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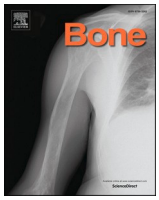

Review Article

Histomorphometric parameters of iliac bone in healthy individuals: Systematic review and meta-analysis

Aníbal Ferreira [a,b,c,*], Luciene Machado dos Reis [d], David Manteigas [e], Aluizio Barbosa Carvalho [f], Vanda Jorgetti [d]

[a] NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Lisbon, Portugal
[b] Department of Nephrology and Renal and Reno-pancreatic Transplantation Unit, Curry Cabral Hospital - Central Lisbon University Hospital Center, Lisbon, Portugal
[c] Centro Clínico Académico de Lisboa (CCAL), Lisbon, Portugal
[d] Nephrology Department, Laboratorio de Fisiopatologia Renal 16 (LIM 16), Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil
[e] Scientific ToolBox Consulting, Lisbon, Portugal
[f] Nephrology Division, Universidade Federal de São Paulo, São Paulo, SP, Brazil

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ABSTRACT

Despite its invasive character, bone biopsy followed by histomorphometry remains the gold standard for diagnosing and classifying many metabolic bone diseases. However, the interpretation of histomorphometric parameters requires comparison with average values obtained from a proper control group, which are only available for some populations, and reference standards still need to be published. Therefore, our objective was to estimate average values for bone histomorphometric parameters overall, by age, gender, and race (White and Black) categories of healthy adult individuals, based on a systematic review and meta-analysis of clinical studies. Relevant studies published in English with available results until December 2020 were identified by PubMed (Medline) search and consulting experts in the field. Out of 447 potentially relevant studies, 37 met the inclusion criteria. Meta-analysis using fixed-effects models was used to pool mean estimates and 95% confidence intervals (CI) for 16 bone histomorphometry parameters.

An age-by-gender trend was observed in most histomorphometry parameters. The mean estimates of bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb.N) decreased. In contrast, trabecular separation (Tb.Sp) increased from the youngest to the oldest age categories in both genders. Osteoblast surface (Ob.S/BS) and osteoclast surface (Oc.S/BS) decreased across all age categories in males. Mineralizing surface (MS/BS) increased from the youngest to the oldest age categories in females, while mineralization lag time (Mlt) increased in both genders. Furthermore, gender and race had a significant effect on several histomorphometry parameters.

In conclusion, this meta-analysis provided mean estimates for normal values of histomorphometric parameters that clinicians may use when evaluating bone biopsies in patients. This enables the direct comparison of patients' histomorphometric values with the suitable reference group regarding age, gender, and race.

1. Introduction

Despite its invasive character, bone biopsy followed by histomorphometry remains the gold standard for diagnosing and classifying many metabolic bone diseases, such as osteoporosis or renal osteodystrophy. Bone histomorphometry [1] provides information that can be obtained from quantitative examination of bone histology, which is not currently available from other approaches such as bone densitometry, high-resolution peripheral computed tomography, and biochemical markers of bone turnover [2]. Moreover, histomorphometry is the only technique able to assess bone turnover and bone mineralization based on the examination of bone cells and their activities in precisely localized regions.

The interpretation of bone histomorphometric parameters in patients

* Corresponding author at: NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, Campo Mártires da Pátria, 130, 1169-056 Lisboa, Portugal.
E-mail address: anibalferreira@netcabo.pt (A. Ferreira).

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with bone disease requires comparison with normal values obtained from an appropriate control group, age- and gender-matched with healthy individuals from the population under study [3–7]. Reference ranges for bone histomorphometric parameters have been derived from control groups in different populations worldwide [3–7]. Publications whose primary objective was establishing bone histomorphometric parameters in healthy populations are scarce, particularly those dependent on tetracycline labelling [8–33]. Normal values of histomorphometric parameters are not available for every population, as they can vary due to genetic background, ethnicity, age, gender, geography, vitamin D serum levels, and diet [1,16,34,35]. Also, these values can vary according to the methods adopted in different laboratories [11,33]. Hence, there is a need to quantitatively summarize normal values of histomorphometric parameters that may be used as reference standards to improve the diagnosis of bone metabolic disease and to guide therapeutic decisions. To the best of our knowledge, this is the first meta-analysis of global data providing an estimation of bone histomorphometric parameters in healthy individuals.

Therefore, we aimed to estimate the overall normal values for bone histomorphometry, as well as per age, gender, and race categories by performing a systematic review and meta-analysis of studies assessing histomorphometric parameters in healthy individuals.

2. Materials and methods

This systematic review was conducted according to the Recommendations in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [36].

2.1. Eligibility criteria

This systematic review and meta-analysis were limited to studies characterizing bone histomorphometry of healthy human subjects as either a primary objective or normal controls. The following inclusion criteria for the studies were used: reported histomorphometric measures of trabecular bone (i.e., including both mineralized bone and osteoid matrix) [37] applied to sections of biopsy samples of the iliac crest obtained from healthy individuals from the general population; conducted on adult (≥18 years old) and healthy subjects (without a history of bone disease, or other diseases, or use of medication or substances which could have affected bone metabolism) of both gender and race. Studies without any numerical data on bone histomorphometric parameters of healthy subjects were excluded.

2.2. Information sources and search strategy

Studies were identified by searching the Medline database (1966-December 2020) via PubMed and consulting with experts in bone histomorphometry. We used the following keywords to search PubMed: iliac AND (histomorphometry OR histomorphometric OR trabecular) AND (reference OR normal OR healthy) AND (human OR subject OR individual OR women OR men OR female OR male) NOT (rat OR mouse OR animal OR dog). No publication date or publication status restrictions were used, whereas language was limited to English-only reports.

2.3. Study selection and data collection

Eligibility assessment was first performed by title and abstract screening by one reviewer and included studies were confirmed by a second reviewer. Disagreements between reviewers were resolved by consensus. Full-text reports were independently reviewed twice by two reviewers.

We developed a data extraction sheet. One review author extracted data from included studies, and the second author checked the extracted data. Disagreements were resolved by discussion between the two review authors. A screening for duplicate publications and data from multiple reports of the same sample of healthy subjects was performed by juxtaposing author names and affiliations, sample sizes and histomorphometric measures. Overlapping reports were excluded if they reported the same histomorphometric parameters.

2.4. Data items

Data on the characteristics of study participants, including age, gender, and race were collected from each study. The following bone histomorphometric parameters were extracted and reported according to the nomenclature and abbreviations proposed by the American Society for Bone and Mineral Research (ASBMR) Histomorphometry Nomenclature Committee [37]: (a) static parameters reflecting bone structure - bone volume (BV/TV; %), trabecular thickness (Tb.Th; μm), trabecular separation (Tb.Sp; μm), and trabecular number (Tb.N; /mm); bone formation – osteoid volume (OV/BV; %), osteoid thickness (O.Th; μm), osteoid surface (OS/BS; %), and osteoblast surface (Ob.S/BS; %); and bone resorption - eroded surface (ES/BS; %), osteoclast surface (Oc.S/BS; %); and (b) dynamic parameters - mineralizing surface (MS/BS; %), mineral apposition rate (MAR; μm/d), bone formation rate (BFR/BS; $\mu m^3 \mu m^2$/d), adjusted apposition rate (Aj.AR; μm/d), mineralization lag time (Mlt; d), and activation frequency (Ac.F; /y).

When not presented as mean and standard deviation, all data used for each parameter were converted to it and then eligible for the analysis. Thirty-seven articles were eligible for the meta-analysis. The data of thirty of them were presented as mean and standard deviation, six as mean and standard error (we converted using the formula: SD = $\sqrt{n}$*SE), and one of them as median, minimum, and maximum, converted to mean and SD according to Hozo et al. [38].

All data for Tb.Th used in this meta-analysis were derived from Parfitt's equation, which is known as the indirect method [30]. For the kinetic parameters MS/BS and BFR/BS, data used considered only studies that used double labels + ½ of single labels for the calculations.

2.5. Risk of bias in individual studies

The risk of bias in individual studies was analyzed at the level of the summarized histomorphometric parameters for each study. Potential outliers were identified by studentized residuals. Studies that provided results with studentized residuals greater than two were individually analyzed by the authors to check for potential inadequacies (e.g., not previously considered differences in demographic characteristics).

2.6. Summary measures and synthesis of results

All histomorphometric parameters were summarized as the mean estimate and 95% confidence interval (CI). Parameter estimates were also calculated by age strata (overall and by gender), gender (male, female), and race (White, Black). In cases when studies reported data for only one class of the categories mentioned (e.g., data only for males), those data were used to calculate the overall estimate for that class but not in the statistical comparison between classes. Therefore, in some analyses, the number of studies included for data comparison was lower compared to the number of studies used for the overall estimate. Meta-analysis estimates by age strata are only calculated when at least two studies have available data for a given stratum.

The meta-analysis of all histomorphometric parameters was performed by computing overall mean estimates by applying a fixed-effects model to the data from categories or individual studies. Weight estimates were obtained by the inverse variance weights. We used I^2 and Cochrane tests to assess the inconsistency of data (i.e., the percentage of total variation across studies due to heterogeneity) across histomorphometric parameters within studies.

Although all estimates presented a high level of heterogeneity, we decided to use a fixed-effects model to calculate the weighted estimates

of each parameter. The high level of heterogeneity found within studies would be translated into almost equal weight estimates for each study if a random-effects model had been used. In this specific case, because the choice of the analysis should not be based solely on a heterogeneity test [39], we decided that studies with higher sample size and less variability should be weighted more and therefore, we used a fixed-effects model.

2.7. Risk of bias across studies

Funnel and radial plots were generated to assess selective reporting or publication bias. Heterogeneity within study size and estimate measure was also assessed.

2.8. Additional analyses

Individual sensitivity analysis was performed by removing each study from the overall estimate and assessing the impact on the parameter estimate and respective 95% CI. The results are presented for each parameter as supplementary data.

3. Results

3.1. Study selection and study characteristics

A total of 37 studies published between 1976 and 2014 met the eligibility criteria and were identified for meta-analysis after the exclusion of 395 full-text articles (Fig. 1). A summary of the characteristics of the included studies is shown in Table 1 [3–33,40–45]. Most studies were observational in design and reported between two and 16 histomorphometric parameters. It is worth pointing out that most of the studies do not report laboratory data. Normal subjects, ranging from 18 to 96 years of age, from European, North American, South American, African, and Asian populations, were included in the meta-analysis. The study from Ballanti et al. [12] was excluded from the calculations for Ob.S/BS and Oc.S/BS due to differences in the technique used for cell count. Data regarding MAR parameter of the study from Parfitt et al. [29] were removed as the sample used in that study was the same as the study from Han et al. [19]. The study from Rehman et al. [9] was excluded from the calculations for BFR/BS because it presented very different values compared to the other studies. The most reported histomorphometric parameter was BV/TV, whereas the least reported parameter was Ac.F.

3.2. Overall estimates of histomorphometric parameters

Overall mean estimates with 95% CI of normal values for BV/TV, Tb.Th, Tb.Sp, Tb.N, OV/BV, O.Th, OS/BS, Ob.S/BS, ES/BS, Oc.S/BS, MS/BS, MAR, BFR/BS, Aj.AR, Mlt, and Ac.F using the fixed effects model are shown in Table 2. Also, overall estimates from the meta-analysis using the random-effects model are provided in Table 3.

Study weights, model fit statistics, and sensitivity analysis of syntheses for each histomorphometric parameter can be found in Appendix A (Tables A.1 to A.16).

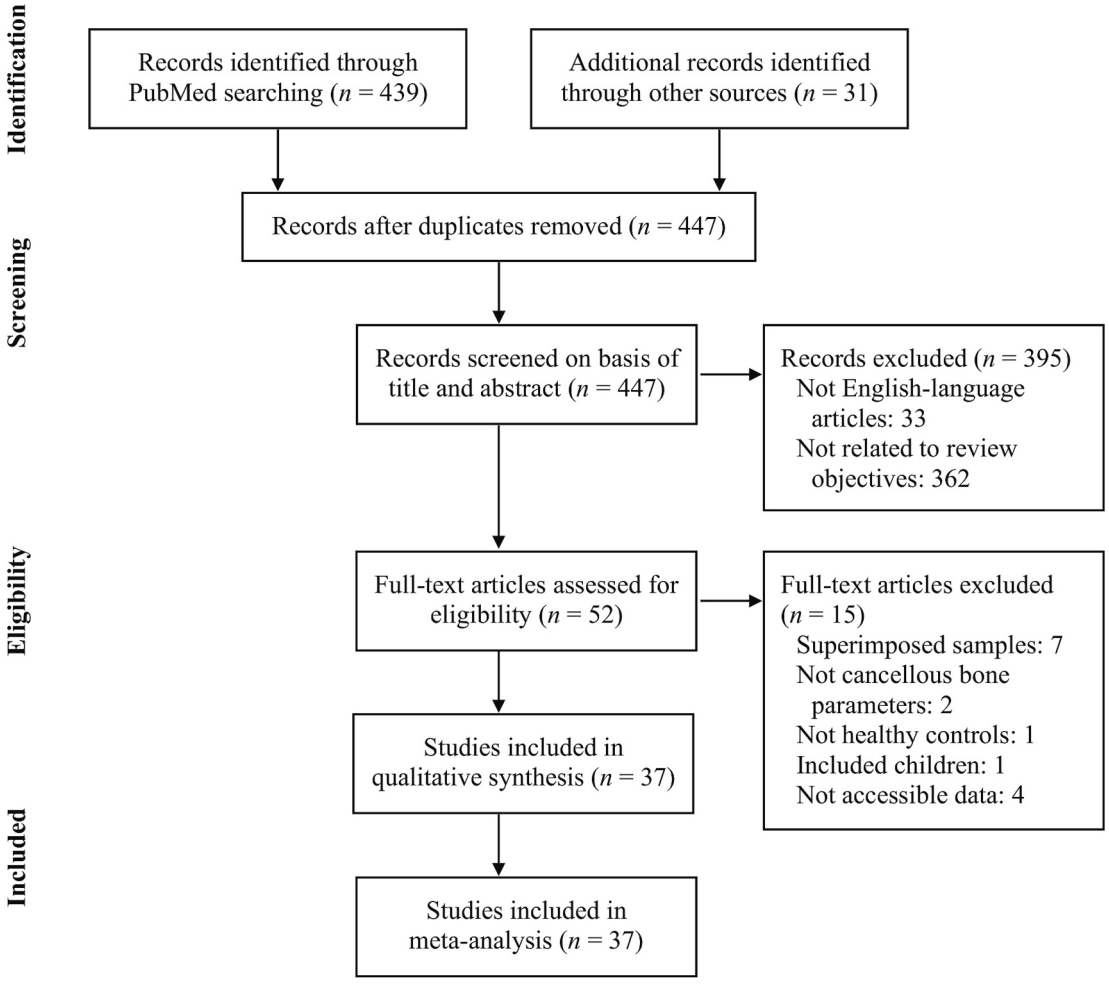

Fig. 1. Flow diagram of study selection.
Adapted from Moher et al. [36].

Table 1
Summary of the characteristics of the included studies, categories, and histomorphometric parameters included in meta-analysis.

Study (year)	N	Age, y[a]	Population	Analyzed categories[b]	Histomorphometric parameters
Arlot et al. [3] (1990)	50	64 (6)	France	None	BV/TV, OS/BS, O.Th, ES/BS, Tb.Th, Tb.N, Tb.Sp, MS/BS, MAR, BFR/BS, Aj.Ar, Mlt
Ballanti et al. [12] (1990)	88	20–89	Italian	Age, gender	BV/TV, OV/BV, OS/BS, O.Th, ES/BS
Ballanti et al. [4] (2001)	64	25(17)	Italian	None	Ac.F
Courpron et al. [17] (1976)	285	20–96	France	Age, gender	BV/TV
Dahl et al. [18] (1988)	72	21–81	Norwegian	Gender	BV/TV, OV/BV, OS/BS, O.Th, ES/BS, MS/BS, BFR/BS, MAR, Aj.Ar, Mlt
Dos Reis et al. [8] (2007)	125	34 (2.7)	Brazilian	Age, gender, race	BV/TV, OV/BV, OS/BS, O.Th, Ob.S/BS, ES/BS, Oc.S/BS, Tb.Th, Tb.N, Tb.Sp
Eastell et al. [40] (1988)	22	51 (4.5)	American	None	BV/TV, OV/BV, OS/BS, O.Th, ES/BS, MS/BS, MAR
Eriksen E.F. et al. [14] (1984)	20	19–60	Danish	None	O.Th, MS/BS, BFR/BS, MAR, Mlt
Fazzalari et al. [41] (1989)	22	18–90	Australian	Gender	BV/TV, OS/BS, ES/BS, Tb.Th, Tb.Sp
Guichelaar et al. [5] (2002)	61	20–50	American	Gender	BV/TV, OV/BV, OS/BS, O.Th, ES/BS, MAR, Aj.AR, Mlt
Han et al. [13](1996)	144	20–74	American	Gender, race	BV/TV, Tb.Th, Tb.N, Tb.Sp
Han et al. [19] (1997)	142	20–74	American	Race	OS/BS, Ob.S/BS, ES/BS, Oc.S/BS, MAR, Ac.F
Hoikka and Arnala [20] (1981)	85	20–70	Finish	Age, gender	BV/TV, OV/BV, OS/BS, ES/BS
Koehne et al. [21] (2014)	152	30–90	German	Gender	BV/TV, OV/BV, OS/BS, O.Th, Tb.Th, Tb.N, Tb.Sp
Malluche and Faugere [22] (1986)	28	20–83	American	Gender	MAR, Mlt
Malluche et al. [11] (1982)	84	20–87	American	Age	BV/TV, OS/BS, O.Th, Ob.S/BS, OV/BV
Mellish et al. [23] (1989)	96	19–90	British	Age, gender	BV/TV, Tb.Th, Tb.N, Tb.Sp
Melsen et al. [24] (1978)	135	19–80	Danish	Age, gender	BV/TV, OV/BV, OS/BS, O.Th, ES/BS
Melsen and Mosekilde [25] (1978)	41	19–56	Danish	Gender	OS/BS, ES/BS, MAR, Aj.AR
Melsen and Mosekilde [26] (1980)	41	19–56	Danish	Gender	Mlt
Meunier et al. [27] (1977)	108	20–80	France	Age, gender	OV/BV, OS/BS, O.Th,
Moore et al. [42] (1989)	21	27–81	Australian	None	BV/TV
Mullender et al. [6] (2005)	34	65 (11)	France	Gender	BV/TV, OV/BV, OS/BS, O.Th, ES/BS, Oc.S/BS, Tb.Th, Tb.N, Tb.Sp, MAR
Nordin et al. [7] (1984)	92	21–96	Australian	None	BV/TV
Ostojic et al. [28] (2006)	46	41–85	Croatian	Age, Gender	BV/TV, Tb.Th, Tb.N, Tb.Sp
Palle et al. [43] (1989)	36	25–73	France	Gender	BV/TV, ES/BS, Oc.S/BS
Parfitt et al. [29] (1997)	142	20–74	American	Race	OV/BV, O.Th, Mlt Aj.AR
Parfitt et al. [30] (1983)	78	48.4 (8.5)	American	Gender	BV/TV, Tb.Th, Tb.N, Tb.Sp
Parisien et al. [31] (1997)	55	33 (1)	American	Race	BV/TV, OV/BV, OS/BS, O.Th, ES/BS, Tb.Th, Tb.N, Tb.Sp, MS/BS, BFR/BS,MAR, Aj.AR, Mlt, Ac.F
Qiu et al. [32] (1989)	49	42.4	Chinese	Gender	BV/TV, Tb.Sp
Recker et al. [10] (1988)	34	45–74	American	Age	BV/TV, OV/BV, OS/BS, O.Th,, Ob.S/BS, ES/BS, Oc.S/BS, Tb.Th, Tb.Sp, Tb.N, MS/BS, BFR/BS, Mlt, MAR, Ac.F
Rehman et al. [9] (1994)	234	31–94	British	Age, gender	BV/TV, OV/BV, OS/BS, O.Th, Ob.S/BS, ES/BS, Oc.S/BS, Tb.Th, Tb.N, Tb.Sp, MS/BS, MAR, Mlt, Aj.AR
Schnitzler et al. [16] (1990)	346	21–71	South African	Age, gender, race	BV/TV, OV/BV, OS/BS, O.Th, ES/BS, Tb.Th, Tb.N, Tb.Sp
Vedi et al. [33] (1982)	57	19–80	British	Age, gender	BV/TV, OV/BV, OS/BS, ES/BS
Vedi et al. [44] (1983)	57	19–80	British	Age, gender	O.Th, MS/BS, MAR, BFR/BS, Aj.AR, Mlt,
Weinstein and Bell [45] (1988)	25	19–46	American	None	BV/TV, OV/BV, OS/BS, O.Th, Ob.S/BS, Oc.S/BS, MS/BS, BFR/BS, MAR, Aj.AR, Mlt
Zhioua et al. [15] (1994)	50	20–80	Tunisian	Age, gender	BV/TV, OV/BV, OS/BS, O.Th, ES/BS, Tb.Th, Tb.N

BV/TV – bone volume; OV/BV – osteoid 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.N – trabecular number; Tb.Sp – trabecular separation; MS/BS – mineralizing surface; MAR – mineral apposition rate; BFR/BS – bone formation rate; Aj.AR – adjusted apposition rate; Mlt – mineralization lag time; Ac.F – activation frequency; N – total number of subjects.

[a] Age in mean (standard deviation) or minimum-maximum.

[b] Age categories considered when age classes by decade were reported.

3.3. Estimates of histomorphometric parameters per gender and age category

Mean estimates with 95% CI from the meta-analysis using the fixed-effects model for each histomorphometric parameter per gender and 10-year age categories are shown in Appendix B. The bone structure parameters BV/TV, Tb.Th, and Tb.N mean estimates decreased, while Tb.Sp increased, from the youngest to the oldest age categories in both males and females. A peak value of BV/TV was observed in females in the decade of 21–30 years. Regarding the bone formation parameters, OV/BV, O.Th, OS/BS, and Ob.S/BS mean estimates showed variation across age categories in both genders. In males, OS/BS mean estimates decreased across age categories, especially between 61 and 90 years, while Ob.S/BS mean estimates decreased across all age categories. In females, there was a tendency for an increase of Ob.S/BS with age, but a peak value was observed between 41 and 50 years. The reabsorption parameter ES/BS mean estimates showed variation across the age categories in both genders. Oc.S/BS mean estimates decreased in males from the youngest to the oldest age categories, while in females, the highest values were observed in the 31–40 years category. Concerning the dynamic parameters, MS/BS mean estimates showed no relevant variation across age categories in males, while an increase from the youngest to the oldest age categories was found in females. There was no relevant variation across age categories of MAR BFR/BS, and Aj.AR mean estimates in both genders. Finally, Mlt mean estimates increased from the youngest to the oldest age categories in both males and females (Appendix B).

3.4. Estimates of histomorphometric parameters per gender and race

Mean estimates with 95% CI from the meta-analysis using the fixed effects model for all histomorphometric parameters per gender and race

Table 2
Overall mean estimates for histomorphometric parameters from meta-analysis fixed effects model.

Parameter	N (n)	M (95% CI)
BV/TV, %	28 (2446)	20.49 (20.36, 20.62)
Tb.Th, μm	14 (1379)	139.32 (137.66, 140.99)
Tb.Sp, μm	14 (1378)	569.4 (561.8, 577)
Tb.N, /mm	13 (1357)	1.4 (1.4, 1.4)
OV/BV, %	19 (1801)	1.65 (1.6, 1.7)
O.Th, μm	20 (1749)	8.67 (8.55, 8.79)
OS/BS, %	22 (1877)	13.75 (13.45, 14.04)
Ob.S/BS, %	6 (580)	3.91 (3.75, 4.06)
ES/BS, %	19 (1588)	3.4 (3.32, 3.47)
Oc.S/BS, %	7 (577)	0.12 (0.1, 0.13)
MS/BS, %	9 (365)	7.57 (7.17, 7.97)
MAR, μm/d	14 (671)	0.57 (0.56, 0.57)
BFR/BS, $\mu m^3/\mu m^2/d$	7 (266)	0.048 (0.045, 0.051)
Aj.AR, μm/d	9 (533)	0.316 (0.302, 0.33)
Mlt, d	12 (615)	34.64 (34.39, 34.9)
Ac.F, /y	4 (237)	0.42 (0.38, 0.45)

BV/TV – bone volume/tissue volume; Tb.Th – trabecular thickness; Tb.Sp – trabecular separation; Tb.N – trabecular number; OV/BV – osteoid volume; O.Th – osteoid thickness; OS/BS – osteoid surface; Ob.S/BS – osteoblast surface; ES/BS – eroded surface; Oc.S/BS – osteoclast surface; MS/BS – mineralizing surface; MAR – mineral apposition rate; BFR/BS – bone formation rate; Aj.AR – adjusted apposition rate; Mlt – mineralization lag time; Ac.F – activation frequency; N – total number of studies; n – total number of subjects; y – year; M – Mean estimate as obtained by fixed effects model; CI – 95% Confidence Interval for mean estimate as obtained by fixed effects model.

Table 3
Overall estimates for histomorphometric parameters from meta-analysis random effects model.

Parameter	N (n)	M (95% CI)
BV/TV, %	28 (2446)	20.46 (19.55–21.36)
Tb.Th, μm	14 (1379)	143.36 (135.2–151.53)
Tb.Sp, μm	14 (1378)	639.1 (578–700.3)
Tb.N, /mm	13 (1357)	1.5 (1.3–1.6)
OV/BV, %	19 (1801)	2.22 (1.83–2.62)
O.Th, μm	20 (1749)	9.87 (8.83–10.91)
OS/BS, %	22 (1877)	14.64 (13.55–15.73)
Ob.S/BS, %	6 (580)	3.62 (2.04–5.2)
ES/BS, %	19 (1588)	4.3 (3.67–4.93)
Oc.S/BS, %	7 (577)	1.04 (0.6–1.49)
MS/BS, %	9 (365)	7.73 (6.81–8.65)
MAR, μm/d	14 (671)	0.6 (0.57–0.64)
BFR/BS, $\mu m^3/\mu m^2/d$	7 (266)	0.059 (0.044–0.075)
Aj.AR, μm/d	9 (533)	0.377 (0.31–0.444)
Mlt, d	12 (615)	27.29 (16.94–37.63)
Ac.F, /y	4 (237)	0.46 (0.37–0.55)

BV/TV – bone volume/tissue volume; Tb.Th – trabecular thickness; Tb.Sp – trabecular separation; Tb.N – trabecular number; OV/BV – osteoid volume; O·Th – osteoid thickness; OS/BS – osteoid surface; Ob.S/BS – osteoblast surface; ES/BS – eroded surface; Oc.S/BS – osteoclast surface; MS/BS – mineralizing surface; MAR – mineral apposition rate; BFR/BS – bone formation rate; Aj.AR – adjusted apposition rate; Mlt – mineralization lag time; Ac.F /y – activation frequency; N – total number of studies; n – total number of subjects; – year; M – Mean estimate as obtained by random effects model; CI – 95% Confidence Interval for mean estimate as obtained by random effects model.

are shown in Appendix C. The forest plots of gender differences are available in Appendix D (Figs. D.1 to D.15) and the forest plots of race differences are available in Appendix E (Figs. E.1 to E.16).

Gender had a significant effect on BV/TV, Tb.Sp, OV/BV, O.Th, OS/BS, MAR, BFR/BS, Aj.Ar, and Mlt mean estimates. Females presented lower mean estimates of BV/TV, Tb.Sp, OS/BS, MAR, BFR/BS, and Aj.Ar compared to males, whereas OV/BV, O.Th, and Mlt mean estimates were higher in females.

Race had a significant effect on BV/TV, Tb.Th, OV/BV, O.Th, MS/BS, MAR, BFR/BS, Aj.Ar, and Mlt mean estimates. Black individuals presented lower mean estimates of MS/BS, MAR, BFR/BS, and Aj.Ar compared to White individuals, whereas BV/TV, Tb.Th, OV/BV, O.Th, and Mlt mean estimates were higher in this category.

4. Discussion

The meta-analysis reported here is the largest study to date that describes primary and derived parameters of trabecular bone histomorphometry of normal or healthy adult individuals per gender, age categories, status from multinational cohorts. To the best of our knowledge, the present study is the first systematic review and meta-analysis that aimed to establish the validity of reference values of bone histomorphometric parameters to be applied in the diagnosis of different metabolic bone diseases.

Bone histomorphometry has been the gold-standard method for evaluating bone tissue in the scenario of metabolic bone diseases [1,2]. This technique has been used for many years to analyze the structure, composition, cells, and remodeling of bone tissue [37]. However, bone histomorphometric analysis is influenced by numerous technique factors, including sample quality, intra- and inter-observer variations, use of different protocols in preparing bone samples, and in vivo tetracycline-derivative labelling for analyzing bone formation and mineralization [46].

The selection of articles that comprise this meta-analysis sought to circumvent those factors, resulting in fewer than 10% of the potential studies described in the literature. Despite this judicious selection, studies of populations from all continents were included. In this meta-analysis, we used the nomenclature proposed by the Bone histomorphometry: standardization of nomenclature, symbols, and units. The report of the ASBMR Histomorphometry Nomenclature Committee [37] standardized the nomenclature of histomorphometry and improved the understanding of the analyzed parameters, as well as the communication among the different professionals involved in the study of metabolic bone diseases. The nomenclature of the histomorphometric parameters used in studies published before that report was adapted, avoiding losses of important information.

There are several bone histomorphometric parameters, some of which are of greater utility to clinicians than others. In this meta-analysis, the most relevant parameters in clinical practice were described. A wide variability of the number of parameters reported among the 37 selected studies [3–33,40–45] was observed, which could be explained by the evolution of the techniques of histomorphometric analysis throughout the years. In the 70s, that analysis was mostly performed with an optical grid manual system, and the measurement of all parameters was a time-consuming and complex process [47]. Later, with the advent of semi-automatic systems coupled into software, some histomorphometric parameters, such as Tb.Th, Tb.Sp and Tb.N, became easily analyzed [48]. For example, in the study of Courpron et al. [17], published in 1976, only BV/TV of 276 individuals had been measured. Moreover, the measurements of osteoblast and osteoclast surfaces (Ob.S/BS and Oc.S/BS, respectively), which depend on the specific staining techniques and the *post-mortem* viability of bone tissue to identify those cells, are scarcely reported [49]. It is worth mentioning that dynamic parameters were the least described, as most of the studies were carried out on *post-mortem* individuals, i.e., those in which in vivo tetracycline labelling is not feasible.

This study provided a comprehensive analysis of histomorphometric parameters broken down by sex over the different decades of life except for childhood and adolescence. These data allowed us to observe a marked decrease in structural parameters with ageing in both men and women. The trabecular volume (BV/TV) decreases by about 60% in men and 51% in women when compared to the first and last decades analyzed. It is well established that bone trabecular volume loss with age is due to the negative balance that occurs in each bone remodeling cycle, e.g., there is a deficit in the amount of bone replaced in relation to that removed.

In this meta-analysis, we also analyzed the histomorphometric parameters by race (White/Black). The values found and described in supplementary tables for both populations will certainly be helpful in studies that need to compare the population under evaluation with the reference values.

4.1. Strength and limitations

The importance of this study lies in the estimation of universal reference standards of commonly used static and dynamic histomorphometric parameters, allowing clinicians and researchers to directly compare the results of their patients with average values of populations of similar age. Another strength of this work is that it complied with the recommendations of the ASBMR [37] regarding the terminology and calculation method of each parameter and its measurement unit. In addition, this work allowed, through histomorphometric means, to corroborate the already supposed mechanisms of physiological loss of bone mass in humans.

This study had several limitations. One was the absence of reporting on the laboratory parameters, which variability could influence the histomorphometric parameters. An example of this is vitamin D, which levels vary with seasonality and latitude and could impact osteoid parameters, as described by Priemel et al. [34]. In fact, overall mineral metabolism, especially balanced calcium homeostasis, is of paramount importance not only for normal osteoid values but also for bone health. Another limitation was the non-inclusion of the values for the cortical bone because they were rarely reported. Other derivative measurements were not included either, which could elucidate further aspects of bone physiology. For example, parameters such as wall thickness and erosion depth could give more information at the basic multicellular unit (BMU) level. Furthermore, most of the bone biopsies performed in the studies included in this meta-analysis were carried out in *post-mortem* individuals, which hinders a better knowledge regarding the lack of cellular (osteoblasts and osteoclasts) parameters and dynamic parameters. These issues limited the reported number of those parameters in each study. Other limitation of the study that one could assume is the different bone histomorphometry techniques used in the included studies. However, Malluche et al. [22] have compared the two histomorphometric methods, i.e. grid and semiautomatic techniques, and found an excellent accuracy between them. Regarding bone biopsy processing, the variability of bone samples could also be a limitation. Despite that, most of the included studies reported all the fixation, embedding and staining process.

Finally, from an individual study perspective, the normality of the data can be assessed, and different summary methods can be used depending on the data distribution. In this work, we performed a meta-analysis of several studies, making the mean the only relevant summary measure we could estimate. In the context of a systematic review with an associated meta-analysis, it is impossible to provide ranges because we need access to subject-level data for all studies considered. As supplemental material, we report an extensive set of tables and figures with all the individual studies considered for each parameter, including the values reported in each study, the weight they provided for each estimate and how they compare to other studies in terms of variability. So, we provide the reader with an accurate estimation of the mean value that can be used to assess the normality of a specific measurement and a detailed set of tables that the reader can refer to identify particular studies that may be closer to the population they are working with, and that may individually provide median and ranges (although the large majority of the studies we considered report only mean and standard deviation). Additionally, we provide some relevant information, such as how, on average, the parameters vary with age, gender, and race considering data from multiple studies.

In conclusion, based on 37 studies, this meta-analysis provided overall mean estimates and 95% CI for values of histomorphometric parameters in healthy and normal subjects. Therefore, we provided reference standards that may be used by clinicians when evaluating bone biopsies in patients, enabling the direct comparison of their histomorphometric values with the suitable reference group regarding gender and age range. These reference standards may ultimately improve the diagnosis of metabolic bone disease and the guidance of therapeutic decisions.

CRediT authorship contribution statement

Aníbal Ferreira: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Luciene Machado dos Reis:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David Manteigas:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Aluizio Barbosa Carvalho:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vanda Jorgetti:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Aníbal Ferreira reports statistical analysis and writing assistance were provided by Center for Research and Development in the Nephrology Area (NIDAN). The remaining authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendices. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2024.117309.

Data availability

Data will be made available on request.

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