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Sex estimation with the total area of the proximal femur: A densitometric approach

Francisco Curate[a,b,c,*], Anabela Albuquerque[d], Izilda Ferreira[d], Eugénia Cunha[b,e]

[a] Research Centre for Anthropology and Health, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[b] Laboratory of Forensic Anthropology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[c] Interdisciplinary Center for Archaeology and Evolution of Human Behavior, University of Algarve, Faro, Portugal
[d] The Coimbra Hospital and University Centre, Coimbra, Portugal
[e] Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

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ABSTRACT

The estimation of sex is a central step to establish the biological profile of an anonymous skeletal individual. Imaging techniques, including bone densitometry, have been used to evaluate sex in remains incompletely skeletonized. In this paper, we present a technique for sex estimation using the total area (TA) of the proximal femur, a two-dimensional areal measurement determined through densitometry. TA was acquired from a training sample (112 females; 112 males) from the Coimbra Identified Skeletal Collection (University of Coimbra, Portugal). Logistic regression (LR), linear discriminant analysis (LDA), reduce error pruning trees (REPTree), and classification and regression trees (CART) were employed in order to obtain models that could predict sex in unidentified skeletal remains. Under cross-validation, the proposed models correctly estimated sex in 90.2–92.0% of cases (bias ranging from 1.8% to 4.5%). The models were evaluated in an independent test sample (30 females; 30 males) from the 21st Century Identified Skeletal Collection (University of Coimbra, Portugal), with a sex allocation accuracy ranging from 90.0% to 91.7% (bias from 3.3% to 10.0%). Overall, data mining classifiers, especially the REPTree, performed better than the traditional classifiers (LR and LDA), maximizing overall accuracy and minimizing bias. This study emphasizes the significant value of bone densitometry to estimate sex in cadaveric remains in diverse states of preservation and completeness, even human remains with soft tissues.

1. Introduction

The assessment of biological sex constitutes a focal research demand in the forensic examination of human skeletal remains, with additional parameters of the biological profile (*e.g.*, stature or age) typically estimated as sex-specific [1,2]. Superlative approaches for the sexual estimation of unknown skeletal individuals usually depend on the recovery and analysis of well-preserved pelvic bones [1–3]. Likewise, the cranium and long bones have been employed to accurately assess sex in human skeletal remains [3–6]. The femur is the longest and, as a rule, the strongest skeletal element, being commonly recovered in both forensic and archeological contexts [5]. As such, it is not surprising that, alongside the cranium and pelvis, the femur has received most of the attention in studies of sexual dimorphism, with several dimensions of the femur employed for the prediction of sex in skeletal remains [4,6–10].

In forensic settings, sex estimation is usually performed in fully skeletonized bodies with the support of standard osteometric techniques, but periodically forensic identification of unknown individuals requires the study of incomplete, partially fleshed or charred remains [11,12]. Medical imaging techniques can be used to observe remains not completely skeletonized in which skeletal preparation (*e.g.*, maceration) is impractical, or even unreasonable from a social or cultural standpoint. Accordingly, imaging techniques, such as computer tomography or projectional radiography, have been extensively used to address the estimation of sex in cranial and postcranial bones [12–18], including the femur [11,19,20].

Dual X-ray absorptiometry (DXA), or bone densitometry, is an application of low energy projectional radiography, generally

* Corresponding author.
E-mail address: fcurate@uc.pt (F. Curate).

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recognized as the gold-standard technique to evaluate bone mineral density (BMD) and diagnose osteoporosis [21,22]. Given that DXA is a two-dimensional scan, real bone density cannot be determined; instead, bone mineral content (BMC, in grams) in a given projected area (in cm^2) is measured. Areal BMD is thus determined by dividing the BMC by area. DXA has been infrequently applied in the forensic sciences, although it can be exploited to estimate sex, age at death and ancestry [10,23–26]. Some advantages of DXA application in the forensic sciences are summarized by Wheatley [23].

The main purpose of this study is to generate and test models for the prediction of sex based on the total area of the proximal femur, a two-dimensional areal measurement performed with DXA. Also, the performance of classical classifiers, such as logistic regression and Fisher's linear discriminant analysis, which have been extensively used for classification of problems where the dependent variable is dichotomous, is compared with that of classification and regression trees and reduce error pruning trees, which are non-parametric decision tree learning techniques.

2. Materials and methods

The samples used in this study were obtained from two Portuguese Identified Skeletal Collections [27,28]. A training set from the Coimbra Identified Skeletal Collection (CISC, University of Coimbra, Portugal), comprising 224 individuals (112 females and 112 males), was used to fit the models for sex estimation. Individual ages at death ranged from 20 to 96 years. Dates of death spanned from 1910 to 1936. A second sample, from the 21st Century Identified Skeletal Collection (ISC/XXI, University of Coimbra, Portugal), included 60 individuals (30 females and 30 males) and was employed to test the predictive value of the models generated in the CISC sample: this is the testing, or holdout, sample. All individuals died between 1995 and 2001. Age at death ranged from 33 to 97 years old. Only individuals with at least one femur showing no macroscopical signs of post-depositional change and lacking significant pathological modifications were included in the samples.

In the domain of densitometry, the proximal femur has been partitioned into distinctive regions of interest. The total area (TA, cm^2) of the proximal femur (also known in the medical literature as total area of the hip) is the sum of three individual areas: femoral neck, trochanteric region, and intertrochanteric/proximal diaphysis regions (Fig. 1) [21,22]. A femur from each individual (as a rule, the bone from the left side) was scanned with a Hologic QDR-4500A densitometer (Hologic, Inc., Bedford, MA) at the Nuclear Medicine Unit (Coimbra Hospital and University Centre, Portugal) and the computer produced the above designated semi-automated regions of interest (if required the technologist made minor adjustments) and the area (cm^2) for each region is calculated. Subsequently TA was automatically determined by the densitometer's software (Fig. 2). Femora were placed in anteroposterior position; with the femoral neck parallel to the plane of the scanner; in a low-density cardboard container with 10 cm depth of dry rice acting as a surrogate for soft tissue (soft tissues and bone marrow slightly influence the reading of bone mineral content but not TA). Fifty femora were scanned in two different days to check repeatability of the DXA measurements. The magnitude of the intraobserver error was assessed with the relative technical error

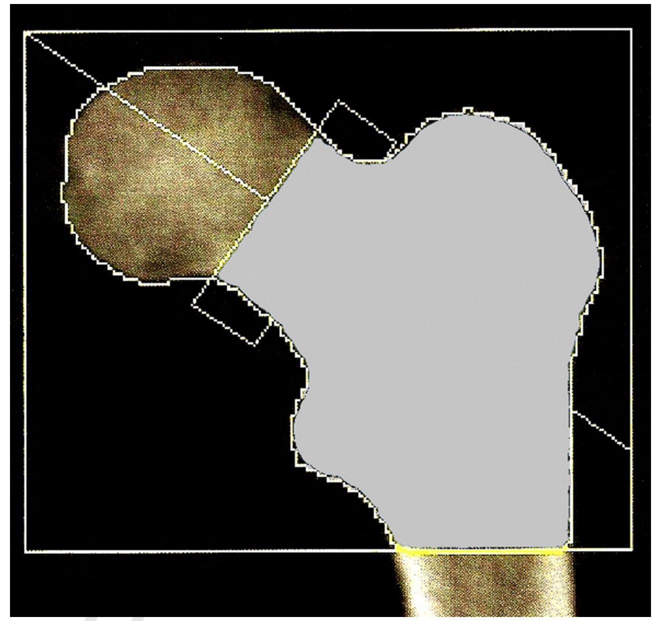

Fig. 1. The total area (cm^2) of the proximal femur (gray color).

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T - score	PR (%)	Z - score	AM (%)
Neck	**4.98**	**2.53**	**0.508**	**-3.1**	**60**	**-0.6**	**88**
Troch	12.48	5.90	0.473	-2.3	67	-0.4	92
Inter	25.77	16.15	0.627	-3.1	57	-1.1	79
Total	43.24	24.58	0.569	-3.1	60	-0.8	85
Ward's	1.12	0.38	0.343	-3.3	47	-0.2	93

Total BMD CV 1.0%

WHO Classification: Osteoporosis

Fracture Risk: High

Fig. 2. Results summary for a DXA scanning (CISC, female, 80 years old). In this example, TA is 43.24 which is the sum of three different areas: neck, trochanteric and intertrochanteric.

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Table 1
Descriptive statistics for TA (cm^2) in both sexes; Coimbra Identified Skeletal Collection (CISC), 21st Century Identified Skeletal Collection (ISC/XXI) and pooled samples.

	♀				♂				
	Mean	SD	95% CI	N	Mean	SD	95% CI	N	Sectioning point
CISC	33.53	3.31	32.91–34.15	112	43.56	3.85	42.84–44.28	112	38.55
ISC/XXI	32.83	3.20	31.63–34.02	30	42.47	3.20	41.28–43.67	30	37.64
Pooled	33.38	3.30	32.83–33.93	142	43.33	3.74	42.70–43.95	142	38.35

SD: standard deviation; 95% CI: 95% confidence interval.

of measurement (rTEM) [29] and it was very low (rTEM = 0.42), suggesting that the positioning of the femur was performed appropriately. Physiological length of the femur was obtained following Martin [30].

Descriptive statistics are presented as group means, standard deviation (SD) and 95% confidence intervals (95% CI) for the mean. Normality of the data was assessed through skewness and kurtosis, and homoscedasticity with a Levene's test [31]. A t-test (independent samples) was used to evaluate the null hypothesis that TA mean in males and females was equal. To assess sexual dimorphism, the ensuing indicator was employed [32]:

$$SD = \frac{\bar{x}_m - \bar{x}_f}{\bar{x}_m} \times 100,$$

where $\bar{x}_m$ and $\bar{x}_f$ are the mean TA values for males and females, respectively.

The models for the mathematical prediction of sex were generated through linear discriminant analysis (LDA), logistic regression (LR), classification and regression trees (CART), and reduce error pruning trees (REPTree). LDA is the oldest classifier still in use and is founded upon the notion of identifying a linear combination of predictor variables that optimally separates mutually exclusive groups. Discriminant analysis then creates a discriminant function that parsimoniously epitomizes the differences between groups and classifies new individuals with unknown group membership [33]. Logistic regression is a non-parametric statistical modeling approach that can be used to describe the relationship of one or more independent variables to a dichotomous dependent variable [34]. Classification and regression trees are binary recursive classifiers that generate hierarchical decision trees by partitioning data among classes of the criterion at a given node, resulting from an "if/then" rule directed to a set of predictors [35,36]. Reduce error pruning trees is the simplest method in decision tree pruning and is founded on the principle of computing the information gain with entropy and minimizing the error that ensues from variance [36,37]. For general reviews of LDA, LR, CART and REPTree see, for example, Maroco et al. [33], Hosmer et al. [34], Wu et al. [35], and Gupta et al. [36]. In order to avoid overfitting and to insure that the results are generalizable to an independent data set, a 10-fold cross-validation approach was followed to train the classifiers.

The performance of the provisional and cross-validated models – as well as the discriminative power of the models in the testing dataset – was evaluated through overall accuracy (a measure of agreement between the documented and the predicted sex), sensitivity (the proportion of males that were correctly recognized), specificity (the proportion of females that were properly predicted), Cohen's Kappa (also a measure of total agreement but adjusting for those that occur by chance alone) and Area Under the Receiver Operating Characteristic Curve (AUC).

All analyses were performed with R programming language [38,39] and Waikato Environment for Knowledge Analysis [40].

3. Results

Descriptive statistics for the Coimbra Identified Skeletal Collection and the 21st Century Identified Skeletal Collection samples are summarized in Table 1. The total area of the proximal femur is statistically different between sexes both in the training (t: −20.907; df = 222; $p < 0.001$) and the testing samples (t: −11.666; df = 58; $p < 0.001$). Kernel density plots show the distribution of TA values per sex (Figs. 3 and 4). TA is 23.0% and 21.0% larger in males in the CISC and ISC/XXI samples, respectively. The total area of the proximal femur is moderately to strongly correlated with femoral physiological length in both samples and sexes (CISC: Pearson's $TA^*FPL_{females}$: 0.578; $p < 0.001$/Pearson's TA^*FPL_{males}: 0.559; $p < 0.001$ | ISC/XXI: Pearson's $TA^*FPL_{females}$: 0.725; $p < 0.001$/Pearson's TA^*FPL_{males}: 0.537; $p < 0.001$) but it is not correlated with age at death (CISC: Pearson's $TA^*age_{females}$: 0.170; p = 0.073/Pearson's TA^*age_{males}: 0.116; p = 0.222 | ISC/XXI: Pearson's $TA^*age_{females}$: −0.195; p = 0.303/Pearson's TA^*age_{males}: 0.253; p = 0.177).

The logistic regression model is summarized in Table 2. It is defined by the ensuing equation (females classified with negative values, males classified with positive values):

$$Sex = 0.800 * TA - 30.498$$

The sex was correctly predicted in 92.0% of all individuals (sensitivity: 91.1%; specificity: 92.9%), with a significant discriminant capability in both the provisional and cross-validation models. In the holdout sample (ISC/XXI), sex was accurately estimated in 91.7% of the cases. The model appropriately identified 96.7% of females and 86.7% of males (Table 3).

Box's M was used to test the equality of the variance–covariance matrices (Box's M: 2.467; p = 0.117). Linear discriminant analysis produced a single discriminant function with a cutoff point equal to zero (scores above zero classified as males and below zero as females):

$$Sex = 0.279 * TA - 10.738$$

In both the provisional and cross-validation models, sex was correctly estimated in 90.6% of individuals (sensitivity: 88.4%; specificity: 92.9%). In the testing sample, sex was correctly assessed in 91.7% of the individuals (sensitivity: 86.7%; specificity: 96.7%; Table 3).

The CART decision tree is utterly simple and straightforward, and provided a sectioning point of 37.31, in which $TA < 37.31$ = FEMALE, and $TA \geq 37.31$ = MALE. The decision rule correctly classified 93.3% of all individuals in the provisional model, with a sensitivity of 95.5% and a specificity of 91.1%. In the cross-validated model, overall accuracy was 90.2% (sensitivity: 92.0%; specificity: 88.4%). In the testing sample, overall accuracy reached 90.0%, with 93.3% males and 86.7% females correctly assigned (Table 3).

The reduced error pruning tree classifier provided a sectioning point of 37.77, in which $TA < 37.77$ = FEMALE, and $TA \geq 37.77$ = MALE. Overall accuracy was 92.9% (with the same sensitivity and

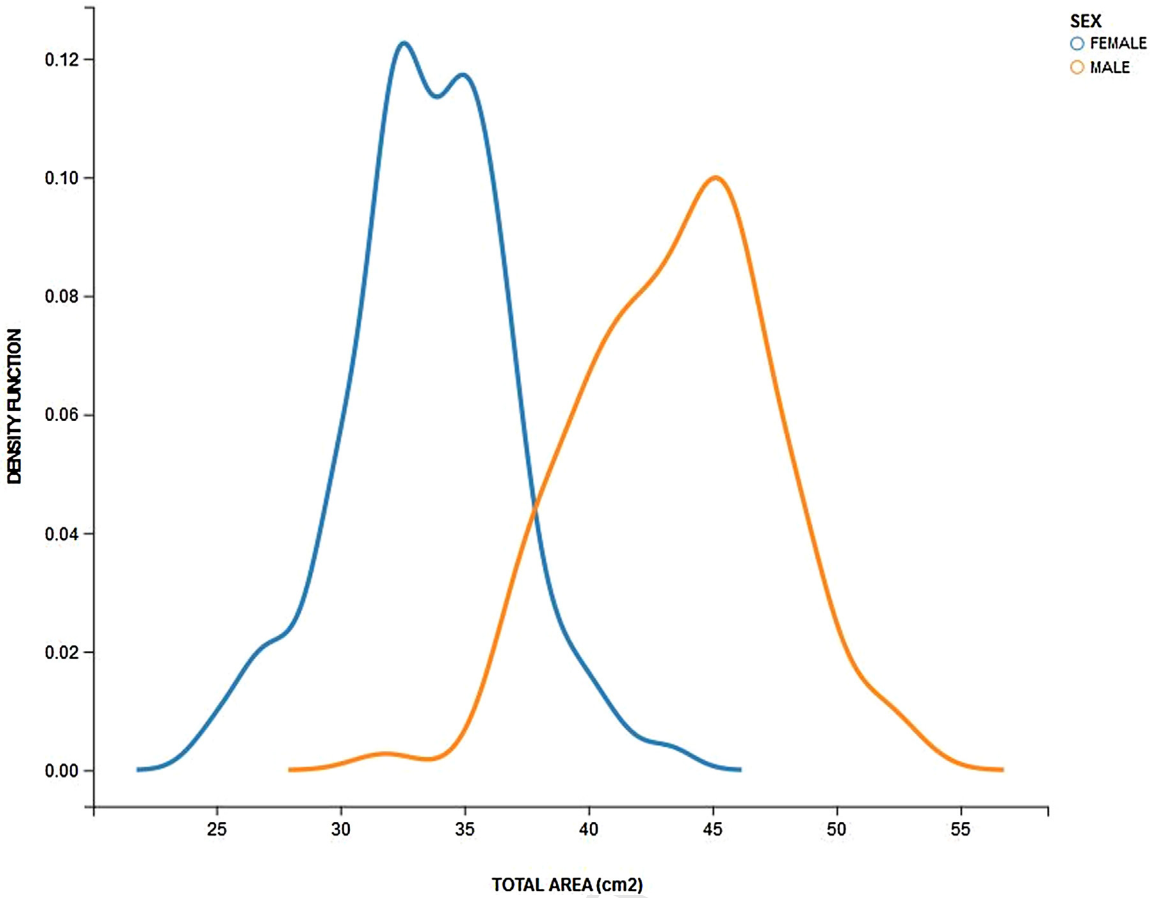

Fig. 3. Kernel density distribution of TA (cm^2) by sex (CISC sample).

specificity) in the provisional model, and 90.6% (sensitivity: 92.0%; specificity: 89.3%) in the cross-validated model. In the ISC/XXI holdout sample, 91.1% of all individuals were correctly classified, with 90.0% females and 93.3% males properly allocated (Table 3).

4. Discussion

Sexual dimorphism in the human skeleton has been classically investigated in the pelvis, cranium and long bones. In cases of commingled, scattered, fractional and/or fragmented human skeletal remains, the pelvis is not always available for forensic analysis. As such, other dimorphic skeletal elements – including the femur [2,4,6] – are widely used in sex determination. Research in forensic anthropology typically involves the analysis of cadaveric remains in different states of preservation and completeness, including human remains with or without soft tissues. Imaging approaches for the assessment of features related with the biological profile should be preferred in cases when skeletal preparation is socially offensive or simply not viable [6,11,12,41]. In such cases, DXA is a suitable technique to estimate sex [10,23,24], and purportedly age at death and ancestry [10,24–26]—even in the case of recovery of a single femur.

The observed sexual dimorphism of the total area of the proximal femur in both the training (CISC) and testing samples (ISC/XXI), as assessed through DXA, was in agreement with the results established in epidemiological studies [42,43]. TA exhibits a slight variation with ancestry; notwithstanding, differences between sexes are large (*circa* 10 cm^2) and consistent within any population (>20% variation between sexes) [42]. Sexual differences in bone size are established early in life, possibly even *in utero*, but are more noticeable after puberty [44,45]. For example, periosteal growth, which expands bone diameter, accelerates during puberty in males; while earlier completion of longitudinal growth and inhibition of periosteal apposition produces smaller bones in females [45,46]. Bone growth and size is influenced by genetic and hormonal factors, mechanical loading and nutrition, among others, and it is probable that the ensuing effect on bone size may be sex-specific [46–49]. The structural phenotype of the proximal femur, in particular, shows high heritability [48,50], also conforming to Wolff's law and Harold Frost's mechanostat model [51,52]. The moderate to strong association of TA with femoral physiological length suggests that sex dimorphism in the

Table 2
Logistic regression model fitting for the training sample (CISC).

	Variable	β	SE	Wald	Sig.	Exp (β)	95% CI for Exp (β)
Training sample (CISC)	TA	0.800	0.118	45.662	<0.001	2.225	1.764–2.805
	Constant	−30.497	4.488	46.185	<0.001	0.000	

TA: total area (cm^2); β: the coefficient for the constant in the null model; SE: standard error; Wald: Wald chi-square test; Exp (β): exponentiation of the β coefficient.

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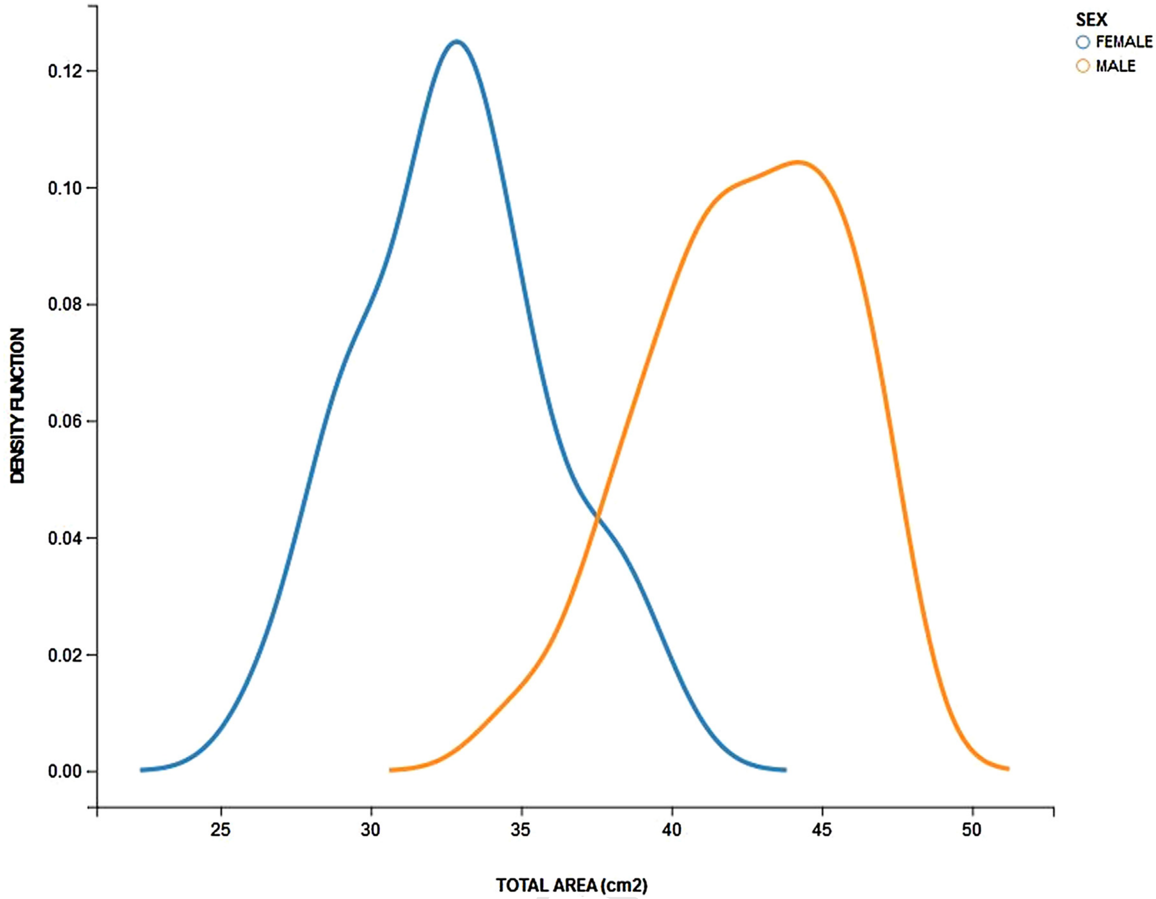

Fig. 4. Kernel density distribution of TA (cm^2) by sex (ISC/XXI sample).

expression of TA has a size effect component. BMD declines during aging in all populations, particularly in females [25], but bone area tends to remain constant or increase marginally with age in adults [42]. Even in the latter case, area increases much less than the degree of sexual dimorphism. In the observed samples, TA was not associated with age at death.

Sex assessment with the total area of the proximal femur in human skeletal remains shows high overall accuracy in the cross-validated models (always exceeding 90%), with an effective performance, independently of the classifier used to create the classification models. The allocation accuracy in a holdout sample not used to develop the models was also very high, suggesting that the results are generalizable to independent datasets. Notwithstanding, classification bias (the difference between properly classified females and males) with the traditional classifiers (LR and LDA, with 13.3% of misclassified females and only 3.3%

Table 3
Classification accuracy with the different classifiers.

	Overall accuracy (%)	Sensitivity (%)	Specificity (%)	Kappa	AUC
LR					
Training set	92.0	91.1	92.9	0.839	0.977
Cross-validation	92.0	91.1	92.9	0.839	0.975
Testing set	91.7	86.7	96.7	0.833	0.979
LDA					
Training set	90.6	88.4	92.9	0.813	0.977
Cross-validation	90.6	88.4	92.9	0.813	0.977
Testing set	91.7	86.7	96.7	0.833	0.979
CART					
Training set	93.3	95.5	91.1	0.866	0.933
Cross-validation	90.2	92.0	88.4	0.804	0.909
Testing set	90.0	93.3	86.7	0.800	0.900
REPTree					
Training set	92.9	92.9	92.9	0.857	0.929
Cross-validation	90.6	92.0	89.3	0.813	0.918
Testing set	91.7	93.3	90.0	0.833	0.917

LR: logistic regression; LDA: linear discriminant analysis; CART: classification and regression trees; REPTree: reduce error pruning trees; AUC: area under the receiver operating characteristic curve.

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misclassified males) and the CART algorithm (6.7% misclassified females and 13.3% misclassified males) was problematic in the testing sample.

Sex specific accuracy is probably related with secular change in bone dimensions [53,54], usually inducing a higher proportion of misclassified females when a model fitted in a chronologically older sample is used to estimate sex. The training sample (CISC) is, on average, composed by individuals that were born much earlier than individuals in the testing sample (ISC/XXI) – with other relevant differences between samples, including socioeconomic status and mortality pattern – but the magnitude of sexual dimorphism in the total area of the proximal femur is very similar in both samples. This is also relevant for the assessment of this method in samples of non-Portuguese origin. Besides the problem of secular change, the selection of the statistical model also seems critical to lower error rate and bias [33,55]. In fact, the decision rule provided by the REPTree classifier maximized the overall accuracy while improving bias: misclassification difference between sexes in the holdout sample was lower than the recommended 5% threshold [12].

Classical statistical techniques, such as LR and LDA, have been widely used to assess sex in forensic contexts [1,6–15,18,19,32,56,57], but the promising performance of data mining methods, with classifiers like support vector machines, random forests or classification trees, has led to a recent research appeal in their application to classification problems in forensic anthropology [6,53,55,58–60]. Results are conflicting about classification accuracy of data mining classifiers as compared to traditional methods [*e.g.*,Refs. 53,58] with the classifiers' performance affected by the different arrangements of predictors, data assumptions, parameters' tuning and sample sizes [33]. In general, our results show that both traditional and decision tree learning techniques perform very well under cross-validation but, except for the REPTree algorithm, the models display unbalanced classification efficiency in the testing sample.

Overall correct classification in this study is comparable to other seemingly highly accurate methods, including techniques using the pelvic region [8,61,62], the cranium [58,63], and different long bones [1,8,9,11,23,59]. The high overall accuracy and low bias obtained in the testing sample with the REPTree model is particularly relevant, since for many published models only resubstitution and cross-validation accuracy rates are reported [32]. Overfitting is often a consequence in the first case, while cross-validation usually estimates well only the likely prediction error [64]. As such, a more valuable approach to assess the generalization error of a classificatory model is to use an independent dataset.

5. Conclusions

The new models for the estimation of sex based on the total area of the proximal femur, a measurement performed with DXA, display great accuracy both in cross-validation and in an independent sample. The model based in a fast decision tree learning algorithm (REPTree) reduces bias in the holdout sample to appropriate levels. The proposed models should endure additional validation in independent skeletal remains (particularly of non-Portuguese origin) to substantiate their reliability in forensic and/or bioarcheological contexts.

Conflict of interest

The authors declare that they have no conflict of interest.

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