Noninvasive Estimation of Bone Mass in Ancient Vertebrae

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KEY WORDS osteopenia; DEXA; bone density; bone histomorphometry; prehistoric Canary Islands; osteoporosis

ABSTRACT Histomorphometry is useful in the assessment of trabecular bone mass (TBM), and thus, in the estimation of the prevalence and intensity of osteopenia in ancient population groups. However, it is a destructive method. It is therefore necessary to explore the accuracy of nondestructive approaches, such as radiography, bone mineral density (BMD) assessed by double-energy X-ray absorptiometry (DEXA), bone density (BD), or optical density (OD) in the diagnosis of osteopenia. We selected 51 vertebrae out of a total sample composed of 333 T12, L1, and L2 vertebrae belonging to adult pre-Hispanic inhabitants from El Hierro. These vertebrae underwent histomorphometrical analysis, a fine-grained film radiography with assessment of trabecular pattern following standard methods, OD, DEXA-assessed BMD, and BD. The presence of biconcave vertebrae and wedge-shaped vertebrae was also assessed by measuring anterior height (a), posterior height (p), and height at the middle point of the vertebral body (m), and further calculating the indices 2m/(a + p) ("spine score") and a/p. Significant correlations were observed between TBM and BMD ($r = 0.43$), TBM and BD ($r = 0.49$), TBM and OD ($r = 0.52$), BMD and OD ($r = 0.51$), and BMD and BD ($r = 0.36$), but not between TBM and the indices 2m/(a + p) and a/p. In the stepwise multiple correlation analysis between TBM and BMD, BD, and OD, OD entered into first place and BD into second place, whereas BMD became displaced; the multiple correlation coefficient was 0.63, with a standard error of 3.78. A BMD greater than 0.60 g/cm^2, or a bone density greater than 0.60 g/cm^3, excluded osteopenia (TBM <15%) with a specificity greater than 90%, whereas a BMD value less than 0.35 g/cm^2, a BD less than 0.35 g/cm^3, or optical density >1.6 excluded a normal bone mass (TBM >20%) with a specificity greater than 90%. Based on radiographic criteria on the total sample, we also conclude that the overall prevalence of vertebral fractures in the adult pre-Hispanic population of El Hierro of any age is 7.5%. Am J Phys Anthropol 125:121–131, 2004. © 2004 Wiley-Liss, Inc.

Osteoporosis is a systemic skeletal condition characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (Levis and Altman, 1998). Currently, the term *osteopenia* denotes decreased bone mass, whereas the term *osteoporosis* requires the presence of bone fractures due to bone fragility. These fractures may affect the femoral neck, the distal forearm, and more frequently, the vertebral bodies (Cummings et al., 1989), leading to the typical wedge-shaped vertebrae, biconcave or "fish vertebrae," and flattened or "pancake" vertebrae.

The assessment of bone mass in ancient skeletal remains is of interest, since it may indicate nutritional stress. For any individual, the observed bone mass is a combination of peak bone mass and any subsequent bone loss. During the first three decades of life bone synthesis predominates, so bone mass progressively increases until it peaks towards the second half of the third decade (Krane and Holick, 1994). Genetics (Johnston and Slemenda, 1995; Garabedian, 1995), physical activity (especially weight-bearing exercise; Smith and Gilligan, 1991; Recker et al., 1992), diet (Eriksen and Langdahl, 1997), and other factors, such as delayed menarche (Anai et al., 2001) and altered reproductive hormone concentrations (Sowers, 2000), influence peak bone mass. After the age at which peak bone mass is achieved, bone synthesis and bone resorption are nearly in equilibrium, so bone mass declines gradually, at a rate of less than 1%/year, except in women during

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Received 9 May 2002; accepted 21 July 2003.

DOI 10.1002/ajpa.10374
Published online 12 January 2004 in Wiley InterScience (www.interscience.wiley.com).

the first 5–10 years after menopause, in whom bone loss is accelerated and bone mass decreases. Besides advanced age, several conditions, such as diabetes, hyperthyroidism, hypogonadism, hyperparathyroidism, Cushing syndrome, chronic alcohol consumption, and chronic pulmonary disease, among many others (Krane and Holick, 1994), may lead to reduced bone mass, but the overall prevalence of all these clinical entities in a nonselected "normal" population is low. Therefore, the finding of a high prevalence of osteopenia in a given population raises the possibility of protein-calorie malnutrition as the underlying cause. Indeed, it is well-known that either protein (Stewart, 1975) or protein-calorie malnutrition (Platt and Stewart, 1962) adversely affects bone development and bone mass. In these situations, bone synthesis is decreased (Bourrin et al., 2000b), and although bone resorption is also decreased (Bourrin et al., 2000a), an imbalance between synthesis and resorption ensues, leading to bone loss. This "nutritional hypothesis" has been widely used to explain the finding of a high proportion of osteopenia, both in ancient (Agarwal and Grynpas, 1996; Martin et al., 1985; Eaton and Nelson, 1991) and modern (Gupta, 1996) population samples, and several experimental data and observational studies (Ponzer et al., 1999; Schurch et al., 1998; Molina-Pérez et al., 2000) support this hypothesis.

The island El Hierro, the smallest (273 km^2) of the seven "big" islands of the Canary Archipelago, was inhabited, in pre-Hispanic times, by people of North African origin whose subsistence was based on goat- and sheep-herding and consumption of marine products (Jiménez Gómez, 1993). Previous studies performed on a small sample of iliac bones (González-Reimers et al., 1988) yielded a relatively low prevalence of osteopenia (12.5%), a figure quite lower than the 29% prevalence observed on a sample composed of 69 pre-Hispanic individuals from Gran Canaria (González-Reimers and Arnay-de-la-Rosa, 1992). This difference was found to be even more striking when trabecular bone mass (TBM) was determined on tibiae. Only one case from El Hierro (2.44%) showed a TBM value in the osteopenic range (below 15%), in contrast with a prevalence of osteopenia of 30.40% among the population of Gran Canaria (Velasco-Vázquez et al., 1999). However, it is well-known that tibial bone mass is strongly affected by weight-bearing exercise. Therefore, the higher tibial TBM values observed among the pre-Hispanic population from El Hierro compared to that of Gran Canaria may be due in part to differences in lifestyle. In a preliminary analysis, severe osteoarthritis was common in the population from El Hierro (Mas Pascual et al., 2000), a fact which could be related to mechanical overload. In any case, the low prevalence of osteopenia observed when iliac crest specimens were analyzed does not support the hypothesis that the population from El Hierro suffered malnutrition, or at least, not as intensely as did the Gran Canaria population.

Thus, the assessment of the prevalence of osteopenia and/or osteoporosis in ancient population groups may provide useful information about skeletal health that reflects economic activities and lifestyle. There are several methods available to determine bone mass. Some of them, such as bone histomorphometry, are destructive and require a specialized laboratory. Currently, the most widely employed method in the assessment of bone mineral density is double-energy X-ray absorptiometry (DEXA) (Larcos and Wahner, 1991; Wahner, 1989; Levis and Altman, 1998), which is accurate, noninvasive, and relatively inexpensive. However, the suitability of DEXA for the evaluation of bone mineral density (BMD) of ancient samples is unclear (Lees et al., 1993; Bennike and Bohr, 1990; Bennike et al., 1993; Hammerl et al., 1991).

Few studies have compared DEXA and other noninvasive procedures with histomorphometry in the analysis of ancient bones. Kneissel et al. (1994) found a poor relationship between DEXA and histomorphometrical analysis performed in the vertebrae and femoral necks of 18 individuals. They attributed these results to the diagenetic changes suffered by the bones, with deposition of mineral salts leading to a denser mineral phase, which would strongly influence X-ray-based density measurements. Histomorphometry would be the appropiate method for the assessment of bone mass for such samples, since diagenetic changes rarely alter the form of trabeculae. Farquharson et al. (1997) found correlation coefficients between BMD assessed by DEXA and bone density of 0.64 for the femur (30 cases) and 0.74 for the fourth lumbar vertebra (25 cases). In a recent study on 95 tibiae, we concluded that DEXA may serve to estimate the intensity of bone loss in a given ancient population, although it is useless for establishing the prevalence of osteopenia, since diagnostic values obtained in modern controls are not applicable to ancient samples (González-Reimers et al., 2002), probably due to the lack of soft tissue and the distorting effect of the air entrapped within the cancellous bone.

Vertebrae are rich in cancellous bone, and may accurately reflect changes in bone mass due to metabolic disturbances. In contrast with hip and forearm fractures, a vertebral fracture is a continuous, insidious process, slowly leading to deformation of vertebral shape. Most epidemiological studies on osteoporosis are based on assessment of the prevalence and incidence of vertebral fractures (Nevitt et al., 1998; Papaioannou et al., 2002; Díaz López et al., 2000; Hasserius et al., 2001; Jackson et al., 2000). However, being a continuous process, it is often difficult to precisely define the degree of vertebral deformity required to classify such a deformity as a vertebral fracture. Bone loss in vertebrae occurs progressively, and is characterized by changes in the radiolucency of the bone, in trabecular pattern, and ultimately in the shape of vertebral bodies. As the rarefaction of transverse trabeculae ensues, it is accompanied by a relative accentuation of the vertical ones, leading to a picture in which sparse, coarse vertical trabeculae and marked cortices are prominent features. It is possible to quantify the intensity of radiolucency with the aid of optical densitometry, and changes in trabecular pattern are easily visible on a radiography. Finally, progressive bone loss is also reflected in bone density, which decreases with time.

All these radiographic changes suggest that analyses of vertebrae with nondestructive radiologic and densitometric methods should yield accurate estimations of the intensity of osteopenia in ancient skeletal remains. This information would help anthropologists interpret economic activities and lifestyles of past populations, as reflected in their skeletal biology. The main objective of the present study is to assess which method (DEXA, optical densitometry, bone density, or trabecular pattern) shows a closer correlation with histomorphometrically determined TBM. In order to fulfill this objective, we selected 51 out of 333 T12, L1, and L2 vertebrae belonging to pre-Hispanic inhabitants from El Hierro. Another objective of this study is to assess the proportion of fractured vertebrae (among the whole sample of vertebrae), based on radiologic criteria. Since, regardless of preexisting osteopenia, vertebral collapse or fracture may even incresase bone density, we also analyzed the ability of histomorphometry, DEXA, or bone density to distinguish between fractured and nonfractured vertebrae.

MATERIALS AND METHODS

Samples

The study was performed on vertebrae belonging to adult pre-Hispanic inhabitants from El Hierro, most of them recovered from the collective burial cave Punta Azul. Punta Azul is the most important pre-Hispanic funerary complex from El Hierro, containing commingled remains of more than 100 individuals. These individuals were not interred, but deposited on the lava floor of a natural volcanic cave, so preservation (aided by the subdesert climatic conditions of the island) was, in general, excellent. Any vertebrae with large cortical disruptions were excluded from the study sample.

Due to the time and expense required to perform histomorphometry on each vertebra (which is, in addition, a destructive procedure), we chose only 19 out of 151 L1 (131 from Punta Azul), 16 out of 91 L2 (79 from Punta Azul), and 16 out of 91 T12 (87 from Punta Azul) vertebrae belonging to adult individuals available from the pre-Hispanic population from El Hierro. Previously, a radiographic analysis was performed on all the vertebrae. We selected the 51 vertebrae destined to fulfill the main objective of this study on the basis of their gross radiological appearance. In order to ensure the inclusion of both severely osteopenic and normal vertebrae, with a wide range of TBM values, the proportion of "osteopenic" cases (those with sparse, coarse, vertical trabeculae, 12/51, or 23.53%) was higher in the study sample than that observed in the total sample (46/333, or 13.81%).

It is impossible to match the vertebrae to any other part of the skeleton. Therefore, age at death and sex are unknown. However, an estimation of age at death was made by applying the criteria of Stewart (1958), based on vertebral body osteophytosis. Following this method, 36 cases fit into category 2 (vertebral lipping under 0.5+), 11 cases fit into category 3 (vertebral lipping, 0.5–1+), 3 in category 4 (1–1.5+), and 1 to category 5. Although this classification does not necessarily imply an age classification, especially considering the very high rates of osteoarthritis among the population of El Hierro (Mas Pascual et al., 2000), it is important to note that inspection of the pubic symphyses recovered from the funerary cave following Brooks and Suchey (1990) yielded an age at death younger than 53 years in the vast majority of cases.

Methods of measurement

We performed the following analyses.

Fine-grained film radiography. Images of vertebrae were projected on mammographic films. All the radiographic analyses were performed at 50 kV and 20 mAs. Lateral projections of all films were exposed at a focus-film distance of 100 cm.

The following variables were obtained from these films:

Degree of preservation of trabecular pattern, classifying the vertebrae into four groups (normal trabecular pattern, slight absence of some horizontal trabeculae, moderate absence of horizontal trabeculae, and preservation of only coarse, vertical trabeculae; Fig. 1).

Optical density of the film (OD), using a digital densitometer (Victoreen, New York).

Presence or absence of central body collapse ("fish vertebrae"). This was assessed by measuring anterior height (a), posterior height (p), and height at the middle point of the vertebral body (m), and calculating the index $2m/(a + p)$ ("spine score").

Presence of wedged vertebrae, by calculating the index a/p.

Although these last variables were measured as continuous variables, we also categorized them as anterior collapse when a was at least 4 mm less than p, following Hurxthal (1968), and defined as biconcave vertebrae those with a "spine score" ≤ 0.80, following Barnett and Nordin (1960).

These measurements could be performed in 282 out of the total 333 vertebrae. Thus, the prevalence of fractured vertebrae (either biconcave or wedge-shaped), which constitutes the second objective of this study, was calculated in relation to the 282 vertebrae on which radiographic measurements

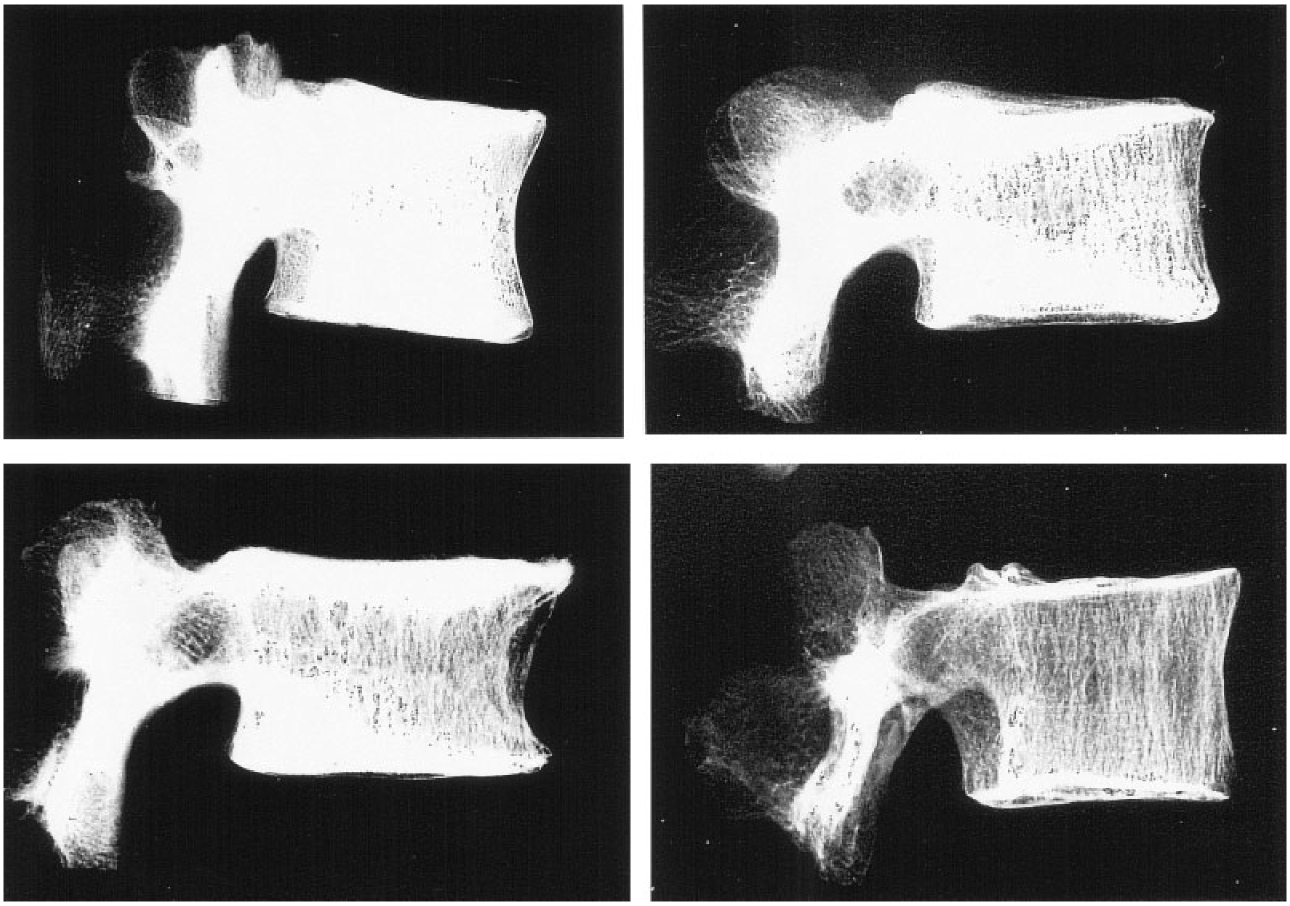

Fig. 1. Radiographic patterns. Normal vertebra is shown in upper left corner; case with severe osteoporosis is shown in lower right corner. Other two cases show mild affectation (upper right) and moderate affectation (lower left).

could be accurately performed. In the remaining vertebrae, poor preservation of the vertebral bodies precluded accurate measurements.

Double x-ray absorptiometry (DEXA). This procedure was performed on the same 51 vertebrae that were also histomorphometrically analyzed. Vertebrae were placed so that the X-ray beam was projected laterally, in order to avoid the distorting effect of vertebral arch and lateral and posterior apophysis. We determined the mean bone mineral density (BMD, g/cm^2) for an area of 1 cm^2 in the central part of the vertebral body. BMD was assessed using a hologic QDR-2000 system (software version 5.54). We did not use any soft-tissue equivalent for DEXA analysis of the prehistoric bones.

Estimation of bone density (BD). This procedure was performed by weighing each vertebra and estimating its volume by measuring the volume of sand displaced, following an already described method (Sabin et al., 1995). Briefly, the vertebrae were introduced into a box filled with sand exactly to its border. The box was placed on a sheet, and after the vertebra was "buried" in the sand, the excess of sand was collected by sliding a ruler along the borders of the box. In two cases, grossly disrupted laminae precluded an accurate estimation of weight (due to contamination with soil material). Bone density was then measured as weight per unit volume.

Bone histomorphometry. This procedure was performed on the 51 vertebrae that had been previously selected on the basis of their gross radiological appearance, as described before, and that were also subjected to DEXA analysis. Vertebral bodies were cut perpendicularly to the vertebral plates, and the central part of the frontal section (Fig. 2) was processed for undecalcified bone sample analysis. Briefly, samples were embedded in methylmetacrylate (Sigma Chemicals, St. Louis, MO), stored for 24 hr at 4°C, and later polymerized at 32–34°C for 3–4 days. Embedded samples were then cut in 9–12-μm-thick slices with a Reichert-Jung microtome and stained with Toluidine blue. Trabecular bone mass (TBM) was determined using an image analyzer equipped with the program Image Measure 4.4a (Microscience, Inc.) at 40×. Results are presented as percent of total area.

Methods of statistical analysis

Normal TBM values for modern populations are about 20%, and values below 15% are in the range of

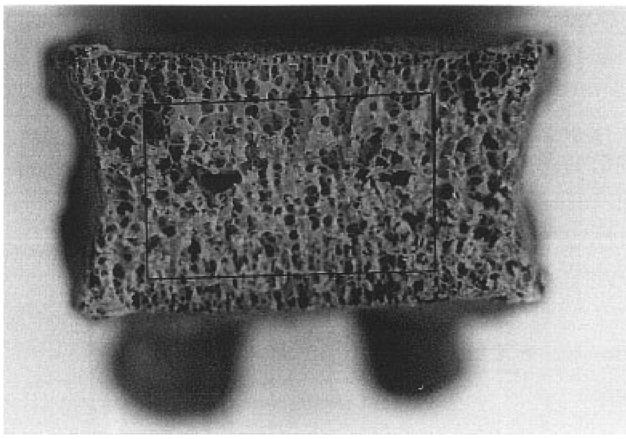

Fig. 2. Section of vertebral body, perpendicular to both cortical plates, indicating area destined for histomorphometrical analysis. Trabeculae are well-preserved.

osteopenia (Velasco-Vázquez et al., 1999). Based on these results, we defined two cutoff points of TBM (20% and 15%), encompassing three categories of TBM values (TBM greater than 20%, between 15–20%, and less than 15%). The first group was considered to have normal bone mass, and the third group, to have osteopenia. We compared BMD, BD, OD, and the indices 2m/(a + p) and a/p among the three groups by one-way analysis of variance and Student's Newmann-Keuls (SNK) post hoc test.

We performed single correlation analysis between BMD, TBM, OD, BD, 2m/(a + p), and a/p . We also performed a stepwise multiple regression analysis between TBM and BMD, OD, and BD (those which kept a significant relationship with TBM using the single correlation analysis).

Considering the aforementioned TBM values as the gold standard for the diagnosis of normal bone mass (>20%) or osteopenia (<15%), we calculated the sensitivity, specificity, and overall accuracy of different arbitrarily defined OD values (1.1, 1, 0.9, and 0.8), BD values (0.60, 0.55. 0.50, and 0.45 g/cm^3), and BMD values (0.6, 0.55, 0.5, and 0.45 g/cm^2) in the diagnosis of TBM greater than 20% (absence of osteopenia), and depicted the corresponding receiver operating characteristic (ROC) curves, plotting the sensitivity and specificity of each cutoff point for each analytical method. Similarly, we also calculated the sensitivity, specificity, and overall accuracy of different arbitrarily defined OD values (1.6, 1.5, 1.4, and 1.3), BD values (0.30, 0.35. 0.40, and 0.45 g/cm^3), and BMD values (0.3, 0.35, 0.4, and 0.45 g/cm^2) in the diagnosis of TBM less than 15% (presence of osteopenia), and depicted the corresponding ROC curves for each analytical procedure. For example, we defined osteopenia when BMD values were below 0.35 g/cm^2. A BMD value for a given sample lower than 0.35 g/cm^2 would be classified by this test as presenting osteopenia. Based on the number of true positives (a) (the test reaches a diagnosis of osteopenia when osteopenia, defined on histomorphometrical grounds, truly exists), true negatives (d), false positives (b), and false negatives (c), we can easily calculate sensitivity (a/(a + c)), specificity (d/(d + b)), and overall accuracy (a + d)/(a + b + c + d). We can repeat this analysis with any other BMD value. Logically, the lower the cutoff point for BMD, the higher the specificity in diagnosing osteopenia, but the lower the sensitivity, i.e., many cases of true osteopenia (according to TBM) would be falsely classified as nonosteopenic by such a low BMD value. On the contrary, if we choose a high BMD value as the cutoff point, probably all osteopenic cases (on histomorphometrical grounds) would also be diagnosed as osteopenia with BMD, but many cases with BMD values below the cutoff point (and thus, osteopenic according to DEXA) would probably have normal TBM values, i.e., sensitivity would be high, but specificity would be low. Plotting the sensitivity and specificity of different BMD values, we can depict the ROC curve for BMD. Similarly, we also depicted the ROC curves for OD and BD (Goldman, 1994).

We compared BMD, OD, TBM, and BD between vertebrae with a "spine score" ≤0.80 (biconcave vertebrae) with those with a score >0.80, between vertebrae with an anterior height >4 mm than the posterior one (wedge-shaped vertebrae) and normal vertebrae, and also between either wedge-shaped or biconcave vertebrae and normal vertebrae, using Student's *t*-test.

We also compared TBM, BMD, BD, OD, and the indices 2m/(a + p) and a/p between the four groups in which vertebrae were radiologically classified according to degree of preservation of the trabecular pattern.

RESULTS

The mean value of TBM was 18.04 ± 4.72%; 14 individuals showed TBM values over 20% (mean ± sd = 24.40 ± 2.54%), 28 between 15–20% (16.82 ± 1.63%), and 9 less than 15% (11.96 ± 1.50%).

BMD, BD, and OD all showed statistically significant differences among the three TBM groups (Table 1), whereas no differences were observed with 2m/(a + p) and a/p between the three groups. Also, significant correlations were observed between TBM and BMD (r = 0.43), TBM and BD (r = 0.49), TBM and OD (r = 0.52), BMD and OD (r = 0.51), and BMD and BD (r = 0.36), but not between TBM and the indices 2m/(a + p) and a/p.

In the stepwise multiple correlation analysis between TBM and BMD, BD, and OD, OD entered into the first place and BD into the second place, whereas BMD became displaced; the multiple correlation coefficient was 0.63, with a standard error of 3.78.

Significant differences were observed among the four groups of vertebrae classified according to the radiologic aspect of the trabeculae regarding TBM (F = 5.47, P = 0.003), BMD (F = 8.12, P < 0.001), and OD (F = 20.59, P < 0.001), but not regarding BD (F = 1.44), a/p (F = 0.53), and 2m/(a + p) (F = 1.36, P > 0.20 in all cases; Table 2).

TABLE 1. Mean values of bone mineral density (BMD), bone density (BD), optical density (OD), and indices 2 × height at midpoint of vertebral body/(anterior + posterior height) (biconcavae vertebrae) and anterior height/posterior height in vertebrae with TBM <15%, between 15–20%, and >20%

	TBM <15% (n = 14)	15% <TBM<20 (n = 28)	TBM >20% (n = 9)	
BMD (g/cm^2)	0.36 ± 0.10	0.45 ± 0.09	0.56 ± 0.12	F = 11.4, $P < 0.001$
BD (g/cm^3)	0.35 ± 0.10	0.46 ± 0.13	0.61 ± 0.22	F = 8, $P = 0.001$
OD	1.57 ± 0.29	1.18 ± 0.40	0.82 ± 0.38	F = 10.8, $P < 0.001$
Biconcave index	0.88 ± 0.06	0.90 ± 0.13	0.90 ± 0.13	F = 0.38, NS
Anterior height posterior height	0.96 ± 0.05	0.94 ± 0.19	0.94 ± 0.09	F = 0.03 NS

TABLE 2. Mean values of bone mineral density (BMD), bone density (BD), optical density (OD), and indices 2 × height at midpoint of vertebral body/(anterior + posterior height) (biconcavae vertebrae) and anterior height/posterior height in vertebrae classified according to radiological preservation of trabecular pattern[1]

	Group 1 (n = 12)	Group 2 (n = 10)	Group 3 (n = 22)	Group 4 (n = 7)	
BMD (g/cm^2)	0.36 ± 0.11	0.41 ± 0.06	0.50 ± 0.09	0.57 ± 0.14	F = 8.2, $p < 0.001$ 1, 2 vs. 3, 4
BD (g/cm^3)	0.40 ± 0.13	0.47 ± 0.26	0.51 ± 0.15	0.55 ± 0.12	F = 1.4, NS
OD	1.62 ± 0.31	1.34 ± 0.24	0.98 ± 0.34	0.61 ± 0.26	F = 20.6, $p < 0.01$ 1 vs. 2–4 2 vs. 3, 4; 3 vs. 4
TBM (%)	14.59 ± 2.53	17.64 ± 4.55	18.71 ± 4.38	22.42 ± 5.28	F = 5.5 $p = 0.003$ 4 vs. 1–3
2m/(a + p)	0.86 ± 0.13	0.93 ± 0.08	0.89 ± 0.09	0.95 ± 0.12	F = 1.36, NS
a/p	0.99 ± 0.15	0.92 ± 0.06	0.95 ± 0.13	0.98 ± 0.09	F = 0.53, NS

[1] Group 1, sparse coarse vertical trabeculae; group 2, vertical + few horizontal trabeculae; group 3, loss of some horizontal trabeculae; group 4, normal.

In Table 3 we show the sensitivity, specificity, and overall accuracy of different BMD, OD, and BD values in diagnosing TBM values greater than 20%. Figure 3 shows the ROC curves obtained by plotting sensitivity and specificity values obtained at different cutoff points of BMD, OD, and BD.

In Table 4 we show the sensitivity, specificity, and overall accuracy of different BMD, RD, and D values in diagnosing TBM values less than 15%. Figure 4 shows the ROC curves obtained by plotting sensitivity and specificity values obtained at different cutoff points of BMD, RD, and D. As shown in Figures 3 and 4, curves corresponding to OD values are situated nearest the left upper corner, i.e., they are the most accurate at detecting both normal TBM values and osteopenia.

In the total sample, biconcave vertebrae were observed in 10 (3.55%) of 282 vertebrae in which anterior, posterior, and medial measurements were possible, whereas wedge-shaped vertebrae were observed in 8 cases (2.84%). Since two vertebrae showed both a spine score <0.80 and anterior collapse, a total of 16 vertebrae (5.67%) fulfilled the criteria for vertebral fracture. Among the 51 verebrae selected for histomorphometry, 5 were wedge-shaped vertebrae, and 3 were biconcavae. No differences were observed regarding BMD, OD, D, or TBM betwen wedge-shaped and nonwedge-shaped, or biconcavae and nonbiconcavae vertebrae. Considering the presence of either wedge-shaped or biconcavae vertebrae as fractured vertebrae, no differences were observed between fractured and nonfractured vertebrae for any of the methods.

DISCUSSION

In addition to biomechanical adaptation, bone plays a key role in calcium-phosphorus homeostasis and acid-base imbalance (Krane and Holick, 1994). It is continuously being remodeled during life, a process which includes bone synthesis, due to osteoblastic activity, and bone resorption, due to osteoclastic activity. Reduced bone mass is called osteopenia. If osteopenia is severe enough, the risk of bone fracture increases (Blake et al., 1997). Currently, the term *osteoporosis* denotes a situation of bone fragility associated with fracture. An important kind of "fragility" fracture is vertebral body fracture, a process frequently suffered by postmenopausal white women (Cooper et al., 1992).

Besides advanced age, several other factors may lead to decreased bone mass. One of them is marasmus-type undernutrition, a situation in which a slow rate of bone synthesis leads to progressive bone loss. Poor nutritional status, therefore, may underlie the finding of a high prevalence of osteopenia and/or osteoporosis observed in a nonsenile population sample, not only through a direct effect on bone synthesis (Bourrin et al., 2000b), but also due to accompanying decreased muscle activity (Duppe et al., 1997). In accordance with others (Gupta, 1996; Eaton and Nelson, 1991; Agarwal and Grynpas, 1996; Martin et al., 1985), this "nutritional hypothesis" was argued by ourselves to explain the high prevalence of osteopenia among the pre-Hispanic population of some islands of the Canary Archipel-

TABLE 3. Sensitivity, specificity, and overall accuracy of different bone mineral density (BMD), bone density (BD), and optical density (OD) values in diagnosing presence or not of TBM values >20%

	Trabecular bone mass (TBM) higher than 20%			
		Yes	No	
BMD	>0.45 g/cm^2	11	13	Sensitivity: 78.57%
BMD	<0.45 g/cm^2	3	24	Specificity: 64.86%
				Overall accuracy: 68.63%
BD	>0.45 g/cm^3	10	15	Sensitivity: 76.92%
BD	<0.45 g/cm^3	3	21	Specificity: 58.33%
				Overall accuracy: 63.27%
OD	<1.1	12	12	Sensitivity: 85.71%
OD	>1.1	2	25	Specificity: 67.51%
				Overall accuracy: 72.55%
BMD	>0.50 g/cm^2	10	9	Sensitivity: 71.43%
BMD	<0.50 g/cm^2	4	28	Specificity: 75.68%
				Overall accuracy: 74.51%
BD	>0.50 g/cm^3	9	11	Sensitivity: 69.23%
BD	<0.50 g/cm^3	4	25	Specificity: 69.44%
				Overall accuracy: 69.39%
OD	<1.0	11	12	Sensitivity: 78.57%
OD	>1.0	3	25	Specificity: 67.57%
				Overall accuracy: 70.59%
BMD	>0.55 g/cm^2	8	5	Sensitivity: 57.14%
BMD	<0.55 g/cm^2	6	32	Specificity: 86.49%
				Overall accuracy: 78.43%
BD	>0.55 g/cm^3	7	4	Sensitivity: 53.85%
BD	<0.55 g/cm^3	6	32	Specificity: 88.89%
				Overall accuracy: 79.59%
OD	<0.9	8	9	Sensitivity: 57.14%
OD	>0.9	6	28	Specificity: 75.68%
				Overall accuracy: 70.59%
BMD	>0.60 g/cm^2	5	1	Sensitivity: 35.71%
BMD	<0.60 g/cm^2	9	36	Specificity: 97.30%
				Overall accuracy: 80.39%
BD	>0.60 g/cm^3	5	3	Sensitivity: 38.46%
BD	<0.60 g/cm^3	8	33	Specificity: 91.67%
				Overall accuracy: 77.55%
OD	<0.8	7	5	Sensitivity: 50%
OD	>0.8	7	32	Specificity: 86.49%
				Overall accuracy: 76.47%

ago (González-Reimers and Arnay de la Rosa, 1992; Velasco-Vázquez et al., 1999).

Besides caloric intake, several nutrients as vitamins A, D, C, and K, zinc, magnesium, and calcium may affect bone formation (Roughead and Kunkel, 1991). However, a chronic *selective* deficiency of any of these nutrients was not likely to occur in the pre-Hispanic inhabitants of any of the Canary Islands, considering that the economy was based on agriculture and goat-herding, and sunshine exposure was intense. In addition to vitamin D intake (a deficiency of which causes defective osteoid mineralization, leading to osteomalacia and ricketts), a dietary ratio of calcium (in milligrams)-to-protein (in grams) greater than 20 is probably necessary for adequate skeletal protection (Heaney, 1998). An excess protein intake related to calcium intake is also unlikely among the pre-Hispanic population of the Canary Archipelago.

In previous studies of the pre-Hispanic population of Gran Canaria we found, both in iliac crest specimens and in tibiae, a high prevalence of osteopenia, in contrast with observations of pre-Hispanic remains on other islands of the Canary Archipelago.

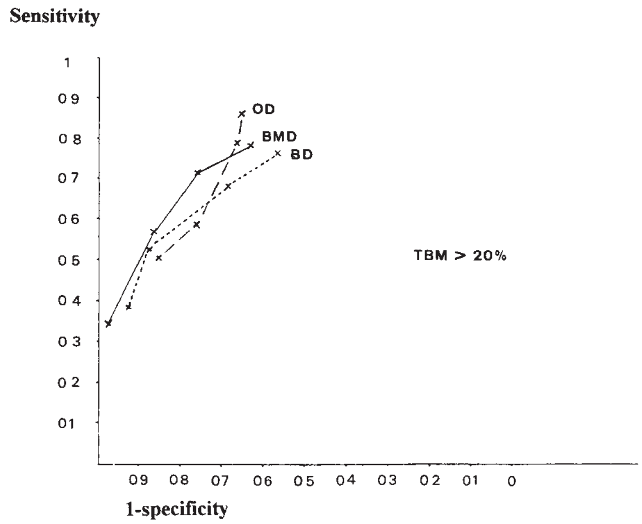

Fig. 3. Receiver-operating characteristic (ROC) curves plotting sensitivity and specificity of different BMD, BD, and OD cutoff points in diagnosis of normal bone mass. Values of BMD, BD, and OD cutoff points are given in Table 3.

TABLE 4. Sensitivity, specificity, and overall accuracy of different bone mineral density (BMD), bone density (BD), and optical density (OD) values in diagnosing presence or not of TBM values <15%

	Trabecular bone mass (TBM) lower than 15%			
		Yes	No	
BMD	<0.30 g/cm^2	3	0	Sensitivity: 33.33%
BMD	>0.30 g/cm^2	6	42	Specificity: 100%
				Overall accuracy: 88.24%
BD	<0.30 g/cm^3	2	1	Sensitivity: 28.57%
BD	>0.30 g/cm^3	7	39	Specificity: 97.62%
				Overall accuracy: 84.31%
OD	>1.6	4	4	Sensitivity: 44.44%
OD	<1.6	5	38	Specificity: 90.48%
				Overall accuracy: 82.35%
BMD	<0.35 g/cm^2	4	3	Sensitivity: 44.44%
BMD	>0.35 g/cm^2	5	39	Specificity: 92.86%
				Overall accuracy: 74.51%
BD	<0.35 g/cm^3	4	4	Sensitivity: 44.44%
BD	>0.35 g/cm^3	5	36	Specificity: 90.00%
				Overall accuracy: 81.63%
OD	>1.5	5	5	Sensitivity: 55.56%
OD	<1.5	4	37	Specificity: 88.10%
				Overall accuracy: 82.35%
BMD	<0.40 g/cm^2	5	12	Sensitivity: 55.56%
BMD	>0.40 g/cm^2	4	30	Specificity: 71.43%
				Overall accuracy: 68.63%
BD	<0.40 g/cm^3	7	12	Sensitivity: 77.78%
BD	>0.40 g/cm^3	2	28	Specificity: 70.00%
				Overall accuracy: 71.43%
OD	>1.4	6	10	Sensitivity: 66.67%
OD	<1.4	3	32	Specificity: 76.19%
				Overall accuracy: 74.51%
BMD	<0.45 g/cm^2	8	19	Sensitivity: 88.89%
BMD	>0.45 g/cm^2	1	23	Specificity: 54.76%
				Overall accuracy: 60.78%
BD	<0.45 g/cm^3	8	16	Sensitivity: 88.89%
BD	>0.45 g/cm^3	1	24	Specificity: 60.00%
OD	>1.3	8	12	Sensitivity: 88.89%
OD	<1.3	1	30	Specificity: 71.43%
				Overall accuracy: 74.51%

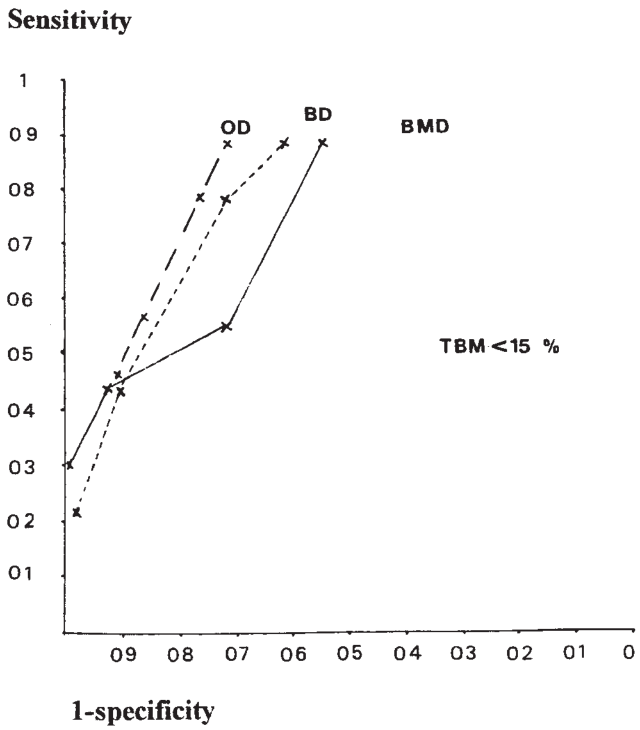

Fig. 4. Receiver-operating characteristic (ROC) curves plotting sensitivity and specificity of different BMD, BD, and OD cutoff points in diagnosis of osteopenia. Values of BMD, BD, and OD cutoff points are given in Table 4.

In the present study, in the total adult population of El Hierro, the proportion of vertebrae with severe osteopenia based on radiologic criteria was 13.81%, a figure similar to that (12.5%) obtained from iliac crest specimens in which TBM was histomorphometrically assessed (González-Reimers et al., 1988). Also, in accordance with these figures, osteoporotic fractures (either wedge-shaped or biconcave vertebrae) were observed in 16 cases of 282 in which measurements of anterior, posterior, and medial height were possible. These results yield a prevalence of 7.5% for T12, 4.72% for L1, and 5.33% for L2, with a global mean of 5.67%. Studies dealing with prevalence of vertebral fractures in modern population samples yield figures between 20–40% (Nevitt et al., 1998; Papaioannou et al., 2002; Díaz López et al., 2000; Hasserius et al., 2001; Jackson et al., 2000). However, most of these studies were performed on individuals older than 50 years. Considering only individuals aged 50–54 years, the prevalence of vertebral fractures ranges between 6–10% (Melton et al., 1989; Díaz López et al., 2000). It is important to keep in mind that these data derive from modern population-based studies. The prevalence of vertebral fractures observed in the present study is comparable to, or somewhat lower than, the figures obtained for the youngest individuals of modern population-based studies, who are, however, older than 50. The lack of information regarding sex and age at death of the pre-Hispanic population of El Hierro constitutes a significant deficiency for the proper interpretation of our results. However, as noted earlier, age at death for this population was less than 53 in the majority of cases. A 7.5% prevalence of osteoporotic vertebral fractures is not a high figure, even for such a relatively young population, and these results do not support the hypothesis that the prevalence of osteoporosis was high among the population from El Hierro. As commented upon elsewhere (Velasco-Vázquez et al., 1999), the striking difference in the prevalence of osteopenia between El Hierro and Gran Canaria may be due to differences in demography and in economic activity among the pre-Hispanic societies of both islands.

Although several reports revealed different degrees of low bone mass in ancient population samples, as reviewed by Agarwal and Grynpas (1996), few studies have been designed to directly assess the prevalence of fragility fractures, and the general conclusion is that they are absent in ancient populations. The underlying reasons for this are not well-understood. It is important to keep in mind that vertebral fracture is more a process which takes place slowly and may not be very disabling, rather than an abrupt event which may be life-threatening (as hip fracture) or not (as distal forearm fracture). But, in contrast with long bone fractures, which may heal completely (as distal foreram fracture) or kill in a few days (as hip fracture), and thus become obscured for the paleoanthropologist, vertebral fractures are easily detected using the radiologic criteria mentioned before; however, large studies assessing the prevalence of vertebral fractures in ancient population groups are lacking.

The main objective of this study was to establish the ability of nondestructive methods to diagnose osteopenia in bare bones. As the gold standard, we defined osteopenia on histomorphometrical grounds. The relatively low prevalence of osteopenia in the population analyzed led us to select a sample for histomorphometrical analysis with a greater proportion of osteopenic vertebrae than that observed in the overall population. Perhaps this contributed to the finding of a lower mean TBM for the vertebrae than that derived from a histomorphometrical analysis of right tibiae previously performed on samples from the same archaeological site. However, the TBM of vertebrae and tibiae are not comparable, TBM in tibiae being more dependent on weight-bearing exercise than TBM in vertebrae (Iwamoto et al., 1998; Casez et al., 1995). Interestingly, severe osteoarthritic changes among the pre-Hispanic population from El Hierro (Mas Pascual et al., 2000) may support the hypothesis that mechanical overload contributed to the high TBM values observed in the tibiae of these people.

In our sample, most cases showed TBM values in the normal range, but in 9 cases (17.65%) these fell clearly below the lower limit of normality, so these cases can be considered osteopenic. A similar result was obtained by Kneissel et al. (1994) in a nonselected 4,000-year BP population. These authors stratified the results obtained by age and sex, with vertebral TBM values ranging from 23.8 ± 7.8% for young men and 19.8 ± 4.5% for young women to 11.3 ± 3.9% for elderly men (40–60 years old) and 14.6 ± 1.1% for elderly women.

Optical density of X-ray films was the method which showed the greatest accuracy in diagnosing both severe osteopenia and normal bone mass, followed by bone density and DEXA. However, correlation was not perfect, and the ROC curves show that, although these methods may roughly estimate bone mass, some inaccuracy does exist. Several reasons may contribute to this finding. Alterations of the radiological appearance of vertebrae only become evident when bone loss reaches 30%. In addition, it is likely that partial vertebral collapse in severely osteoporotic individuals may lead to falsely high TBM values if the sample destined for histomorphometry was obtained from a collapsed area, thus distorting the results, not only those obtained with histomorphometry, but also with DEXA, bone density, and optical density. In this regard, it is noteworthy that the TBM values of the three cases of biconcavae vertebrae were 24.96%, 18.23%, and 12.59%, while their BD values were 0.35, 0.60, and 0.36 g/cm^3, and OD values were 0.85, 1.40, and 1.64. Only their BMD/DEXA values, which were 0.38, 0.31, and 0.17 g/cm^2, were very low. But even selecting only those cases without any evidence of fracture (and thus eliminating the possibility of distorted results due to vertebral collapse), correlations between TBM and the other parameters were no better than those observed in the whole sample: $r = 0.45$ for the correlation between TBM and BMD, 0.52 between TBM and BD, 0.55 between TBM and OD, and 0.57 between BMD and OD ($P < 0.01$ in all cases). The reason(s) for the nonperfect correlation between TBM and the noninvasive methods remain(s) elusive. Although in clinical settings DEXA is the standard method for the diagnosis and follow-up of osteoporosis, with several studies supporting the excellent relationship between DEXA-assessed BMD and bone mass (Marshall et al., 1996), the results are not as good when the method is applied to prehistoric samples. Kneissel et al. (1994) obtained a relatively poor correlation between DEXA-assessed BMD and histomorphometrically assessed trabecular bone mass in 18 vertebrae and femoral necks ($r^2 = 0.34$ and 0.50, respectively). In a study of 95 cases comparing DEXA with TBM, the correlation coefficient was 0.52, which is similar to that obtained in the present study as well as other results reported in the literature. Perhaps the lack of soft tissue and bone marrow account for the nonperfect correlation, although it is noteworthy that other authors, such as Kneissel et al. (1994), introduced the bones into a waterbath in order to eliminate distortion introduced by air bubbles, without obtaining better results. Diagenesis may exist, and may distort the results. Indeed, Kneissel et al. (1994) stated that the relatively poor correlation between histomorphometry and DEXA may have been due to diagenetic changes of bone composition. It is important to bear in mind that diagenesis was probably not very important in our series. The skeletal remains found at Punta Azul were not interred, simply lying on the lava flour. The subdesert climatic conditions of the island should have aided in the good preservation of the bone remains.

Multiple correlation analysis shows that although the correlation is highly significant, the standard error is too high to allow an accurate estimation of TBM using only densitometric methods. Interestingly, optical density shows a closer correlation with TBM than that obtained with the more sophisticated DEXA absorptiometry. It is also noteworthy that a fine-grained film radiography permits not only optical density to be determined but also the anterior, posterior, and medial height to be measured, thus allowing us to identify those cases of fractured vertebrae which could distort the densitometry, histomorphometry, or optical density results. In addition, marked differences were observed among the four groups based on trabecular pattern regarding TBM and DEXA, although, as shown in Table 2, differences for TBM were only significant between normal specimens and any of the other three groups, whereas differences for DEXA were significant in cases with either normal or near-normal trabecular pattern compared with those of any of the other two groups. Indeed, if we calculate confidence intervals for the values of DEXA or TBM observed in the best and worst groups of trabecular pattern, a significant overlapping does occur. In any case, our study strengthens the value of the mere inspection of a fine-grained film radiography of vertebrae, followed by measurement of optical density and vertebral height, in the evaluation of bone mass of prehistoric samples.

CONCLUSIONS

We conclude that several noninvasive approaches, such as fine-grained film radiography, optical density, DEXA, or merely bone density, permit the severity of osteopenia to be estimated. In fact, a BMD greater than 0.60 g/cm^2, or a bone density greater than 0.60 g/cm^3, exclude osteopenia with a specificity greater than 90%, whereas a BMD value less than 0.35 g/cm^2, or a BD less than 0.35 g/cm^3 or optical density >1.6, exclude normal bone mass with a specificity greater than 90%.

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