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Original Article

Reference Curve of Bone Ultrasound Measurements in Proximal Phalanges in Normal Spanish Women

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Abstract

There are clear discrepancies in how the different measurements of phalangeal bone ultrasound, such as the amplitude-dependent speed of bone ultrasound (Ad-SoS), correlate with age, given their dependence on gonadal status and other anthropometric variables. In order to contribute to clarifying these discrepancies, we evaluated the phalangeal Ad-SoS in healthy women—295 postmenopausal, 59 perimenopausal, and 270 premenopausal. Phalanges (II–V) of the nondominant hand were measured and the mean Ad-SoS was computed. There were significant differences between groups ($p < 0.0001$ in all cases), with the perimenopausal group presenting the intermediate values. For the overall group of women, the Ad-SoS was significantly and negatively correlated with age, weight, and body mass index (BMI), and positively correlated with height ($p < 0.0001$ in all cases). By gonadal status group, the premenopausal women showed the three significant negative correlations of Ad-SoS with age, weight, and BMI (each, $p < 0.0001$), the perimenopausal group only with BMI ($p < 0.007$), and the postmenopausal group with age and BMI ($p < 0.0061$ to $p < 0.0001$) and also with years since menopause ($p < 0.0001$). The premenopausal decline in AD-SoS requires further longitudinal studies, although in our experience it may depend on dietary habits and/or a diminished quality, though not quantity, of bone in this period of a woman's fertile life.

Key Words: Body mass index; bone ultrasound; bone ultrasound and weight; healthy women; premenopausal, perimenopausal, and postemenopausal.

Introduction

The early scepticism about the role of ultrasound in osteoporosis is decreasing as a result of the growing body of evidence, particularly from prospective studies, of the utility of quantitative ultrasound for the assessment of bone *(1).* Much recent work indicates that quantitative ultrasound scanning (QUS) could be more suitable for assessing fracture risk in a large population than conventional dual-energy X-ray absorptiometry (DXA) bone densitometry *(2–6).* QUS is a simple, inexpensive, and radiation-free technique with which to study the skeletal status of normal and osteoporotic subjects *(1,7,8).* Indirect and in vitro experiments have shown that quantitative

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[1] The authors dedicate this article to the memory of Prof. Horacio Rico, who died while this article was in press.

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ultrasound may provide information not only about the bone mass but also about bone tissue architecture and elasticity *(9,10).*

Peripheral measurement techniques were the first to be developed for the assessment of osteoporosis, and they remain useful. A longitudinal study has shown that radiogrammetry of the metacarpals can predict hip fractures *(11),* and a cross-sectional trial has demonstrated that radiographic absorptiometry of the phalanges discriminates between osteoporotic and healthy postmenopausal women *(12).* In a comparative study it has been observed that the phalanges of elderly women had higher deviations from peak adult bone mass compared with other techniques such as spine DXA, spine quantitative computed tomography (QCT), femoral neck DXA, and forearm DXA, and that it therefore seems to be an appropriate technique for evaluating the risk of fracture *(13).*

In this sense phalangeal ultrasound measurements *(14–16),* both because of the ultrasound technique itself and because of the location of the measurement in the phalanges, seem to be ideal for the evaluation of bone mass in normal and in pathological circumstances. This already quite widely used technique is low in cost, and the results are readily reproducible *(14,17).* Nonetheless, there are clear discrepancies between some of these studies. Thus, Ventura et al. *(14),* in a study of 417 normal women, found no correlation between the amplitude-dependent speed of bone ultrasound (Ad-SoS) and age in premenopausal women, while Pluskiewicz and Drozdzowska *(15),* in a study of 460 premenopausal women, did observe such a correlation. Other discrepancies noted by these and other workers refer to the correlation of Ad-SoS with weight and body mass index (BMI).

In an attempt to clarify the controversy, we used the same technique to study the Ad-SoS in a large group of women, considered as normal, separating them according to their gonadal status into premenopausal, perimenopausal, and postmenopausal.

Material and Methods

Subjects

A total of 624 women were studied. They were grouped by age every 5 yr, from 16 yr old through older than 85 yr, and also according to their gonadal status: 295 were postmenopausal (mean age 63.9 ± 10.6 yr, all naturally), defined as not having menstruated for at least 12 mo; 59 (mean age 47.4 ± 3.7 yr) were perimenopausal, defined as three to six missed menstrual periods with widely varying menstrual cycles, with episodes of menstrual bleeding occurring at intervals of more than 35 and less than 90 d; and 270 were healthy premenopausal (mean age 33.3 ± 9.7 yr). In all the premenopausal women, menstrual histories indicated current and prior menstrual regularity (11–13 cycles/yr).

All subjects were from the health district of Cáceres, Spain. In this geographic area, the mean annual hours of sun in the decade 1991–2000 was 2972.9 h/yr, with 3006.5 h in the year 2000. The premenopausal and perimenopausal women had previously visited the clinic of the Rheumatology Department for nonspecific pain for which no organic cause had been found. The postmenopausal women were referred randomly by a cohort of general physicians skilled at identifying osteoporosis risk factors, following a protocol used in our public health district. All subjects gave written informed consent. The investigation was approved by the Office for Protection from Research Risks of the Extremadura University and the Ethical Committee of the Hospital San Pedro de Alcántara in Cáceres.

Before a candidate was enrolled in the study, a complete medical history was taken and a physical examination was made. Normality was established on the basis of an interview and biochemical measurements of blood glucose, transaminases, γ–glutamyltranspeptidase (GGT), creatinine, calcium, phosphorus, total proteins, bilirubin, alkaline phosphatase, tartrate-resistant acid phosphatase (TRAP), and a coagulation study. In all cases, the calcium level was corrected for proteins. Radiological studies, based on A-P and lateral Rx of the thoracic and lumbar spine to exclude vertebral deformities, defined as the loss of more than 25% of the height of the anterior, middle, or posterior vertebral body, compared with normal reference values in subjects matched for age and sex *(18).* The women in the study were not taking any medication that could interfere with calcium metabolism. All lived active lives but did not practice sports. They responded to a questionnaire with items concerning physical activity, including their current daily activity (the amount of time typically spent

Table 1
Characteristics of the Groups of Premenopausal, Perimenopausal, and Postmenopausal Normal Women Studied

	Pre-	Peri-	Post-
n	270	59	295
Age (yr)	33.3 ± 9.7	47.4 ± 3.7	63.9 ± 10.6
Years since menopause			13.9 ± 10.2
Height (m)	1.62 ± 0.06	1.60 ± 0.06	1.57 ± 0.07
Weight (kg)	59.5 ± 7.8	63.6 ± 7.5	65.2 ± 8.6
Body mass index (kg/m^2)	22.8 ± 3.1	24.8 ± 2.6	26.3 ± 3.1
Ad-SoS (m/s)	2123 ± 48	2092 ± 53	2001 ± 73
Calcium intake (mg)	1124 ± 509	1103 ± 445	1153 ± 489
Phosphorus intake (mg)	1444 ± 572	1494 ± 435	1554 ± 640
Calcium/phosphorus (mg/mg)	0.77 ± 0.15	0.73 ± 0.18	0.74 ± 0.16
Physical activity	1.722 ± 0.833	1.750 ± 0.737	1.711 ± 0.831

either walking or on a bicycle out of doors each day). According to their physical activity, the women were classified as: 1) habitually doing no physical exercise; 2) doing 15–30 min/d of exercise; and 3) doing >30 min/d. Only 5% of the women smoked, but none smoked more than 10 cigarettes/d. Nutrient intake was quantified using dietetic scales, measuring cups, and spoons as we have described elsewhere, based on 7 d of dietary records *(19,20)*. Their alcohol intake was sporadic, and coffee intake in no case exceeded 100 mL/d. Height was measured using a Harpenden stadiometer with mandible plane parallel to the floor, and weight using a biomedical precision balance—both measurements were with the subject in pyjamas and without shoes. The BMI was calculated according to weight in kilograms divided by the square of the height in meters (BMI kg/m^2). The characteristics of the groups (number, age, years since menopause, anthropometric data, etc.) are summarized in Table 1.

Ultrasound Studies

In women with possible metacarpophalangical deformities, conventional X-rays of both hands were taken to exclude pathological alterations at the measurement sites, as described elsewhere *(21)*. The morphological properties of the phalanges were measured on a digitized X-ray image of the hand acquired using industrial film. Bone status was assessed as in previous studies *(19,22)*: for all the women, an ultrasound study was made of the 2nd to 5th proximal phalanges of the nondominant hand. The results were expressed as the mean ± SD of all the ultrasound measurements for each individual. The instrument used was a model DBM Sonic 1200R® (Igea, Capri, Italy) equipped with a caliper that closes tangentially on the phalanx and that measures the amplitude-dependent speed of sound through the phalanx in meters/second. Positioning and repositioning the instrument is easy because it uses the prominences of the lower phalangeal epiphysis as reference for placing the clip just behind the prominences. The instrument transmits with 22 W power at 1.2 MHz. Its precision was determined from five measurements made on eight women at time intervals not exceeding 21 d; the coefficient of variation was 1.1%. The interobserver coefficient of variation was 0.77%. All measurements were made at the room temperature of 22°C, kept constant throughout the year.

Analytical Studies

No smoking, coffee, tea, or alcohol intake, or exercise was permitted for 24 h before the day of investigation. Urine samples were collected in the morning after an overnight fast. Hematological and biochemical studies were made on venous blood samples obtained in the fasting state at 8:00 AM. Blood samples were centrifuged and the serum was stored at –20°C until assay. Biochemical concentrations were measured in serum using a BM/Hitachi system 717 automated analyser (Boehringer, Mannheim, Germany). The 24-h urinary calcium excretion was determined by atomic absorption spectroscopy using a Perkin Elmer model 5000 spectrophotometer (Perkin Elmer, Norfolk, CT, USA). All samples from each woman were analyzed in the same assay to reduce interassay variation. Assay reproducibility was determined by assaying four samples five times in five different runs at two laboratories. The CVs between runs and between laboratories were determined by components of variance, which give a statistical estimate of the variation of replicates of one or multiple assay runs. In every case, the CV was less than 6%. A biochemical study was made of 24-h urine to confirm the normality of calcium excretion and tubular phosphate resorption.

Statistical Studies

All the values were expressed as mean ± SD. The normal distribution of the data was confirmed by calculating the skewness and kurtosis before applying standard tests. The parameters studied (continuous variables) in each group (nominal variables, premenopausal, perimenopausal, and postmenopausal) were compared using analysis of variance (ANOVA) and covariance to determine the effects of nominal variables; data were analyzed by analysis of variance with a post-hoc test of differences between groups using the Bonferroni/Dunn test. A p-value of <0.05 was taken as the necessary condition for statistical significance. Relationships between continuous variables were examined by single regression analysis as appropriate, and by partial correlations adjusted for age and weight. Data were processed on a Macintosh computer using the StatView 5.01 statistical package (Abacus Concepts, Berkeley, CA, USA).

Results

The characteristics of the groups of pre-, peri-, and postmenopausal normal women studied are listed in Table 1. Apart from age, as is logical, the most significant data are those of weight and BMI, which were less in the premenopausal group compared with the other two groups ($p = 0.004$ to $p < 0.0001$). The Ad-SoS differed significantly between groups ($p < 0.0001$ in all cases), with the intermediate values belonging to the perimenopausal group. Table 2 gives the results for the Ad-SoS of the women according to age group.

Table 2
Bone Amplitude-Dependent Speed of Sound (Ad-SoS m/s) in Women by Age Group

Age group (yr)	n	Mean
16–20	40	2130 ± 37[a]
21–25	40	2148 ± 66[b]
26–30	30	2132 ± 42[a]
31–35	34	2114 ± 47[c]
36–40	43	2112 ± 43[c]
41–45	69	2119 ± 41[c]
46–50	75	2084 ± 46[d]
51–55	80	2042 ± 69[e]
56–60	43	2032 ± 44[f]
61–65	53	1997 ± 68[g]
66–70	34	1999 ± 64[h]
71–75	33	1972 ± 59[i]
76–80	24	1932 ± 89
81–85	16	1949 ± 29
>85	10	1914 ± 65

[a] $p < 0.0001$ vs from 46–50 to >85.
[b] $p < 0.01$ vs from 31–35 to >85.
[c] $p < 0.01$ vs from 46–50 to >85.
[d] $p < 0.0001$ vs from 51–55 to >85.
[e] $p < 0.001$ vs from 61–65 to >85.
[f] $p < 0.05$ vs from 61–65 to >85.
[g] $p < 0.05$ vs from 71–75 to >85.
[h] $p < 0.01$ vs from 76–80 to >85.
[i] $p < 0.01$ vs 76–80 and >85.

Table 3 gives the results of the correlation study (Fisher's r- to z-test) in the overall group of women. All the correlations between the parameters were found to be significant ($p < 0.0001$ in all cases). The correlations between the Ad-SoS and age (Fig. 1), weight, and BMI were negative, and positive with height ($p < 0.0001$ in all cases). Age had significant positive correlations with weight and BMI, and negative with height ($p < 0.0001$ in all cases). With the data separated according to gonadal status, in the premenopausal group the correlations were still significant and negative for the Ad-SoS with age ($r = –0.23$, $p < 0.0001$) (Fig. 2), weight ($r = –0.29$, $p < 0.0001$), and BMI ($r = –0.38$, $p < 0.0001$). In the perimenopausal group, there was only observed a correlation of the Ad-SoS with BMI ($r = –0.35$, $p = 0.007$), and in the postmenopausal group there were correlations of Ad-SoS with age ($r = –0.50$, $p < 0.0001$) and BMI ($r = –0.166$, $p = 0.0061$), and also with years since menopause (YSM) ($r = –0.452$, $p < 0.0001$).

Table 3
Correlation Matrix in the Overall Group of Women Studied Between Anthropometric Factors, Amplitude-Dependent Speed of Sound in Bone (Ad-SoS m/s), and Body Mass Index (BMI kg/m^2)

	Ad-Sos (m/s)	Age (yr)	Weight (kg)	Height (m)	BMI (kg/m^2)
Ad-Sos (m/s)	1.00	–0.73	–0.32	0.31	–0.49
Age (yr)	–0.71	1.00	0.32	–0.44	0.57
Weight (kg)	–0.32	0.32	1.00	0.24	0.81
Height (m)	0.31	–0.44	0.24	1.00	–0.36
BMI (kg/m^2)	–0.49	0.57	0.81	–0.36	1.00

$p < 0.0001$ in all, according to Fisher's r- to z-test.

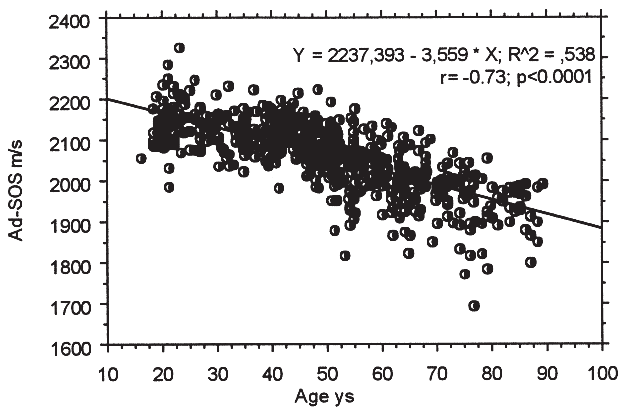

Fig. 1. Simple linear regression between age and amplitude-dependent speed of sound in bone (Ad-Sos), in the overall group of the women studied.

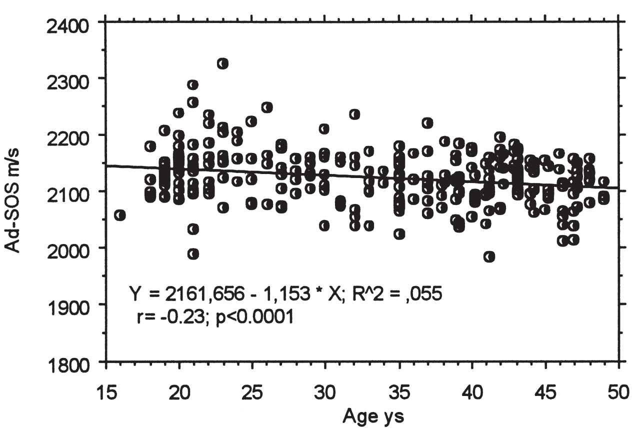

Fig. 2. Simple linear regression between age and amplitude-dependent speed of sound in bone (Ad-SoS), in only the premenopausal group of the women studied.

In an age-adjusted partial correlations study, for the overall group of women the correlation between Ad-SoS and BMI was still significant ($r = –0.48$, $p < 0.0001$), and, separating the groups according to gonadal status, we still observed this inverse correlation, although at a lower level of significance, in the pre-, peri-, and postmenopausal women ($r = –0.38$, $r = –0.346$, and $r = –0.17$; $p < 0.0001$, $p = 0.0070$, and $p = 0.0067$, respectively).

In the study of the analytical parameters (data not shown), there was a significant elevation of TRAP levels in the postmenopausal women compared with the pre- and perimenopausal. The remainder of the parameters did not differ.

Discussion

The basic principle of ultrasound measurement of the skeleton is that the speed at which ultrasound propagates in bone is determined by the mass density and by the "elastic modulus" (inherent material quality) of the bone. Ultrasound is quickly becoming the technique of choice in the measurement of bone mass since it is a cheaper and more portable method than previous techniques, and also has the advantage of not using ionizing radiation. With Ad-SoS, a widely used technique in studies of bone mass *(14–16),* we here observed a significantly negative correlation between this parameter and age in the women as an overall group and also when they were separated into premenopausal and postmenopausal groups. The premenopausal data agree with those of Pluskiewicz and Drozdzowska *(15),* but disagree with those of Ventura et al. *(14),* with a previous study of ours *(16),* and with those of Duboeuf et al. *(23),* which found that in separate analyses of pre- and postmenopausal women the AD-SoS remained unchanged before menopause. These differences can not be put down to sample sizes, which were almost the same in the study of Ventura et al. *(14)* and in the present study (n = 253 vs 270 normal premenopausal women, respectively). Neither do they seem to depend on the individual's weight or her BMI: in this case, both Ventura et al. *(14)* and we, here and in a previous work *(16),* observed a significant inverse correlation with the Ad-SoS *(16).* The reason may lie in nutritional differences, and indeed various factors could be involved including low Ca intake, low or high protein intake, and high phosphorus or caffeine intake, among others *(24).* It may be, as has been suggested by Heaney *(25),* that the effect of protein on bone mass depends on the relationship between calcium and protein intake. In our geographic area, normal women have a high ingestion of proteins *(20),* so that imbalances in Ca/P and Ca/Pr ratios might be the explanation for this difference in premenopausal women. It is obvious that this circumstance requires deeper study and analysis, which will have to be taken into account in future work with Ad-SoS.

Alenfeld et al. *(26)* report that a simple regression analysis applied to the overall group of the women that they studied showed a negative correlation of Ad-SoS with age (r = –0.73, p < 0.001), but a positive correlation with the anthropometric factors BMI (r = 0.37, p < 0.001), height (r = 0.40, p < 0.001), and weight (r = 0.23, p < 0.05). While the age results are in agreement with others in the literature, the BMI correlation is in clear disagreement with our own *(16)* and others' *(14)* findings in which there was an inverse correlation. We have confirmed the dependence on fatty tissue *(27,28),* and it is obvious that the greater the BMI the more body fat there is, and hence one has the inverse relationship between Ad-SoS and BMI. Nevertheless, neither can one say that this relationship is totally clear-cut: it depends on several factors including the techniques of evaluation of bone mass, the anatomical area evaluated, the BMI, etc. We consider that weight is one of the main determinants of bone mass in white women, as we have reported previously using dual-energy X-ray absorptiometry *(29).* Furthermore, we have observed that, as determined by peripheral quantitative computed tomography (pQCT), bone mass is unaffected by the weight *(30),* and, when ultrasound techniques are used, we and other workers have observed the Ad-SoS to be negatively correlated with the weight *(14,28).* Kleerekoper et al. *(31)* report a positive correlation between BMI and bone mineral density in the spine (L1) for postmenopausal women as measured by quantitative computed tomography (r = 0.127, p = 0.053, in white women with a mean BMI of 29.24 kg/m^2; and r = 0.295, p = 0.002, in black women with a mean BMI of 32.04 kg/m^2). It must be borne in mind, however, that they evaluate axial bone mass by means of QCT in spine (cancellous bone), whereas in the present study we evaluated peripheral bone mass in phalanges (cortical thickness) in postmenopausal white women with a mean BMI of 26.34 kg/m^2. These data therefore strongly suggest that the results, which differ substantially between techniques, are specific to the evaluation method and cannot be extrapolated to other measurement procedures.

We believe it to be important that in this study, as was noted by Ventura et al. *(14)* and also observed in our previous work *(16),* the Ad-SoS differed significantly among the three groups of women (pre-, peri-, and postmenopausal). This indicates the sensitivity of the Ad-SoS to small changes in gonadal status. There is also support for this in the study of de Aloysio et al. *(32),* who observed significant changes in the Ad-SoS in postmenopausal women according to whether or not they were receiving substitutive hormone treatment.

In conclusion, first, the premenopausal decline in AD-SoS observed in the present study requires further longitudinal investigations to determine which factors affect the skeleton before menopause. Our own experience is that we observe no changes before postmenopause when measuring bone mass with DXA *(33),* whereas in the present study using Ad-SoS we did see such changes. Given the QUS techniques indicate bone quality and quantity *(34),* one might presume that the decrease of the Ad-SoS with age in the premenopausal women could be a sign of deterioration in bone quality. This obviously merits particular attention and will have to be studied in depth. Second, the sensitivity in detecting gonadal status-dependent changes and the inverse relationship observed between the phalangeal Ad-SoS and BMI, an aspect of especial relevance in studies of the evolution of bone quality with age, above all in women, makes this a useful and promising technique for future studies.

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References

1. Njeh CF, Boivin CM, Langton CM. 1997 The role of ultrasound in the assessment of osteoporosis: a review. Osteoporos Int 7(1):7–22.
2. Miller PD, Siris ES, Barrett-Connor E, et al. 2002 Prediction of fracture risk in postmenopausal white women with peripheral bone densitometry: evidence from the National Osteoporosis Risk Assessment. J Bone Miner Res 17(12):2222–2230.
3. Frost ML, Blake GM, Fogelman I. 2002 A comparison of fracture discrimination using calcaneal quantitative ultrasound and dual X-ray absorptiometry in women with a history of fracture at sites other than the spine and hip. Calcif Tissue Int 71(3):207–211.
4. Hartl F, Tyndall A, Kraenzlin M, et al. 2002 Discriminatory ability of quantitative ultrasound parameters and bone mineral density in a population-based sample of postmenopausal women with vertebral fractures: results of the Basel Osteoporosis Study. J Bone Miner Res 17(2):321–330.
5. Montagnani A, Gonnelli S, Cepollaro C, et al. 2001 Usefulness of bone quantitative ultrasound in management of osteoporosis in men. J Clin Densitom 4(3):231–237.
6. Giardino R, Rotini R, Noia F, et al. 2002 Phalangeal ultrasonography in forearm fracture discrimination. Biomed Pharmacother 56(7):332–338.
7. Wu CY, Gluer CC, Jergas M, Bendavid E, Genant HK. 1995 Related. The impact of bone size on broadband ultrasound attenuation. Bone 16(1):137–141.
8. Gluer CC, Cummings SR, Bauer DC, et al. 1996 Osteoporosis: association of recent fractures with quantitative US findings. Radiology 199(3):725–732.
9. Langton CM, Njeh CF, Hodgskinson R, Currey JD. 1996 Prediction of mechanical properties of the human calcaneus by broadband ultrasonic attenuation. Bone 18(6):495–503.
10. Gluer CC, Wu CY, Jergas M, Goldstein SA, Genant HK. 1994 Three quantitative ultrasound parameters reflect bone structure. Calcif Tissue Int 55(1):46–52.
11. Jergas M, San Valentin R, Black M, et al. 1995 Radiogrammetry of the metacarpals predicts future hip fracture: a prospective study. J Bone Miner Res 10(suppl 1):475.
12. Takada M, Engelke K, Hagiwara S, et al. 1997 Assessment of osteoporosis: comparison of radiographic absorptiometry of the phalanges and dual X-ray absorptiometry of the radius and lumbar spine. Radiology 202(3):759–763.
13. Blake GM, Wahner HW, Fogelman IMD. 1999 The Evaluation of Osteoporosis: Dual Energy X-Ray Absorptiometry and Ultrasound in Clinical Practice, 2nd ed. Martin Dunitz, London.
14. Ventura V, Mauloni M, Mura M, Paltrinieri F, de Aloysio D. 1996 Ultrasound velocity changes at the proximal phalanxes of the hand in pre-, peri- and postmenopausal women. Osteoporos Int 6(5):368–375.
15. Pluskiewicz W, Drozdzowska B. 1998 Ultrasound measurement of proximal phalanges in a normal Polish female population. Osteoporos Int 8(4):349–354.
16. Aguado F, Revilla M, Hernandez ER, Villa LF, Rico H. 1997 Ultrasound bone velocity on proximal phalanges in premenopausal, perimenopausal, and postmenopausal healthy women. Invest Radiol 32(1):66–70.
17. Benitez CL, Schneider DL, Barrett-Connor E, Sartoris DJ. 2000 Hand ultrasound for osteoporosis screening in postmenopausal women. Osteoporos Int 11(3):203–210.
18. Revilla M, Fraile E, Aguado F, Hernandez ER, Villa LF, Rico H. 1997 Vertebral and metacarpal morphometry as indicators of nutritional improvement. Clin Rheumatol 16(3):279–283.
19. Rico H, Canal ML, Manas P, Lavado JM, Costa C, Pedrera JD. 2002 Effects of caffeine, vitamin D, and other nutrients on quantitative phalangeal bone ultrasound in postmenopausal women. Nutrition 18(2):189–193.
20. Pedrera JD, Canal ML, Postigo S, Lavado J, Hernandez ER, Rico H. 2001 Phalangeal bone ultrasound and its possible correlation with nutrient in an area of high protein intake. Ann Nutr Metab 45(2):86–90.
21. Montalban J, Rico H, Cortes J, Pedrera JD. 2001 Cortical bone mass and risk factors for osteoporosis among postmenopausal women in our environment. Rev Clin Esp 201(1):16–20.
22. Rico H, Aguado F, Revilla M, Villa LF, Martin J. 1994 Ultrasound bone velocity and metacarpal radiogrametry in hemodialyzed patients. Miner Electrolyte Metab 20(3):103–106.
23. Duboeuf F, Hans D, Schott AM, Giraud S, Delmas PD, Meunier PJ. 1996 Ultrasound velocity measured at the proximal phalanges: precision and age-related changes in normal females. Rev Rhum Engl Ed 63(6):427–434.
24. Dodd J. 1996 Nutrition and bone health. In: Mahan LK, Escott-Stump S (eds.). Krause's Food, Nutrition and Diet Therapy. Saunders. Philadelphia, 583–596.
25. Heaney RP. 1998 Excess dietary protein may not adversely affect bone. J Nutr 128(6):1054–1057.
26. Alenfeld FE, Wuster C, Funck C, et al. 1998 Ultrasound measurements at the proximal phalanges in healthy women and patients with hip fractures. Osteoporos Int 8(5):393–398.
27. Gomez M, Aguado F, Manuel J, et al. 1997 Influence of soft tissue (fat and fat-free mass) on ultrasound bone velocity: an in vivo study. Invest Radiol 32(10):609–612.
28. Rico H, Gomez M, Aguado F, Villa LF, Hernandez ER, Cortes J. 1999 Impact of weight in obese subjects on bone speed of sound. Invest Radiol 34(9):596–599.
29. Rico H, Arribas I, Casanova FJ, Duce AM, Hernandez ER, Cortes-Prieto J. 2002 Bone mass, bone metabolism, gonadal status and body mass index. Osteoporos Int 13(5):379–387.

30. Hernandez ER, Seco C, Cortes-Prieto J, Villa LF, Revilla M, Rico H. 2000 Gynecological factors and body mass index as determinants of bone mass in normal postmenopausal women. A study with peripheral quantitative computed tomography (pQCT). Eur J Obstet Gynecol Reprod Biol 92(2):193–198.
31. Kleerekoper M, Nelson DA, Peterson EL, et al. 1994 Reference data for bone mass, calciotropic hormones, and biochemical markers of bone remodeling in older (55–75) postmenopausal white and black women. J Bone Miner Res 9(8):1267–1276.
32. de Aloysio D, Rovati LC, Cadossi R, et al. 1997 Bone effects of transdermal hormone replacement therapy in postmenopausal women as evaluated by means of ultrasound: an open one-year prospective study. Maturitas 27(1):61–68.
33. Rico H, Revilla M, Villa LF, Alvarez de Buergo M. 1993 Age-related differences in total and regional bone mass: a cross-sectional study with DXA in 429 normal women. Osteoporos Int 3(3):154–159.
34. Abendschein W, Hyatt GW. 1970 Ultrasonics and selected physical properties of bone. Clin Orthop 69:294–301.