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Double-Energy X-Ray Absorptiometry in the Diagnosis of Osteopenia in Ancient Skeletal Remains

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ABSTRACT Bone mineral density (BMD) assessed by double-energy X-ray absorptiometry (DEXA) accurately estimates the bone mass in living individuals, and is thus the method usually employed in the diagnosis and follow-up of osteopenia. It is preferred, in clinical settings, to the more invasive and destructive histomorphometrical assessment of trabecular bone mass in undecalcified bone samples. This study was performed in order to examine the value of DEXA-assessed BMD at the proximal end of the right tibia, either alone or in combination with the cortico-medullary index at the midshaft point of the right tibia (CMI), in the diagnosis of osteopenia in a prehistoric sample composed of 95 pre-Hispanic individuals from Gran Canaria. Age at death could be estimated in 34 cases. Diagnosis of osteopenia was performed by histomorphometrical assessment of trabecular bone mass (TBM) in an undecalcified bone section of a small portion of the proximal epiphysis of the right tibia. A high prevalence of osteopenia was found among the population of Gran Canaria. Both TBM and BMD were significantly lower in the older individuals than in younger ones, and BMD was also significantly lower in female individuals. BMD was moderately correlated with TBM ($r = +0.51$); the correlation was higher if CMI was included (multiple $r = +0.615$). BMD values lower than 0.7 g/cm^2 showed a high specificity (>93%) at excluding normal TBM values. These methods were prospectively applied in a further sample of 21 right tibiae from Gran Canaria, Tenerife, and El Hierro. The results were similar to those obtained in the larger sample. Thus, DEXA-assessed BMD combined with CMI (noninvasive procedures) may be useful in detecting osteopenia in ancient populations. Am J Phys Anthropol 118:134–145, 2002.

Several kinds of analysis may provide information about nutritional status, stressful episodes, and dietary habits of past populations. These analyses include assessment of dental attrition and enamel hypoplasia (Powell, 1985; Corrucini et al., 1985), bone trace elements (Price and Kavanagh, 1982; Gilbert, 1985; Francalacci, 1989; Burton and Price, 1990), bone stable isotopes of carbon, nitrogen, and other elements (Schoeninger, 1983; Klepinger, 1984), and prevalence of osteopenia and/or osteoporosis (Huss-Ashmore, 1978; Martin et al., 1985), among others. The finding of a high prevalence of the latter entity in a population that is not comprised solely of aged individuals may be interpreted as an indicator of protein-calorie malnutrition (Huss-Ashmore, 1978; Velasco-Vázquez et al., 1999).

A modern definition of osteoporosis describes 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. In osteoporosis there is always a decrease in bone mass relative to age. Currently, the term osteopenia denotes the decrease in bone mass, whereas the term osteoporosis requires the presence of bone fractures due to bone fragility. Bone mass may be accurately assessed by histomorphometrical measurement of trabecular bone mass (TBM), usually of iliac crest biopsy specimens (Bordier and Tun Chot, 1972). However, the invasiveness of this procedure may hamper its clinical value, and its destructive nature may preclude its application to anthropological remains. Therefore, other methods have been developed, including cortico-medullary indices, computed tomography, and assessment of bone mineral density (BMD) by double-energy X-ray absorptiometry (DEXA), among others. This last procedure, due to its accuracy, low cost, and simplicity, is the standard method employed today in the clinical evaluation and follow-up

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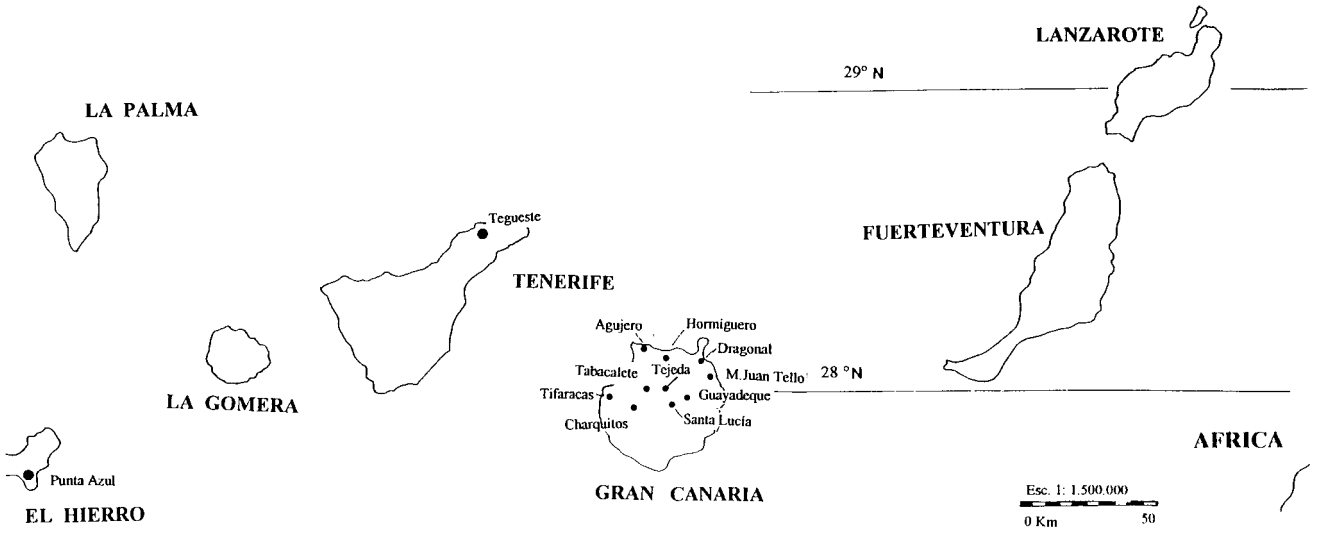

Fig. 1. Geographical location of archaeological sites mentioned in this study.

of osteopenia (Levis and Altman, 1998). Indeed, several studies support its primacy in establishing an accurate diagnosis of osteopenia in clinical settings (Larcos and Wahner, 1991; Wahner, 1989), as it is the most accurate predictor of the risk of bone fracture. Lacking invasiveness, it may be repeated several times in a patient, and thus may serve to evaluate therapeutic efficacy. It would be also an excellent method to estimate the prevalence of osteopenia in ancient population groups, due its nondestructive nature.

Several investigators have applied this method to the analysis of ancient bones (Lees et al., 1993; Bennike and Bohr, 1990; Farquharson et al., 1997; Hammerl et al., 1991). Some authors (Kneissel et al., 1995) do not recommend DEXA for the evaluation of archaeological skeletal material, due to the poor relation between DEXA and histomorphometrical analysis performed in the vertebrae and femoral necks of 18 individuals. Other authors, however, emphasize the ease and noninvasiveness of DEXA analysis (Farquharson et al., 1997; Lees et al., 1993). Farquharson et al. (1997) found correlation coefficients between BMD assessed by DEXA and bone mineral density of +0.64 for the femur (30 cases) and +0.74 for the fourth lumbar vertebra (25 cases); although correlation coefficients are acceptable, inaccuracies may exist in BMD determinations due to the absence of bone marrow in dry bones.

We performed the present study to estimate the sensitivity, specificity, and overall accuracy of DEXA-assessed BMD in the diagnosis of different degrees of osteopenia (as defined on histomorphometrical grounds) in 95 samples of pre-Hispanic individuals from Gran Canaria. Since the measurement of the cortico-medullary index at the midshaft of the right tibia (CMI) is a useful tool in the diagnosis of osteopenia in ancient bones (González-Reimers et al., 1998), we also analysed the relationship between the two noninvasive procedures (BMD and CMI) and TBM. Later, we prospectively applied these methods in a further "test" sample composed of 21 pre-Hispanic individuals from Gran Canaria, Tenerife, and El Hierro.

MATERIALS AND METHODS

Samples

The study was performed on 95 right tibiae belonging to pre-Hispanic inhabitants from Gran Canaria. In 27 cases, most of the remaining skeleton was preserved. The vast majority of these samples were included in a study comparing prevalence of osteoporosis between the prehistoric population of Gran Canaria and that of El Hierro (Velasco-Vázquez et al., 1999). The samples were obtained from the following mass burials: Guayadeque (68 cases), Tifaracas (1 case), El Agujero (13 cases), Hormiguero (5 cases), Dragonal (1 case), Charquitos (1 case), Santa Lucía (1 case), Andén de Tabacalete (2 cases), Tejeda (2 cases), and Montaña de Juan Tello (1 case) (Fig. 1). Guayadeque is the most important of these archaeological sites, with several huge collective burials, each of them containing the remains of several hundred individuals. These individuals were not interred, but deposited on plant or stony layers in natural volcanic caves, avoiding direct contact with soil, so that preservation (aided by the subdesertic climatic conditions) was excellent. Radiocarbon dating on some individuals buried in these caves (although not those included in this study) yielded a time depth ranging from 1405 ± 60 BP to 1213 ± 60 BP. In contrast, El Agujero is a tumulus burial complex containing a few dozen well-preserved skeletons, located in the coastal region of Gran Canaria, with a time depth of 875 ± 60 BP. This material belongs to the anthropological collection of the Museo Canario (Las Palmas).

In the 27 complete skeletons, sex was determined by inspection of the pelvis. In the remaining cases, sex was estimated by adapting the discriminant functions analysis of Iscan and Miller-Shaivitz

(1984) to the population of Gran Canaria, as previously described (Velasco-Vázquez et al, 1999). In total, 27 tibiae were classified as belonging to female individuals, and 65 to males. In 3 cases, values obtained did not allow unambiguous sexing.

Age at death was estimated in 34 cases. In 7 tibiae, the epiphyseal closure line was partially fused to the bone diaphysis, and so these individuals were classified as "very young" (Bennett, 1987). In 27 further complete skeletons, age at death was estimated by inspection of the pubic symphysis following the method of Brooks and Suchey (1990): 4 cases were "very young" (symphyseal stage I; approximate age at death, 19 ± 2.5 years); 16 cases were "young" (symphyseal stage II; approximate age at death, 25 ± 4 years); and 7 were "mature" (symphyseal phase III; approximate age at death, 30 ± 7 years). No cases were included with symphyseal phases IV–VI. Hence, an estimation of the approximate age at death was possible in 34 individuals from Gran Canaria: 11 of them died very young, 16 young, and 7 at a mature age.

Methods of measurement

We performed the following analyses.

Bone histomorphometry. A small portion of the medial part of the posterior aspect of the proximal epiphysis was removed and processed for undecalcified bone sample analysis. Briefly, samples were embedded in methylmetacrylate (Sigma Chemical Co., 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 (so that the resulting sections were perpendicular to the long axis of the tibiae) 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 given as percent of total area.

We compared TBM of the pre-Hispanic population with that of our own control group, which consisted of 12 modern male individuals aged 17–44 years, from whom a bone specimen was obtained from tibiae during surgical operations on the right knee.

Plain x-ray film of the tibiae. We also calculated the cortico-medullary index (CMI) at the midpoint of the shaft (González-Reimers et al., 1998). This index was also calculated in 16 healthcare workers (11 men, 5 women, age range 28–51 years) who served as controls.

Double x-ray absorptiometry (DEXA). This procedure was performed on all bone samples at the proximal end of the right tibia. Three subareas were defined: a proximal one, rich in trabecular bone, a distal one, which includes cortical bone, and an intermediate one (Fig. 2). We determined the mean bone mineral density (BMD, g/cm^2) of each of these subareas, and also calculated BMD for the whole

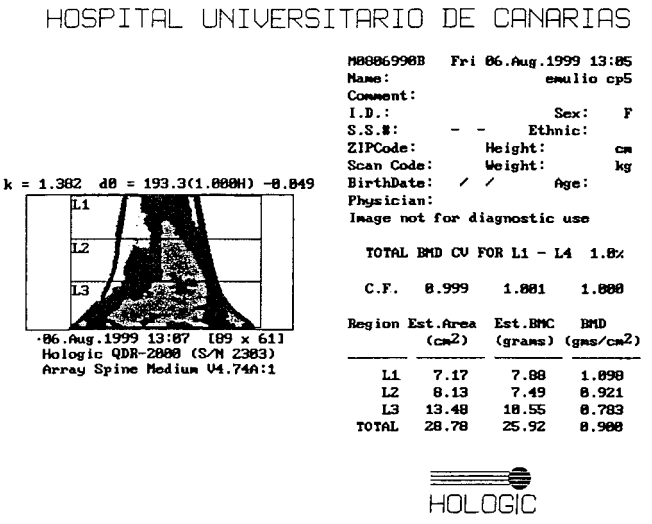

Fig. 2. Area of proximal end of right tibiae examined by DEXA, specifying three subareas L1, L2, and L3.

area (BMDWA). This last parameter was used in multiple correlation studies and contingency tables to calculate sensitivity and specificity of different BMDWA values in the diagnosis of different degrees of osteopenia (see below). BMD was assessed using a hologic QDR-2000 system (software version 5.54). Precision analysis of the technique was performed, measuring four times, on different days, BMDWA of three tibiae. Values obtained were (in g/cm^2): 0.586, 0.590, 0.582, and 0.578 for tibia 1; 0.850, 0.855, 0.848, and 0.837 for tibia 2; and 0.386, 0.363, 0.371, and 0.356 for tibia 3 . We did not use any soft-tissue equivalent for DEXA analysis of the prehistoric bones.

Using exactly the same technique, the same parameter was also determined in 20 healthcare workers aged 24–50 years (11 men and 9 women), who also underwent a DEXA analysis of the femoral neck and second, third, and fourth lumbar vertebrae, in order to exclude osteopenia.

Methods of statistical analysis

We first compared BMD (and TBM and CMI) between males and females and between very young, young, and mature individuals, using Student's *t*-test and variance analysis, respectively, and then the Student's Newmann-Keuls (SNK) test. We also compared these parameters between the study group and the test groups, and also TBM and CMI of ancient bones with those of the modern ones, using Student's *t*-test.

Both in the whole sample and in those with estimated sex and age at death, we performed single-correlation analysis between BMD, TBM, and CMI. In the whole sample, we also performed stepwise multiple correlation analysis between TBM (as the dependent variable) and BMDWA and CMI.

Arbitrarily, we classified our population into four groups according to TBM values (more than 17.5%; between 17.5–15%, between 15–12.5%; and less than 12.5%), and compared the mean values of

TABLE 1. Mean values of trabecular bone mass (TBM), cortico-medullary index (CMI), and bone mineral density (BMDWA) in males and females

Group	TBM (%)	CMI	BMDWA (g/cm^2)
Males			
N	65.0	65.0	65.0
Mean	18.48	0.3217	0.9045
Standard deviation	5.27	0.0979	0.2211
Females			
N	27	27	27
Mean	16.52	0.3321	0.8021
Standard deviation	5.24	0.0919	0.2104
Student's-*t*; *P*	t = 1.63; NS	t = 0.46; NS	t = 2.05; *P* = 0.04

TABLE 2. Mean values of bone mineral density (BMDWA), trabecular bone mass (TBM), and cortico-medullary index (CMI) in very young, young, and mature individuals

Group	TBM (%)	CMI	BMDWA (g/cm^2)
Very young (1)	11	11	11
Mean	21.95	0.37	1.1030
Standard deviation	5.30	0.1053	0.2117
Young (2)			
N	16	16	16
Mean	18.03	0.3007	0.8848
Standard deviation	5.79	0.0979	0.2327
Mature (3)			
N	7	7	7
Mean	14.88	0.3161	0.7543
Standard deviation	5.91	0.1400	0.1721
ANOVA	F = 3.51; *P* = 0.042	F = 1.42; NS	F = 6.23; *P* = 0.005
SNK test	1 vs. 3		1 vs. 2, 3
	2 vs. 3		

BMDWA and CMI in these four groups by analysis of variance (ANOVA).

Finally, we calculated sensitivity, specificity, and overall accuracy of different arbitrarily defined BMDWA values (1, 0.9, 0.8, 0.7 g/cm^2) in the diagnosis of TBM less than 12.5%, less than 15%, less than 17.5%, and less than 20%.

Similarly, we performed a stepwise multiple correlation analysis between BMDWA (as the dependent variable) and TBM and CMI.

We also classified our population into four groups according to BMDWA values (more than 1, between 0.9–1, between 0.8–0.899, between 0.7–0.799, and less than 0.7 g/cm^2), and compared the mean values of TBM and CMI in these four groups by ANOVA.

We prospectively applied these analyses in a test group composed of 21 individuals: 4 from El Hierro (Punta Azul), 3 from Tenerife (Tegueste), and 14 from Gran Canaria (13 from Guayadeque, and 1 from Santa Lucía). Following the same method described previously for the study group, sex was estimated in 20 cases of the test sample (9 males), whereas age at death was only estimated in 5 cases by inspection of the pubic symphysis (4 young, and 1 mature individual).

RESULTS

Study group

The mean value of TBM was 17.93 ± 5.26% (for the controls, 24.58 ± 5.30, $t = 4.12$, $P < 0.001$); 44 individuals showed TBM values over 17.5%, 21 between 15–17.5%, 15 between 12.5–15%, and 15 below 12.5%. Differences of TBM, BMDWA, and CMI between males and females are shown in Table 1, and the values obtained in the very young, young, and mature individuals are shown in Table 2. Table 3 records the mean values of TBM, BMDWA, and CMI in men and women of different age groups. As shown, both BMDWA and TBM were significantly different between the three different groups of individuals, although differences were not statistically significant among the women, probably because of the small number of cases . Also, we compared TBM, BMDWA, and CMI values between individuals of known age at death and those with unknown age at death. The latter showed TBM values of 17.53 ± 4.74%, BMDWA values of 0.8386 ± 0.1973, and CMI values of 0.323 ± 0.087, similar to the results obtained in individuals with known age at death. Among individuals with known age at death, correlations between TBM and CMI (r = +0.61), between TBM and BMDWA (r = +0.66), and between BMDWA and CMI (r = +0.57) were all highly significant.

The mean value of BMDWA at the tibial epiphysis was 0.8708 ± 0.2195. Twenty-six individuals showed BMDWA values over 1, 40 over 0.90, 55 over 0.80, 71 over 0.70, and 24 less than 0.70. Those individuals with TBM higher than 17.5% showed BMDWA values (0.9807 ± 0.2001) significantly higher than those with TBM values lower than 17.5% (0.7759 ± 0.1906, $t = 5.1$, $P < 0.001$). The same is valid for BMD values at the proximal (trabecular bone) end of the tibia ($t = 4.14$), at the cortical bone area ($t = 5.36$), and at the intermediate area ($t = 5.16$, $P < 0.001$ in all cases).

TABLE 3. Mean values of bone mineral density (BMDWA), trabecular bone mass (TBM), and cortico-medullary index (CMI) in very young, young, and mature individuals according to sex[1]

Group	TBM (%)	CMI	BMDWA (g/cm^2)
Very young			
Men			
N	9	9	9
Mean	21.28	0.3636	1.1211
SD	5.34	0.1103	0.2290
Women			
N	2	2	2
Mean	24.98	0.3995	1.0217
SD	5.51	0.1082	0.1108
Young			
Men			
N	12	12	12
Mean	19.21	0.2963	0.8881
SD	6.29	0.0821	0.2735
Women			
N	4	4	4
Mean	14.47	0.3138	0.8750
SD	2.47	0.1237	0.2621
Mature			
Men			
N	4	4	4
Mean	13.71	0.3095	0.7625
SD	5.47	0.1781	0.2023
Women			
N	3	3	3
Mean	16.46	0.3250	0.7433
SD	7.31	0.1048	0.1648
ANOVA			
Men	F = 2.3, NS	F = 1, NS	F = 3.6, P = 0.043
Women	F = 2.9, NS	F = 0.4, NS	F = 1, NS
SNK			
Men			1 vs. 2, 3 2 vs. 3

[1] SD, standard deviation.

The mean value of CMI was 0.3243 ± 0.0958, whereas that of controls was 0.3909 ± 0.0590 (t = 2.69, $P < 0.001$). Thirty-three out of the 95 pre-Hispanic individuals (34.74%) showed CMI values below 0.275 (a figure which approximates the mean-1.96*standard deviation of the control population) . Among those individuals with known age at death, the proportion of those with CMI below 0.275 was 35.29% (12 cases).

Twenty further individuals showed CMI values below 0.33 (approximately, mean of the controls minus standard deviation), whereas 42 showed CMI values in the range of that of the controls. Both TBM (F = 11.5) and BMDWA (F = 4.01) were significantly different in the three groups of individuals classified according to CMI (Table 4). Similar differences were observed when only those with known age at death were considered (Table 5).

The mean BMDWA value of the controls was 0.5436 ± 0.0542 for the whole group, 0.5493 ± 0.053 for men, and 0.5312 ± 0.0606 for women. All controls also underwent a DEXA analysis of the second, third, and fourth lumbar vertebrae and at the femoral neck. None of them was either osteopenic or osteoporotic.

Significant correlations were observed between TBM and CMI (r = +0.52, $P < 0.001$), and between TBM and BMDWA (r = +0.51, $P < 0.001$) (Fig. 3). CMI was also significantly related to BMDWA (r = 0.35, $P < 0.001$). Also, significant correlations were observed between BMD of the distal (cortical bone) area and both TBM (r = +0.52) and CMI (r = +0.38); between BMD of the intermediate area and both TBM (r = +0.51) and CMI (r = +0.31, P = 0.002); and between the BMD of the proximal (trabecular bone) area and TBM (r = +0.44) and CMI (r = +0.33, $P < 0.001$ in all cases unless otherwise specified).

In the stepwise multiple correlation analysis between TBM and CMI and BMDWA, CMI entered in the first place and BMDWA in the second; the multiple correlation coefficient was +0.624, with a standard error of 4.15.

BMDWA was significantly different when compared between individuals with TBM values greater or lower than 17.5%, 15%, and 12.5% (Table 6); also, CMI was significantly different among these four groups. Conversely, when we classified our population according to BMDWA values, TBM and CMI showed, in general, progressively decreasing values with progressively decreasing BMDWA values (Table 7).

In Table 8, we show the sensitivity, specificity, and overall accuracy of different BMDWA values in diagnosing TBM values less than 20%, less than 17.5%, less than 15%, and less than 12.5%. Figure 4 shows the receiver-operating characteristic curves (ROC curves) obtained by plotting sensitivity and specificity values obtained at different cutoff points of BMDWA.

Test group

The test group was composed of 21 prehistoric individuals from Tenerife (Tegueste, 3 cases), El Hierro (Punta Azul, 4 cases), and Gran Canaria (Guayadeque, 13 cases; Santa Lucía, 1 case), including 9 males and 11 females. No differences existed between the study and test groups regarding TBM (mean value = 18.63 ± 5.67%), CMI (mean value = 0.3634 ± 0.0996), and DEXA (BMDWA mean value = 0.8638 ± 0.1806) values, or between males and females in the test group. Ten of them showed TBM values over 20%, 4 between 17.5–20%, 4 between 15–17.5%, and 3 less than 12.5%. Mean values of TBM, CMI, and BMDWA of these individuals are shown in Table 9. It is also noteworthy that all the individuals from Tenerife (TBM = 26.23%, 21.25%, and 21.99%; BMDWA = 1, 0.95, and 1.02, respectively; the first two were women, and the third was male) and El Hierro (TBM = 24.85%, 25.71%, and 21.34% for 3 males and 20.75% for 1 woman; BMDWA = 0.7, 0.97, 0.93, and 0.82, respectively) showed TBM values over 20%, i.e., in the normal range, in contrast with those from Gran Canaria.

A significant correlation was observed between TBM and CMI (r = +0.56, P = 0.009) and between TBM and BMDWA (r = +0.72, $P < 0.001$) (Fig. 5). Stepwise multiple correlation analysis showed that BMDWA was the first parameter which entered the final equation and CMI the second one; multiple r was 0.795, and the standard error was 3.63.

TABLE 4. Mean values of trabecular bone mass (TBM) and bone mineral density in the whole population (BMDWA), classified according to different values of cortico-medullary index (CMI)

	Number of cases	TBM (%)	BMDWA (g/cm^2)
CMI over 0.33	42	20.25 ± 4.99	0.9242 ± 0.2239
CMI between 0.275–0.33	20	18.01 ± 4.70	0.9190 ± 0.2225
CMI below 0.275	33	14.94 ± 4.50	0.7901 ± 0.2013
ANOVA		F = 11.5, $P < 0.001$	F = 4.011, $P = 0.021$
SNK		3 vs. 1, 2	

TABLE 5. Mean values of trabecular bone mass (TBM) and bone mineral density (BMDWA) in population with known age at death, classified according to different values of cortico-medullary index (CMI)

	Number of cases	TBM (%)	BMDWA (g/cm^2)
CMI over 0.33	16	21.66 ± 5.28	1.0527 ± 0.2337
CMI between 0.275–0.33	6	20.24 ± 4.22	0.9683 ± 0.1499
CMI below 0.275	12	13.84 ± 5.15	0.7428 ± 0.2396
ANOVA		F = 8.49, $P < 0.001$	F = 6.65, $P = 0.004$
SNK		3 vs. 1, 2	3 vs. 1, 2

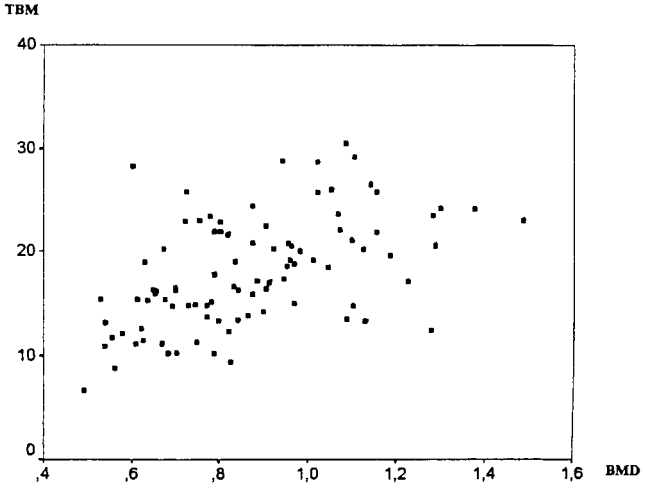

Fig. 3. Correlation between BMDWA and TBM in study group.

In Table 10, we show the sensitivity, specificity, and overall accuracy of different BMDWA values in diagnosing the presence of TBM values less than 20%, less than 17.5%, less than 15%, and less than 12.5% in the test group. Figure 6 shows the ROC curves obtained by plotting sensitivity and specificity values obtained at different cutoff points of BMDWA.

DISCUSSION

Bone is constantly remodelled throughout life. This remodelling process includes bone synthesis, due to osteoblastic activity, and bone resorption, due to osteoclastic activity. During infancy, adolescence, and early adulthood, bone synthesis predominates, so bone mass progressively increases until it peaks towards the second half of the third decade of life, and then declines gradually. The rate of bone loss in normal adults is less than 1%/year, except in women during the first 5–10 years after menopause, in whom bone loss is accelerated. Genetic factors (Johnston and Slemenda, 1995; Garabedian, 1995), physical activity (Smith and Gilligan, 1991; Recker et al., 1992), and diet (Eriksen and Langdahl, 1997) influence peak bone mass.

A decrease in bone mass is termed osteopenia. If osteopenia is severe enough, the risk of bone fracture increases (Blake et al., 1997).

Osteopenia on Gran Canaria

Although age at death cannot be established with confidence in our sample, it does seem that bone mass, assessed by either histomorphometry or by DEXA, decreases with age in prehistoric populations, a result fully in accordance with the previous statement and with the findings of other authors, who also found differences in bone mineral density (BMD) in different age groups. A decrease in DEXA-assessed BMD was observed in postmenopausal women from the 18th century (Lees et al., 1993). Similar results were obtained by other authors using similar, although not exactly the same (Bohr and Schaadt, 1987), methods in other population groups (Erikson, 1976; Dewey et al., 1969).

Both DEXA and histomorphometry yield differences between men and women, which are statistically significant when using DEXA. A similar result was obtained in the study mentioned previously (Velasco-Vázquez et al., 1999). Although speculative, it is possible that differences in physical activities might explain these observations. In any case, in the modern population, there is a trend towards higher TBM in men than in women.

In addition to this result, the overall prevalence of osteopenia in our sample is high, i.e., 31.58% (30 out of 95; 11 out of 34 with known age at death, or 32.35%) of individuals showing TBM values below 15% (a figure far below the mean value of TBM in normal population groups aged 20–59 years; Velasco-Vázquez et al., 1999). These figures are similar to those obtained when osteopenia is defined as a CMI value of 0.275 or less, both in the group with known age at death and in those without known age at death. Although several diseases may lead to osteopenia, this finding was already reported in the pre-Hispanic population of Gran Canaria and may

TABLE 6. Mean values of bone mineral density (BMDWA), cortico-medullary index (CMI), and trabecular bone mass (TBM) in four groups of individuals according to trabecular bone mass

Group	TBM (%)	CMI	BMDWA (g/cm^2)
1. TBM > 17.5%			
N	44	44	44
Mean	22.64	0.3690	0.9807
Standard deviation	3.26	0.0744	0.2001
2. TBM between 15–17.5%			
N	21	21	21
Mean	16.19	0.3028	0.8038
Standard deviation	0.73	0.0602	0.1886
3. TBM between 12.5–15%			
N	15	15	15
Mean	13.83	0.3394	0.8597
Standard deviation	0.83	0.1094	0.2065
4. TBM < 12.5%			
N	15	15	15
Mean	10.65	0.2080	0.6531
Standard deviation	1.48	0.0761	0.1069
F value	124.86	16.51	F = 12.72
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$

TABLE 7. Mean values of trabecular bone mass (TBM) and cortico-medullary index (CMI) in five groups by whole-area bone mineral density (BMDWA) values

	TBM (%; x ± SD)	CMI (x ± SD)
BMDWA greater than 1 (n = 26)	21.63 ± 4.96	0.3766 ± 0.0770
BMDWA between 0.90–0.99 (n = 14)	19.25 ± 3.61	0.3389 ± 0.0696
BMDWA between 0.80–0.89 (n = 15)	17.25 ± 4.40	0.3027 ± 0.0968
BMDWA between 0.70–0.79 (n = 16)	17.04 ± 4.95	0.3133 ± 0.0966
BMDWA less than 0.70 (n = 24)	14.18 ± 4.35	0.2798 ± 0.1043
	F = 8.92, $P < 0.001$	F = 4.007, $P = 0.005$

be due to widespread protein-calorie malnutrition (Velasco-Vázquez et al., 1999; González Reimers and Arnay-de-la-Rosa, 1992). 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. A decrease in bone mass ensues due to either reduced bone synthesis or increased bone destruction. Bone synthesis includes the formation of a protein and collagen matrix, termed osteoid, which later becomes calcified. In situations of prolonged starvation and/or in the so-called kwashiorkor-like malnutrition, the liver utilizes amino acids for the synthesis of important proteins such as albumin, transferrin, coagulation factors, and others, and, in the case of kwashiorkor-like malnutrition, acute-phase reactants. These amino acids derive from muscle breakdown (muscle is the main protein reserve), so that muscle atrophy ensues (in the marasmus type of malnutrition; in a situation of kwashiorkor-like malnutrition, as in sepsis or other inflammatory situations, muscle catabolism is much more intense, although over a shorter time). Muscle mass is a major determinant of bone mass, and hence, muscle activity is related to bone mass (Bendavid et al., 1996; Duppe et al., 1997). Synthesis of osteoid tissue also requires amino acids, but the amino-acid pool is mainly utilized by the liver in situations of malnutrition. Bone synthesis is, therefore, decreased (Bourrin et al., 2000b), not only because there are probably few amino acids available, but also because there is a decreased stimulus for bone synthesis due to decreased muscle strength. Although in situations of protein restriction, bone breakdown is also decreased (Bourrin et al., 2000a), an imbalance between synthesis and resorption ensues, leading to bone loss.

The "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 groups, and the relationship between osteopenia and undernutrition has been pointed out both in clinical settings (Ponzer et al., 1999; Schurch et al., 1998; Santolaria et al., 2000) and in experimental studies (Molina-Pérez et al., 2000).

Prehistoric Gran Canaria was densely populated (35,000–50,000 inhabitants in an area of 1,532 km^2), the economy was based mainly on agriculture (although some fishing and herding was also present), and the social structure was strongly hierarchical, at least at the time of the Spanish conquest, during the 15th century (Abreu Galindo, 1977). Chroniclers wrote that the pre-Hispanic population practiced female infanticide in order to control for population overgrowth. Moreover, the climate is irregular, with some years of very low rainfall. Locust plagues probably arrived at times from the African continent. Chroniclers also wrote that the surplus of good agricultural years was kept in huge silos to be distributed by the landlords in years of bad yield (Morales Padrón, 1994). Possibly during the dry years with low agricultural production, and perhaps also coinciding with locust plagues, malnutrition became widespread among

TABLE 8. Sensitivity, specificity and overall accuracy of different whole area bone mineral density (BMDWA) values in diagnosing the presence of different degrees of osteopenia defined on histomorphometrical grounds

	Trabecular bone mass (TBM) less than 20%		
	Yes	No	
BMDWA <0.7	22	2	Sensitivity: 36.07%
BMDWA >0.7	39	32	Specificity: 94.12%
			Overall accuracy: 56.84%
BMDWA <0.8	33	7	Sensitivity: 54.1%
BMDWA >0.8	28	27	Specificity: 79.41%
			Overall accuracy: 63.16%
BMDWA <0.9	43	12	Sensitivity: 70.49%
BMDWA >0.9	18	22	Specificity: 64.71
			Overall accuracy: 68.42%
BMDWA <1	52	17	Sensitivity: 85.25%
BMDWA >1	9	17	Specificity: 50%
			Overall accuracy: 72.63%
	TBM less than 17.5%		
	Yes	No	
BMDWA <0.7	21	3	Sensitivity: 41.18%
BMDWA >0.7	30	41	Specificity: 93.18%
			Overall accuracy: 65.26%
BMDWA <0.8	31	9	Sensitivity: 60.78%
BMDWA >0.8	20	35	Specificity: 79.55%
			Overall accuracy: 69.47%
BMDWA <0.9	40	15	Sensitivity: 78.43%
BMDWA >0.9	11	29	Specificity: 65.91%
			Overall accuracy: 72.63%
BMDWA <1	45	24	Sensitivity: 88.24%
BMDWA >1	6	20	Specificity: 45.45%
			Overall accuracy: 68.42%
	TBM less than 15%		
	Yes	No	
BMDWA <0.7	13	11	Sensitivity: 40.63%
BMDWA >0.7	19	52	Specificity: 82.54%
			Overall accuracy: 68.42%
BMDWA <0.8	23	17	Sensitivity: 71.88%
BMDWA >0.8	9	46	Specificity: 73.02%
			Overall accuracy: 72.63%
BMDWA <0.9	26	29	Sensitivity: 81.25%
BMDWA >0.9	6	34	Specificity: 53.97%
			Overall accuracy: 63.16%
BMDWA <1	27	42	Sensitivity: 84.38%
BMDWA >1	5	21	Specificity: 33.33%
			Overall accuracy: 50.53%
	TBM less than 12.5%		
	Yes	No	
BMDWA <0.7	11	13	Sensitivity: 73.33%
BMDWA >0.7	4	67	Specificity: 83.75%
			Overall accuracy: 82.11%
BMDWA <0.8	13	27	Sensitivity: 86.67%
BMDWA >0.8	2	53	Specificity: 66.25%
			Overall accuracy: 69.47%
BMDWA <0.9	15	40	Sensitivity: 100%
BMDWA >0.9	0	40	Specificity: 50%
			Overall accuracy: 57.89%
BMDWA <1	15	54	Sensitivity: 100%
BMDWA >1	0	26	Specificity: 32.5%
			Overall accuracy: 43.16%

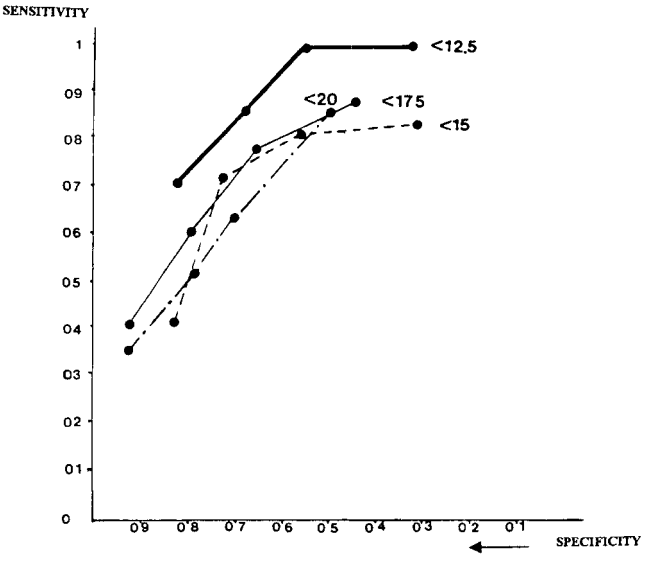

Fig. 4. ROC curves plotting sensitivity and specificity of different BMD values in diagnosis of different degrees of osteopenia in study group.

the lower social classes, leading to osteopenia. Although we cannot be sure about this, in Figure 3 it is evident that several individuals showed normal (and even high) TBM values, whereas many others showed TBM values clearly in the osteoporotic range. Thus, only some individuals were affected by osteopenia, a result that agrees with previous studies (Velasco-Vázquez et al, 1999; González-Reimers and Arnay-de-la-Rosa, 1992).

In the test group, we included some individuals from Tenerife and El Hierro. It is remarkable that none of these 7 individuals showed TBM values below 20%. We have already pointed out (Velasco-Vázquez et al., 1999) that osteoporosis in the pre-Hispanic population of El Hierro was only rarely observed, probably because of low population density and a greater reliance on herding and shellfishing. Also, no osteoporosis was observed among the 3 cases from Tenerife, a result in agreement with previous preliminary data derived from the analysis of a few iliac crest specimens (González-Reimers et al., 1988) and several dozen right tibiae recently analyzed (unpublished observations). Tenerife is the largest island of the Archipelago, and although inhabited by about 20,000 people (approximately 10 inhabitants/km^2) at the time of the Spanish conquest (it was the last island conquered by the Spaniards, after tenacious resistance), the island offers a relatively large, fertile northern side, and the economy was based on less developed agriculture than in Gran Canaria, but had an important goat-herding and shellfishing subsistence base. It is therefore likely that the impact of natural catastrophes on the economy of the islanders from Tenerife was less devastating than on Gran Canaria.

Double-energy X-ray absorptiometry for assessing prehistoric bone

Bone mass may be accurately assessed by histomorphometrical methods. However, this is an invasive procedure, and should not be repeated many

TABLE 9. Mean values of whole-area bone mineral density (BMDWA), cortico-medullary index (CMI), and trabecular bone mass (TBM) in four groups of individuals according to different values of trabecular bone mass in test group

Group	TBM (%)	CMI	BMDWA (g/cm^2)
1. TBM > 17.5%			
N	10	10	10
Mean	23.67	0.4134	0.9879
Standard deviation	3.37	0.0770	0.0971
2. TBM between 15–17.5%			
N	4	4	4
Mean	16.22	0.3888	0.7275
Standard deviation	0.68	0.0676	0.1070
3. TBM between 12.5–15%			
N	4	4	4
Mean	14.40	0.3333	0.7583
Standard deviation	0.62	0.0966	0.1889
4. TBM <12.5%			
N	3	3	3
Mean	10.73	0.2033	0.7722
Standard deviation	1.20	0.0428	0.2529
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$

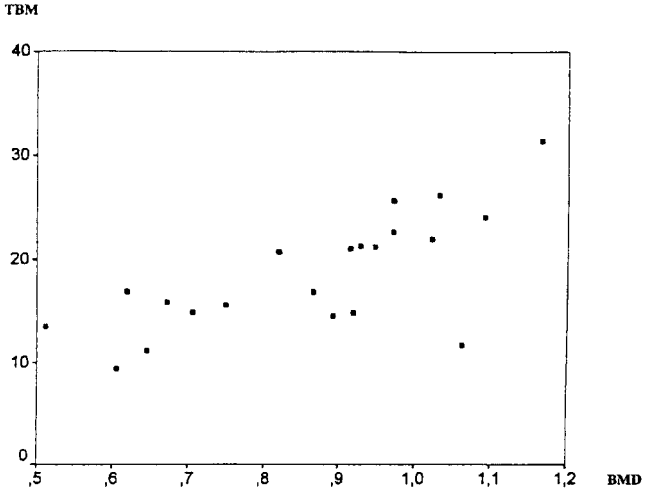

Fig. 5. Correlation between BMDWA and TBM in test group.

times in the same patient. Fortunately, new methods have been developed, including double-energy X-ray absorptiometry (DEXA). In this procedure, a low-energy X-ray beam is attenuated both by the soft tissue and bone of a certain part of the body, whereas a high-energy beam is practically not absorbed by the soft tissues but is absorbed by bones. After measuring the absorption of each of the two X-rays, two simultaneous absorption curves are generated, which are then used to calculate the attenuation caused by the bone and to cancel out the effect of soft tissue. In clinical settings, DEXA is the standard method for the diagnosis and follow-up of osteoporosis. Several studies support the excellent relationship between DEXA-assessed BMD and bone mass (Marshall et al., 1996). Moreover, DEXA-assessed BMD is the strongest known predictor of fracture risk (Levis and Altman, 1998), although accuracy of DEXA scans is limited by the variable composition of soft tissue. For instance, due to its higher hydrogen content, the attenuation coefficient of fat is different from that of lean tissue, and uneven fat distribution may affect the accuracy of DEXA measurements (Svendsen et al., 1995; Tothill and Pye, 1992).

Prehistoric bones not only lack surrounding soft tissue, but also bone marrow and fat in the trabecular spaces. Therefore, several authors perform DEXA analyses after introducing the bones into a water bath. Kneissel et al. (1994) obtained a relatively poor correlation between DEXA-assessed BMD and histomorphometrically-assessed trabecular bone mass both in 18 vertebrae and femoral necks (r^2 = 0.335 and 0.504, respectively). Postmenopausal women showed a marked decrease of BMD, so the authors concluded that bone loss due to aging occurred to a similar extent in a 4000 BP population and in the modern one. This finding contrasts with the observations of Lees et al. (1993), who analyzed 87 left femora from female subjects and found that postmenopausal bone loss in Ward's triangle region was significantly greater in the modern population than in the ancient one. Kneissel et al. (1994) concluded that DEXA analysis should not be encouraged for the evaluation of archaeological skeletal material, since diagenetic changes could influence X-ray absorptiometry, but would not affect the results obtained with invasive histomorphometric methods.

There may also be other reasons that may distort the BMD results in ancient bones. In a study (unpublished) to analyze the relationship between quantitative computerized tomography and histomorphometry, we observed that even by introducing the bones (well-preserved tibiae) for more than 1 hr in a water bath, we could not completely remove the entrapped air bubbles within the cancellous bone. This problem probably does not occur when using bone sections, but this is a destructive procedure.

However, even with entrapped air bubbles, and without surrounding soft tissue or water, bone attenuates the X-ray energy, so densitometric methods should be of value in the analysis of ancient bone specimens and in the estimation of bone mass, provided there is a similar degree of diagenetic change in the individuals studied. It is possible that mo-

TABLE 10. Sensitivity, specificity, and overall accuracy of different whole-area bone mineral density (BMDWA) values in diagnosing presence of different degrees of osteopenia in test group

	Trabecular bone mass (TBM) less than 20%		
	Yes	No	
BMDWA <0.7	5	0	Sensitivity: 45.45%
BMDWA >0.7	6	10	Specificity: 100%
			Overall accuracy: 71.43%
BMDWA <0.8	7	0	Sensitivity: 63.64%
BMDWA >0.8	4	10	Specificity: 100%
			Overall accuracy: 80.95%
BMDWA <0.9	9	1	Sensitivity: 81.82%
BMDWA >0.9	2	9	Specificity: 90%
			Overall accuracy: 85.71%
BMDWA <1	10	6	Sensitivity: 90.91%
BMDWA >1	1	4	Specificity: 40%
			Overall accuracy: 66.66%
	TBM less than 17.5%		
	Yes	No	
BMDWA <0.7	5	0	Sensitivity: 45.45%
BMDWA >0.7	6	10	Specificity: 100%
			Overall accuracy: 71.43%
BMDWA <0.8	7	0	Sensitivity: 63.64%
BMDWA >0.8	4	10	Specificity: 100%
			Overall accuracy: 80.95%
BMDWA <0.9	9	1	Sensitivity: 81.82%
BMDWA >0.9	2	9	Specificity: 90%
			Overall accuracy: 85.71%
BMDWA <1	10	6	Sensitivity: 90.91%
BMDWA >1	1	4	Specificity: 40%
			Overall accuracy: 85.71%
	TBM less than 15%		
	Yes	No	
BMDWA <0.7	3	2	Sensitivity: 42.86%
BMDWA >0.7	4	12	Specificity: 85.71%
			Overall accuracy: 71.43%
BMDWA <0.8	4	3	Sensitivity: 57.14%
BMDWA >0.8	3	11	Specificity: 78.57%
			Overall accuracy: 71.43%
BMDWA <0.9	5	5	Sensitivity: 71.43%
BMDWA >0.9	2	9	Specificity: 64.29%
			Overall accuracy: 66.66%
BMDWA <1	6	10	Sensitivity: 85.71%
BMDWA >1	1	4	Specificity: 28.57%
			Overall accuracy: 47.62%
	TBM less than 12.5%		
	Yes	No	
BMDWA <0.7	2	3	Sensitivity: 66.67%
BMDWA >0.7	1	15	Specificity: 83.33%
			Overall accuracy: 80.95%
BMDWA <0.8	2	5	Sensitivity: 66.67%
BMDWA >0.8	1	13	Specificity: 72.22%
			Overall accuracy: 71.43%
BMDWA <0.9	2	8	Sensitivity: 66.67%
BMDWA >0.9	1	10	Specificity: 55.56%
			Overall accuracy: 57.14%
BMDWA <1	2	14	Sensitivity: 66.67%
BMDWA >1	1	4	Specificity: 22.22%
			Overall accuracy: 28.57%

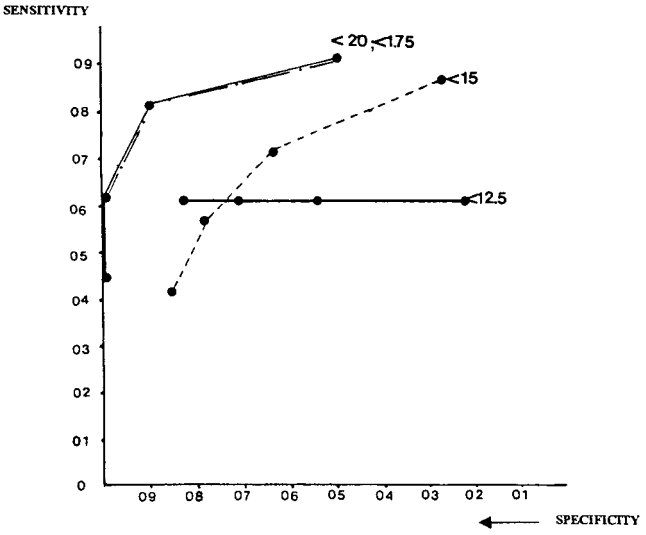

Fig. 6. ROC curves plotting sensitivity and specificity of different BMD values in diagnosis of different degrees of osteopenia in test group.

noenergetic photonic absorptiometry and dual-photon absorptiometry would be at least as useful as DEXA, due to the lack of soft tissue (in fact, dual-photon absorptiometry seems to be highly accurate in estimating bone mineral content in archaeological bone sections; Bennike et al., 1993). However, these methods were rapidly substituted by DEXA in a clinical setting so it is more useful to test the value of DEXA, a commonly used and rapidly available method, always taking into account that comparisons with living bone may be invalid (Lees et al., 1993). Indeed, the controls in our study show much lower BMDWA values than the prehistoric ones, despite the fact that none of them was osteopenic or osteoporotic, as assessed by BMD at the femoral neck and lumbar vertebrae. In contrast, a high proportion of the pre-Hispanic sample showed TBM and CMI values totally within the osteopenic range. Therefore, we cannot estimate the prevalence of osteopenia using BMD alone (we cannot define a normal range for a given prehistoric population), but we can estimate the intensity of osteopenia by comparing BMDWA values between different individuals of this population, since differences in BMDWA parallel differences in TBM, even in prehistoric bones, as shown in this work.

There is a good correlation between TBM and BMDWA, so that lower DEXA values correspond to lower TBM values. It is noteworthy that a similar number of cases show TBM values lower than 15% (30 cases) and BMDWA values lower than 0.7 (24 cases). Indeed, ROC curves plotting sensitivity and specificity of different cutoff points of BMDWA in the diagnosis of different cutoff points of TBM values are significantly displaced to the upper left-hand corner, suggesting that BMDWA, assessed as described in this study, is quite useful in the diagnosis of different degrees of osteopenia.

In a previous study, on samples different from those used in this work, we showed that the corticomedullary index (CMI) at the midshaft of the right tibia is also useful in the diagnosis of osteoporosis in

prehistoric bones. In this sample, CMI values were slightly higher than those previously reported, although there is still a good correlation between TBM and CMI as well as betwen CMI and BMDWA. Interestingly, when TBM falls below 12.5% or BMDWA falls below 0.7, CMI values also drop markedly, and if we consider individuals with CMI values below the mean minus 1.96 standard deviations of the control population to be osteopenic, the proportion of osteopenia detected with CMI is similar to that diagnosed by histomorphometry, and also similar to the proportion of individuals with BMDWA values below 0.7. This finding is consistent with the known ability of CMI to detect advanced stages of osteoporosis but not early ones (Jorge et al., 1988).

Possibly, diagenesis could introduce errors in the interpretation of our results. As pointed out by several authors (Pfeiffer, 2000), diagenetic changes may take place not only due to the dynamics of physical chemistry, such as ground water ionic exchange or mineral salt deposition, but also because of bacterial or fungal invasion. Deposition of calcium salts would surely affect not only TBM determination but, especially, DEXA analysis. However, these arguments do not apply in this case. As mentioned previously, the vast majority of samples analyzed belong to individuals who were deposited on stony layers either in volcanic caves or in tumuli. These caves are located in the cliffs of ravines; they are large enough to contain several hundred individuals, and the dry climatic conditions of the island favor preservation of the bones. It is important to bear in mind that many of the skeletons still preserve soft tissue. Although some calcium or other mineral salts could reach the bones, it is more difficult to accept that they would affect to a similar degree cortical bone (assessed by CMI), trabecular bone (assessed by histomorphometry), and bone mineral content (assessed by DEXA), and that these effects would be more marked in older individuals than in younger ones, or in women than in men, despite similar burial conditions.

Multiple regression analysis between TBM and BMDWA and CMI yields a highly significant multiple r of +0.625. However, the standard error is too high to allow accurate estimation of TBM using the last two parameters.

We repeated the analysis in a test group composed of 21 further individuals. This test group includes some individuals from Tenerife and El Hierro (with normal TBM values), in order to test the validity of the results of the study group in other population groups. As shown in Figures 5 and 6 and Tables 9 and 10, the results obtained in the test group were similar to those from the study group (except for the group with TBM less than 12.5%, due to the smaller number of cases). Indeed, the r value between BMDWA and TBM is +0.715, and when we also introduce CMI, the multiple r nears +0.8, although, as with the study group, the standard error is too high to permit the precise estimation of TBM using only CMI and BMDWA.

CONCLUSIONS

In addition to the differences concerning the prevalence of osteopenia in Gran Canaria, El Hierro, and Tenerife, we conclude that BMD may serve to detect differences in bone mass between different individuals of a given prehistoric population. In the population studied, BMDWA values (assessed at the proximal end of the right tibia) lower than 0.7 exclude the presence of normal TBM values (higher than 17.5%), with a specificity higher than 93%. However, since diagenesis surely affects DEXA-assessed BMDWA measurement, these values should not be extrapolated to other populations. On the other hand, the presence of entrapped air bubbles within the cancellous bone and the lack of bone marrow and soft tissue in ancient bones distort the comparison of DEXA-assessed BMD between ancient individuals and living ones. Therefore, with DEXA alone we cannot define a range of normal BMD values for prehistoric individuals, and thus, we cannot assess the prevalence of osteopenia in a given prehistoric population.

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