in Brazilian subjects, as well as to investigate the bone structure of healthy females and males of different races and ages.

Materials and methods

Study design

We analyzed 125 iliac crest bone samples obtained from healthy individuals residing in the urban and periurban areas of the city of Sao Paulo, located in southeast Brazil. Of the 125 individuals evaluated, 56 were female (mean age, 35.4 ± 2.9 years), 69 were male (mean age, 32.9 ± 2.4 years), 8.8% were of Asian (Japanese or other) descendant, 12% were black, 19.2% were mulatto (also mulato; a person of mixed ancestry with an African and a European parent, half black and half white, the offspring of two mulatto parents, or a person with a mixture of African-European ancestry), and 60% were Caucasian. A total of 119 (95.2%) of the individuals from whom samples were obtained had died unexpectedly. All samples were obtained concomitantly with the post mortem examination performed by a pathologist. The majority (56.8%) were victims of gunshot wounds, knife wounds, trauma, or traffic accidents. Other causes of death included myocardial infarction (30.4%) and intracranial hemorrhage (4.8%). In some cases (3.2%), the cause of death was undetermined. All the samples were collected within the first 24 h after death. The remaining 6 individuals (4.8%) were live kidney donors from whom samples were obtained at the time of surgery.

Particularly in samples obtained from females, we decided to study the menopause effect in women. To that end, we compared females aged 50 or above with those between 30 and 50 years old, and with those below the age of 30.

Exclusion criteria were gastrointestinal disease, intestinal surgery, liver disease, renal disease, metabolic bone disease, and history of prolonged bed rest, as well as the use of anticonvulsant drugs, corticosteroids, or any medication known to interfere with bone metabolism.

The study protocol was approved by the ethics committee of the local institution.

Bone biopsy

The bone sample specimens were taken with a Bordier trephine (7 mm in diameter), concomitantly with the autopsy, from a standardized location 2 cm inferior and posterior to the anterior superior iliac spine. The specimens were fixed in 70% ethanol, dehydrated, and embedded in methylmethacrylate. Undecalcified 5-μm-thick sections were cut using a Polycut S equipped with a tungsten carbide knife (Leica, Heidelberg, Germany), and the sections were stained with 0.1% toluidine blue, pH 6.4.

Bone histomorphometry

Structural and static histomorphometric data were obtained using an Osteomeasure semiautomatic image analyzer (Osteometrics, Atlanta, GA, USA), a digitizing tablet with a cursor, and a camera lucida. We started the measurements below the corticomedullary differentiation. Using this criteria, all parameters were determined in at least three nonconsecutive sections per sample, at a magnification of 125×. The mean tissue area (T.Ar) analyzed was 20.01 ± 4.96 mm^2.

All histomorphometric parameters are reported according to the nomenclature recommended by the American Society of Bone and Mineral Research (ASBMR) [11].

Structural histomorphometric parameters. Bone volume (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm), and trabecular number (Tb.N, /mm).

Static histomorphometric parameters of bone formation. Osteoid volume (OV/BV, %), osteoid surface (OS/BS, %), osteoblast surface (Ob.S/BS, %), and osteoid thickness (O.Th, μm).

Static histomorphometric parameters of bone resorption. Eroded surface (ES/BS, %) and osteoclast surface (Oc.S/BS).

Statistical analysis

The data in the table are expressed as mean ± standard error (SE), and the figures were expressed as median, minimum and maximum, and lower and upper quartile. A logarithmic transformation was performed for data not presenting normal distribution. Thus, log(x) was used for Tb.Sp and ES/BS and log x + 1 was used for Tb.N.

The analysis of variance (ANOVA) technique was applied to analyze the effect of sex (females and males), race (Asian, Caucasian, mulatto, or black) and age (arranged by age bracket) on each histomorphometric parameter. The effect of the interaction between any two factors (sex and age, sex and race, or age and race) was also analyzed. When such interaction was found to have a significant effect, multiple comparisons among categories were performed using the Wilks test. To verify the effect of menopause in the female group, the same model was used. However, age, and race were rearranged. Race was determined as Caucasian and non-Caucasian, and age was divided as follows: <30, 30–50, and >50 years. If any interaction was found to have a significant effect, Duncan's post hoc test among categories was performed.

The level of statistical significance was set at 5% (P = 0.05). The SAS and SPSS systems were used to perform statistical calculations.

Table 1. General static histomorphometric data according to race and sex

Race	Sex	BV/TV (%)[a]		OV/BV (%)		OS/BS (%)		O.Th (µm)		Ob.S/BS (%)		ES/BS (%)		Oc.S/BS (%)		Tb.Th (µm)		Tb.Sp (µm)		Tb.N (/mm)[b]	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Asian	Female $n = 3$	18.90	3.55	2.10	1.32	13.50	7.26	9.97	0.94	2.33	1.35	1.17	0.82	0.03	0.03	137.00	15.00	611.87	78.59	1.33	0.09
	Male $n = 7$	23.93	1.82	2.44	0.66	16.10	4.50	10.37	0.75	1.26	0.58	2.62	0.90	0.00	0.00	121.37	9.17	391.59	26.53	1.96	0.14
Caucasian	Female $n = 35$	20.71	1.11	1.57	0.36	9.51	1.57	9.96	0.62	1.29	0.59	4.33	1.25	0.03	0.01	113.43	5.26	480.56	36.40	1.92	0.13
	Male $n = 39$	26.98	1.24	2.83	0.39	15.98	2.13	10.91	0.62	2.20	0.61	2.89	0.74	0.05	0.02	129.65	5.14	370.86	18.18	2.11	0.11
Mulatto	Female $n = 15$	24.72	2.05	1.51	0.29	9.92	2.04	11.06	0.66	1.52	0.62	4.90	2.21	0.07	0.03	131.30	9.50	431.94	38.65	1.96	0.20
	Male $n = 12$	24.69	3.03	3.34	1.35	16.51	3.91	9.89	0.72	1.74	0.95	3.50	1.50	0.14	0.13	119.81	8.38	451.30	74.52	2.07	0.27
Black	Female $n = 3$	28.00	5.72	1.41	0.25	8.67	0.19	11.47	1.87	0.50	0.10	2.83	0.77	0.00	0.00	127.53	8.25	362.97	86.38	2.60	0.26
	Male $n = 11$	22.42	2.36	4.78	1.07	21.85	3.40	12.75	1.17	2.90	1.63	4.04	1.98	0.11	0.07	126.17	11.31	469.71	52.43	1.85	0.25
Total	Female $n = 56$	22.08	0.97	1.58	0.24	9.79	1.16	10.34	0.44	1.36	0.41	4.23	0.97	0.04	0.01	120.24	4.34	468.27	26.06	1.94	0.10
	Male $n = 69$	25.55	0.98	3.19	0.37	17.02	1.54	10.97	0.43	2.13	0.46	3.15	0.58	0.06	0.03	126.54	3.78	402.71	18.88	2.05	0.09

BV/TV, bone volume; OV/BV, osteiod volume; OS/BS, osteoid surface; O.Th, osteoid thickness; Ob.S/BS, osteoblast surface; ES/BS, eroded surface; Oc.S/BS, osteoclast surface; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; Tb.N, trabecular number; data are reported as mean ± SE

[a]BV/TV: There was an interaction between race and sex ($P < 0.04$)
Black females have a BV/TV higher than Caucasian females ($P = 0.03$) and slightly higher than Asian females ($P = 0.06$)
Caucasian males have a BV/TV higher than Caucasian females ($P < 0.001$)

[b]Tb.N: There was an interaction between race and sex ($P = 0.009$)
Black females have a Tb.N higher than black males ($P = 0.009$), Caucasian females ($P = 0.007$), and mulatto females ($P = 0.012$)
Asian males have a Tb.N higher than Asian females ($P = 0.032$)
Asian females have a Tb.N lower than Caucasian females ($P = 0.028$), black females ($P < 0.001$), and Mulatto females ($P = 0.028$)

Results

Age had a significant impact on BV/TV ($P < 0.001$) when we analyzed females and males together. Individuals over the age of 50 presented markedly lower BV/TV values than did those in any other age bracket ($P < 0.05$; Fig. 1). Table 1 summarizes the results of static bone histomorphometric parameters according to race divided by sex. The interaction between race and sex had a significant effect on BV/TV ($P < 0.04$). However, purely race-related differences were seen only among females. Black females presented significantly higher BV/TV in comparison to Caucasian females ($P = 0.03$) and slightly higher BV/TV in comparison to Asian females ($P = 0.06$). In addition, only Caucasian females presented BV/TV values that were significantly lower than those found for Caucasian males ($P < 0.001$; Table 1; Fig. 2). When we verified the effect of menopause in the females group, there was an interaction between race and age ($P = 0.003$), where Caucasian (17.50 ± 1.45) and non-Caucasian (20.23 ± 1.92) females aged 50 and over presented lower BV/TV than Caucasian (22.52 ± 1.21) and non-Caucasian (26.26 ± 2.9) below the age of 30, and Caucasian (22.13 ± 3.49) and non-Caucasian (26.72 ± 4.16) between 30 and 50 years old ($P < 0.05$).

Interactions among age, sex, and race had no effect on Tb.Th in males or in females. However, age was found to have a significant effect on Tb.Th ($P < 0.001$). Individuals in the first decade of life (0–10 years of age) presented significantly lower Tb.Th values than did those in any other age bracket ($P < 0.05$; Fig. 3). No sex-related or race-related differences were observed in Tb.Th. Also, no effect of menopause was observed in this parameter.

Similarly, interactions among age, sex, and race had no effect on Tb.Sp, which was, however, found to be significantly affected by age ($P < 0.001$). Individuals over the age of 40 presented higher Tb.Sp values than did those in the other age brackets ($P < 0.005$; Fig. 4). Overall, males presented significantly lower Tb.Sp values than did females

Fig. 1. Impact of age on bone volume (*BV/TV*) ($P < 0.001$) in 125 bone biopsies from normal subjects. Individuals over the age of 50 presented lower values of BV/TV than did those in the lower age brackets ($P < 0.05$). Results are expressed as median, minimum, and maximum, lower and upper quartile

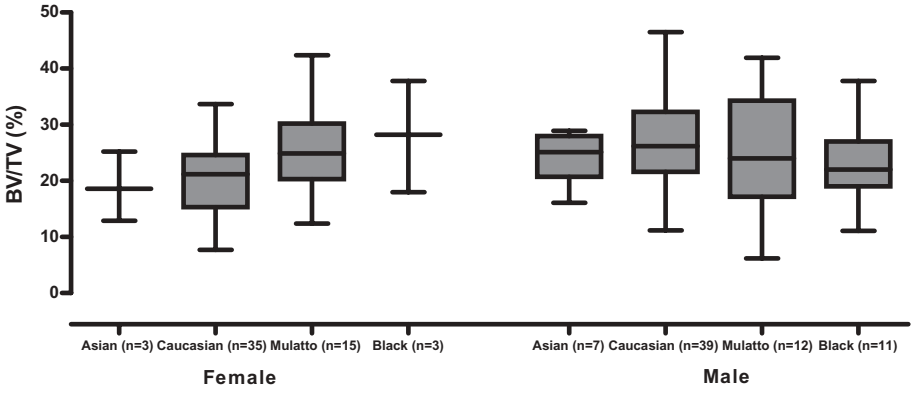


Fig. 2. Effect of race and sex on *BV/TV* ($P < 0.04$). BV/TV differed by race only among females. Black females presented higher BV/TV values than did their Caucasian counterparts ($P = 0.03$) and slightly higher BV/TV than did their Asian counterparts ($P = 0.06$). Only Caucasian females presented BV/TV that was lower than those found for their male counterparts ($P < 0.001$). Results are expressed as median, minimum, and maximum, lower and upper quartile

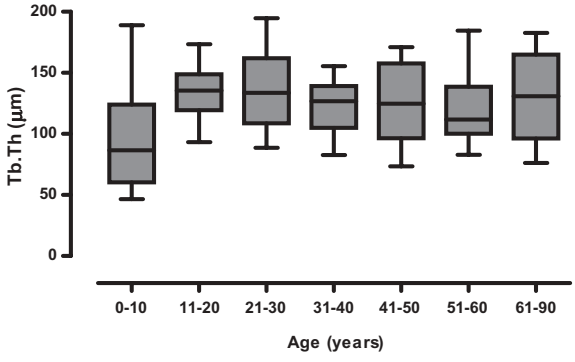


Fig. 3. Effect of age on trabecular thickness (*Tb.Th*) ($P < 0.001$). Children up to 10 years of age presented lower values of Tb.Th than did individuals in any other age bracket ($P < 0.05$). Results are expressed as median, minimum, and maximum, lower and upper quartile

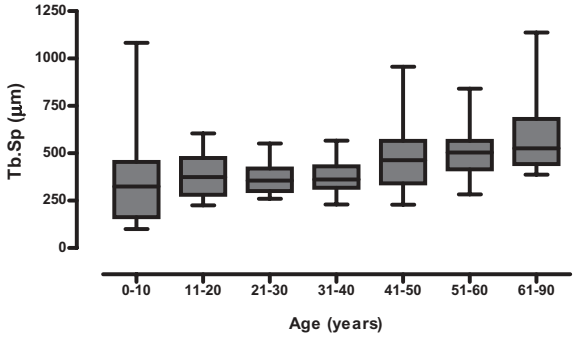


Fig. 4. Effect of age on trabecular separation (*Tb.Sp*) ($P < 0.001$). Subjects older than 40 presented higher values of Tb.Sp than did those in the lower age brackets ($P < 0.05$). Results are expressed as median, minimum and maximum, lower and upper quartile

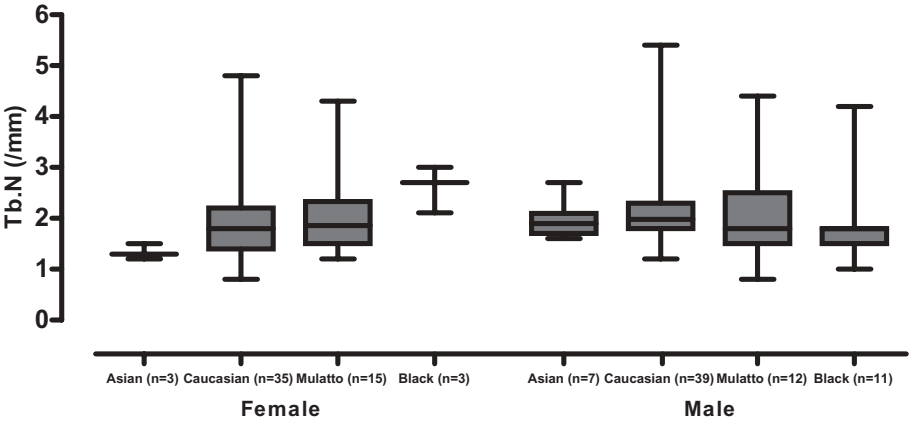
(402.71 ± 18.88 vs. 468.27 ± 26.06; $P = 0.04$). There was an interaction between race and age in Tb.Sp ($P = 0.0001$). The effect of menopause in the female group was clearly observed, where Caucasian (645.37 ± 61.09) and non-Caucasian (559.06 ± 29.41) aged 50 and over presented higher Tb.Sp than Caucasian (350.94 ± 27.64) and non-Caucasian (400.44 ± 61.54) below the age of 30, and non-Caucasian (377.26 ± 55.27) aged 30–50 years old ($P < 0.05$).

The interaction between sex and race had a significant effect on Tb.N ($P = 0.009$; Fig. 5), as did that between sex and age ($P = 0.01$; Fig. 6). Black females presented higher Tb.N than did Black males ($P = 0.009$), whereas Asian males presented higher Tb.N than did Asian females ($P = 0.032$). Comparing all the females as a group, we found that Tb.N values were significantly lower in Asians than in Caucasians ($P = 0.028$), blacks ($P < 0.001$), or mulattos ($P = 0.028$). The Tb.N values found for Caucasian females were lower than those found for black females ($P = 0.007$), as were those found for mulatto females ($P = 0.012$; see Fig. 5). No significant race-related differences were found among the males. Figure 6 depicts the effects that age and sex have on Tb.N ($P = 0.01$). Females in the first decade of life presented Tb.N values that were significantly higher than those found for females in any other age bracket ($P < 0.05$). Females in the 41–50 age bracket presented significantly lower Tb.N than did those in the age brackets 11–20 ($P = 0.03$), 21–30 ($P < 0.001$), and 31–40 ($P = 0.01$). There was an interaction between race and age in Tb.N ($P = 0.0007$). The effect of menopause in the female group was confirmed as in the first ANOVA analysis. The post hoc test analysis showed that Caucasian (1.38 ± 0.09) and non-Caucasian (1.65 ± 0.23) females aged 50 or above presented lower Tb.N than Caucasian (2.41 ± 0.22) and non-Caucasian (2.16 ± 0.31) below the age of 30 ($P < 0.05$). In males in the first decade of life, Tb.N was significantly higher than that seen in males in the 51–60 age bracket ($P = 0.01$) and older than 60 ($P = 0.03$).

Regarding bone formation parameters, interactions between age, sex, and race had no significant effect on OV/BV, OS/BS, or O.Th. Mean OV/BV values were significantly higher in males than in females (3.19 ± 0.37 vs. 1.58 ± 0.24; $P = 0.001$), as were mean OS/BS values (17.02 ± 1.54 vs. 9.79 ± 1.16; $P = 0.005$). Age had a significant effect on O.Th ($P = 0.019$), although sex and race did not ($P = 0.383$ and $P = 0.225$, respectively). Individuals in the first decade of life presented lower O.Th than did those in any other age bracket ($P < 0.05$; Fig. 7). No effect of menopause was observed in bone formation parameters.

Interactions among age, sex, and race also had no significant effect on ES/BS, although there was a significant age-related difference in ES/BS ($P < 0.001$). Individuals in the first decade of life presented higher ES/BS values than did those in any other age bracket ($P < 0.05$; Fig. 8). No effect of menopause was observed in ES/BS.

Fig. 5. Effect of sex and race on trabecular number (*Tb.N*) ($P = 0.009$). Black females presented greater Tb.N than did Black males ($P = 0.009$), although Asian males presented greater Tb.N than did Asian females ($P = 0.032$). Among females, Tb. N values were lower in Asians than in Caucasians ($P < 0.028$), in Blacks ($P < 0.001$), and in Mulattos ($P = 0.028$). In females, Tb.N values in Caucasians were lower than for Blacks ($P = 0.007$) and in Blacks than in Mulattos ($P = 0.012$). Results are expressed as median, minimum, and maximum, lower and upper quartile



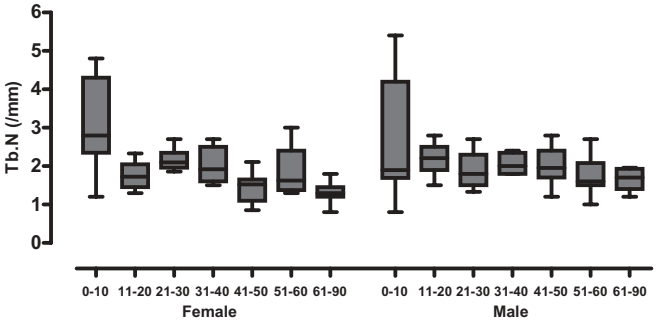


Fig. 6. Effect of age and sex on trabecular number (*Tb.N*) ($P = 0.011$). Male children up to 10 years of age presented higher Tb.N than did males from 51 to 60 years of age and males older than 60 ($P = 0.03$). A similar relationship was observed between females up to 10 years of age and those in all other age brackets ($P < 0.05$). Females from 41 to 50 years of age presented lower Tb.N than did females from 11 to 20 ($P = 0.038$), 21 to 30 ($P < 0.001$), and 31 to 40 ($P = 0.011$) years of age. Results are expressed as median, minimum, and maximum, lower and upper quartile

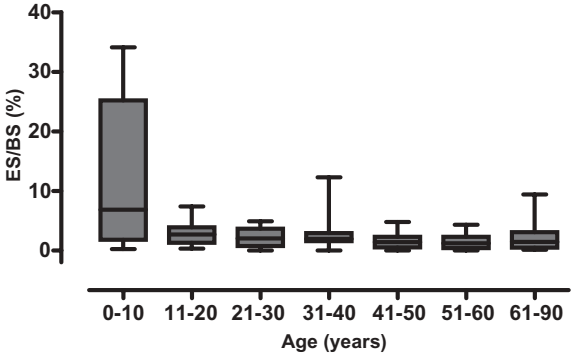


Fig. 8. Effect of age on eroded surface (*ES/BS*) ($P < 0.001$). ES/BS values were higher in children up to 10 years of age than in older individuals ($P < 0.05$). Results are expressed as median, minimum, and maximum, lower and upper quartile

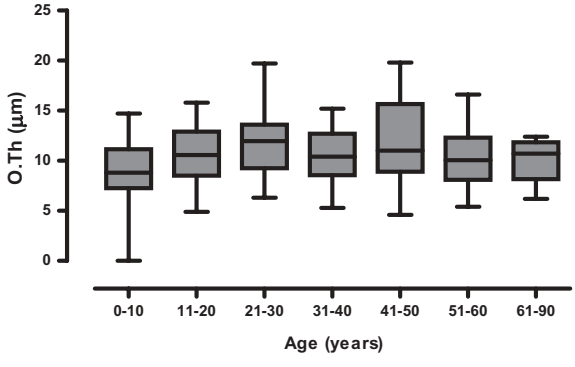


Fig. 7. Effect of age on osteoid thickness (*O.Th*) ($P = 0.019$). Children up to 10 years of age presented lower O.Th values than did individuals in other age brackets ($P < 0.05$). Results are expressed as median, minimum, and maximum, lower and upper quartile

Parameters as Ob.S/BS and Oc.S/BS presented in Table 1 are not included in the ANOVA because they did not present normal distribution, although we applied many transformation steps to normalize them.

Discussion

In the present study, normal values for static histomorphometric parameters were determined in bone samples of iliac crest obtained from 125 Brazilian individuals. Unfortunately, one limitation of the present study was the lack of dynamic parameters, those measured by tetracycline labeling. As in most similar studies, bone samples were obtained *post mortem* [12–20]. This limitation hampers the comprehension of normal bone remodeling over lifetime.

Various authors have shown that BV/TV decreases with age [12,14,15,16,18–22]. In our study, we also found that age has a significant influence on BV/TV, irrespective of sex or race. As expected, BV/TV increased progressively, albeit slightly, until the third decade of life (peak bone mass), and decreased thereafter. Among females in particular, there was a trend toward a reduction in BV/TV after the age of 50, probably corresponding to the onset of menopause. In males, there was a continuous loss of bone volume, although this difference was also not significant. We could speculate that this occurred as a result of the greater periosteal apposition and endosteal resorption in men compared to women [3].

According to the race-related differences in bone formation and resorption, there are few and conflicting studies in

the literature. Schnitzler et al. [12], studying blacks and Caucasians from South Africa, observed that black males presented higher BV/TV than did Caucasian males, and that Tb.Th was greater in black males and females than in their Caucasian counterparts. The authors also speculated that the higher values found for blacks in osteoid and erosion surfaces reflect prolonged remodeling periods, i.e., a larger number of remodeling units, also suggesting a greater bone turnover. In contrast, Parisien et al. [23], in a comparative study of healthy premenopausal black and Caucasian females, reported that bone volume and micro architecture were similar between the two races, although bone formation was significantly higher in black females than in Caucasian ones. The authors argued that the longer bone formation period, observed in blacks, would favor more time for secondary mineralization, leading to higher mineral density and the consequent reduced bone fragility. Han et al. [24], studying healthy black and Caucasian females, obtained similar results, although BV/TV, Tb.Th, Tb.Sp, and Tb.N were higher in blacks than in Caucasians, independently of menopausal status [25].

In our study, BV/TV was influenced by both race and sex. Black females and Caucasian males presented greater bone volume than did Caucasian females, in agreement with other studies [12,25]. Regarding microarchitecture, we found that black females presented higher Tb.N than did Caucasian and mulatto females and black males. To the best of our knowledge, this is the first time that such a difference has been demonstrated. We also found that Tb.N was lower in Asian females than in females or males of any other ethnic group. It is well known that Asian individuals present lower bone mineral density than do individuals of other races [26–28]. However, at least in the Western literature, there have been no histomorphometric studies of Asian individuals.

Our findings show that age had an effect on Tb.Th. Individuals in the first decade of life presented significantly lower values than did those in the other age brackets. This finding is in agreement with Glorieux et al. [29] and could be attributable to the highest bone resorption in the first period of life.

In males and females alike, we observed that, over the course of a lifetime, Tb.Sp increases and Tb.N decreases. These changes were more marked in females, especially in those over the age of 50, and coincided with a reduction in BV/TV. Our findings regarding microarchitecture were similar to those reported by others [19,20,22,30], confirming the bone loss following the peak bone mass [31–36]. In addition, bone loss is also present in males, albeit in a different manner, presenting as thinning of the trabeculae rather than the complete loss of connectivity seen in females [4].

Evaluation of bone formation parameters revealed that OV/BV and OS/BS were significantly lower in females than in males. We also found an age-related difference in O.Th. The lower OV/BV and OS/BS observed in females appear to be related to a negative balance that results in reduced bone formation at certain ages. Females in the 21–30 age bracket, as well as those in the 31–40 age bracket (when bone mass peaks), presented slight reductions in OV/BV and in OS/BS. Those reductions probably contributed to the drop in BV/TV seen in premenopausal period. In males, OV/BV and OS/BS were high in the first decade of life and stabilized thereafter. It is likely that the stabilization of bone formation has a protective effect on BV/TV in males. According to Melsen et al. [18], changes in the amount of osteoid reflect variations in formation, mineralization rate, or both. However, these changes might be better understood through dynamic analysis.

Some authors found that neither age nor sex correlates with bone formation parameters [14,16,20]. On the other hand, others have demonstrated age-related decreases in OV/BV and OS/BS [18,31,32]. In contrast, Schinitzler et al. [12] and Vedi et al. [9] observed that OV/BV and OS/BS increase in both sexes with advancing age. Schinitzler et al. [12] drew no conclusions regarding this finding, although Vedi et al. attributed it to the vitamin D deficiency observed in the elderly English population [9].

Regarding resorption parameters, we observed no significant sex- or race-related differences, although age had a significant impact on ES/BS, a result of the high ES/BS values seen for both sexes in the first decade of life. In children, especially those less than 1 year of age, bone histomorphometric analyses and densitometric data are scarce [33,34]. Some authors have observed that resorption increases in parallel with aging [14,16], whereas others established no correlation at all [13]. These discrepancies could be attributed to differences in technical evaluation and subjective interpretation of ES/BS [29,35]. Many such studies evaluated only individuals over the age of 10, which could explain the differences between our results and those of others, according to bone resorption as well as bone formation.

Schulz et al. [33] demonstrated that ES/BS and Oc.S/BS decrease with aging. Their study included individuals in the first decade of life, in particular those aged less than 1 year, as in our study. Recently, Glorieux et al. [29] established the normative data for histomorphometric parameters in growing children. The authors observed no age-related variations in resorption parameters. However, their findings for the first decade of life were similar to ours.

It is well known that changes in bone mass depend on two major factors: the balance between bone resorption and formation and the number of remodeling units [36]. We observed that both bone resorption and formation decreased with age, and that there was a consequent reduction in the number of remodeling units. In addition, the decreased Tb.N and increased Tb.Sp indirectly suggest that there was an imbalance between bone resorption and formation, contributing to the loss of bone mass with age. These effects were more marked in Caucasian and Asian females.

In conclusion, sex, race, and age, as well as interactions among these three parameters, were found to affect some static histomorphometric indexes in Brazilian healthy subjects. This present study may contribute to the better knowledge of normal bone measurements, although further studies regarding dynamic analysis are necessary.

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