PRELIMINARY REPORT

Effects of Caffeine, Vitamin D, and Other Nutrients on Quantitative Phalangeal Bone Ultrasound in Postmenopausal Women

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OBJECTIVE: We investigated the controversial effects of coffee and other nutrients on bone mass.

METHODS: In a study of 93 healthy postmenopausal women (mean ± standard deviation: 57.3 ± 7.1 y old and 8.9 ± 7.5 y since menopause) selected on the basis of not having changed their eating habits since premenopause, not smoking, not exercising, not receiving hormone-replacement therapy, and having a weight in the range of 70% to 130% of their ideal weights, amplitude-dependent speed of sound (Ad-SOS) was determined by quantitative bone ultrasound, and a prospective 7-d diet survey evaluated the intake of caffeine and nutrients involved in calcium metabolism. Women were stratified according to their caffeine, calcium, and vitamin D intakes and ratios of calcium to phosphorus and to protein. Ad-SOS differed only with vitamin D intake and was greater in the group taking at leasst 400 IU/d ($P < 0.0001$).

RESULTS: In simple and multiple regression analyses, the only significant variable that affected Ad-SOS and nutrient intake was vitamin D ($P < 0.0001$). Phalangeal bone Ad-SOS was influenced only by the intake of vitamin D, not of caffeine or other nutrients.

CONCLUSIONS: This lack of effect of caffeine and protein may be related to good nutritional intake or the low levels of caffeine consumed. *Nutrition* 2002;18:189–193. ©Elsevier Science Inc. 2002

KEY WORDS: calcium, calcium and phosphorus ratio, calcium and protein ratio, coffee, folate, minerals, normal women

INTRODUCTION

With regard to the effect of coffee on bone mass, the literature suggests only confusion and controversy. In a recent analysis of 11 studies published since 1990, Lloyd et al.[1] found that most reported no association between caffeine (1,3,7-trimethylxanthine) intake and the frequency of fractures or changes in bone mineral density. Conversely, other studies have reported significant increases in the frequency of fractures or enhanced bone loss associated with caffeine intake.[1] This discrepancy may be due to differences in the methods used to determine bone mass, the population groups studied, and the way caffeine intake was evaluated. These factors are subject to different influences, which makes it difficult to calculate precisely the amount of caffeine ingested.

Among the different methods used to calculate caffeine intake, the food-frequency questionnaire is often used. However, because the caffeine content of brewed beverages can vary greatly, from 60 to 180 mg/180 mL per cup of coffee and from 20 to 100 mg/180 mL per cup of tea,[2,3] it is still difficult to quantify caffeine intake. Moreover, coffee and tea are not the only sources of caffeine. In the United States, 80% of caffeine intake is from coffee,[2] to which caffeine-containing soft drinks and chocolate must be added to obtain a good estimate of total caffeine intake. All these points should be taken into account to minimize errors. Other sources of errors, such as the mode of preparation of the beverages, are more difficult to evaluate.

In addition, to determine as exactly as possible the real long-term effects of caffeine and other nutrients on bone in the women studied, there should be no variables that can confuse results, such as major changes in dietary habits, hormone-replacement therapy, smoking, and exercise. Weight should be within the range of 70% to 130% of ideal weight. Therefore, in a group of postmenopausal women selected in accordance with the criteria mentioned, we measured bone mass by quantitative ultrasound propagation in phalangeal bone (Ad-SOS, amplitude-dependent speed of sound in meters per second), a technique that can evaluate different aspects in the bone that dual-energy x-ray absorptiometry (DXA) cannot and that is more interrelated with the quality than with the quantity of bone.[4] We also asked participants to complete a prospective 7-d dietary questionnaire, as in previous studies,[5,6] to evaluate caffeine intakes in coffee, tea, and other caffeine-containing beverages and intakes of other nutrients involved in calcium metabolism.

MATERIALS AND METHODS

Subjects

The study was carried during January and February of 1999 and 2000. Each month had an average of 219 h of sunlight. Ninety-three healthy postmenopausal women (mean ± standard deviation: 57.3 ± 7.1 y old and 8.9 ± 7.5 y since menopause) were studied. All had natural onset of menopause, defined as the absence of a menstrual period for at least 12 mo and serum follicle-stimulating hormone levels above 50 U/L. They were referred by a cohort of general physicians skilled at identifying osteoporosis risk factors according to a protocol in our public health district and randomly assigned to groups after the criteria were met. Before the candidate was enrolled in the study, a complete medical history was taken

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0899-9007/02/$22.00
PII S0899-9007(01)00718-3

and a physical examination was done. Normal weight was established as being in the range of 70% to 130% of ideal body weight for height and the results of a biochemical study. Radiologic study of the thoracic and lumbar spine was used to exclude those with 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 persons matched for age and sex previously published by our group[7]; 12 women were excluded on that basis. Subjects were not taking any medication and had no disease known to affect mineral metabolism (diabetes mellitus, liver disease, renal osteodystrophy, or parathyroid, thyroid, adrenal, or ovarian disease) that could interfere with calcium metabolism. All subjects were from the health district of Cáceres, in Spain. All subjects gave written informed consent. The Office for Protection Against Research Risks, University of Extremadura, approved the study in accordance with the Helsinki Declaration of 1975. Sample size was calculated in a pilot study by determining the variability of Ad-SOS measurements. Forty-five women were needed to attain statistical power with 95% confidence intervals, with a one-sided test of significance.

Heights were measured with a Harpender stadiometer, with the mandible plane parallel to the floor, and patients were weighed on a biomedical balance. Both measurements were made with subjects wearing pajamas and no shoes. The body mass index was calculated by dividing weight (kg) by height squared (m^2). Ideal weight was calculated in relation to height. The characteristics of the group are presented in Table I.

Based on 7 d of diet records,[5,6] and the study by Hasling et al.,[8] which reported that 112 mg of caffeine increases calcium loss by 0.24 mM/d (~10 mg), the women were stratified according to their reported current and lifelong caffeine use into one of two groups: 1) low caffeine intake, equivalent to no more than 100 mg of caffeine per day; and 2) high caffeine intake, equivalent to more than 100 mg of caffeine per day. Also, according to the most generalized recommendations (European Community and Spanish recommended daily allowance [RDA]), women were classified as having low (<800 mg/d) or normal (≥800 mg daily) calcium intake and as low (<400 IU/d) or normal (≥400 IU/d) vitamin D intake. The amount of caffeine in food and beverages was calculated according to the method of Barone and Roberts[9] and modified (Table II) for the serving sizes of coffee, tea, or chocolate usually consumed in our region. Food was quantified with the use of a dietetic scale, measuring cups, cans, small bottles, and spoons. Dietary calcium and caffeine levels were measured in duplicate of all the meals served. The survey on foods included 135 commonly consumed foods and others that were added by the subjects. The relation of food characteristic and percentages of intake by food type is shown in Table III.

TABLE I.

NUMBER, ANTHROPOMETRIC CHARACTERISTICS, YSM, AD-SOS, AND DAILY NUTRIENT INTAKE

	Intakes‡	RDA in EU and Spain
N	93	
Age (y)	57.3 ± 7.1	
YSm	8.4 ± 7.4	
Weight (kg)	60.3 ± 5.6	
%Ideal body weight	120 ± 7	
Height (m)	1.59 ± 0.06	
BMI (kg/m^2)	23.8 ± 1.5	
Ad-SOS (m/s)	2057 ± 45	
Total caffeine (mg)	62.8 ± 43.7	
Ca (mg/d)*	1234 ± 471	800
P (mg/d)	1665 ± 675	800
Ca:P (mg/mg)	0.76 ± 0.16	≥1[19]
Fe (mg/d)	18.2 ± 9.8	17–21
Zn (μg/d)	13.0 ± 8.3	15
Mg (mg/d)	352 ± 190	350
F (μg/d)*	983 ± 416	1500–3000
Cu (mg/d)	1.9 ± 2.1	2
Folates (μg/d)	224 ± 113	200
Vitamin D (IU)	363 ± 122	200†
Vitamin E (mg)	3.5 ± 2.1	8
Protein (g)	107 ± 49	47
Ca:protein (mg/g)	12.6 ± 5.7	≥20[18]
Fat (g)	85 ± 39	90
Carbohydrates (g)	271 ± 109	330
kJ	9590 ± 3399	9485

* The calcium and fluoride content of drinking water was not considered.
† 400 IU in Spain.
‡ Data are presented as mean ± standard deviation.
Ad-SOS, amplitude-dependent speed of sound (m/s); BMI, body mass index; EU, European Union; RDA; recommended daily allowance; YSM, years since menopause

Analytical Studies

No coffee, tea, smoking, alcohol, or exercise was permited for 24 h before the day of study. Fasting venous blood and urinary samples were collected in the morning, at 8 AM. The hematologic and biochemical studies were performed on fasted blood samples. Biochemical measurements were taken: blood glucose, transaminases, gamma-glutamyltransferase (GGT), creatinine, calcium, phosphorus, total proteins, bilirubin, alkaline phosphatase, tartrate-resistant acid phosphatase, and a coagulation study. In all cases calcium was corrected for proteins. A biochemical study was made of 24-h urine samples to confirm the normality of calcium excretion and tubular phosphate resorption. The biochemical studies were measured in serum with a BM/Hitachi automated analyzer system 717 (Boehringer, Mannheim, Germany). The excretion of calcium in 24-h urinary samples was determined with an atomic absorption spectroscopy (Model 5000 spectrophotometer, Perkin Elmer, Norfolk, CT, USA). Blood samples were centrifuged, and serum was stored at −20°C until analyzed. All samples were analyzed in the same assay to eliminate interassay variation. Assay reproducibility was determined by assaying four samples five times in five different runs at two laboratories. The coefficients of variation between runs and between laboratories were determined by components of variance, which give a statistical estimate of the variation of replicates of one for multiple assay runs. In every case, coefficients of variation was less than 6%.

Ultrasound Studies

As in previous studies,[6,10] conventional radiographs of both hands for each subject were made before the study to exclude pathologic alterations at the measurement sites. Bone status was assessed with an ultrasound device (DBM Sonic 1200, IGEA, Carpi, Italy) that measures Ad-SOS. Phalanges (II–V) of the non-dominant hand were measured and an average value was computed. Coupling was achieved using standard ultrasound gel. Two 16-mm–diameter, 1.25-MHz transducers assembled on a high-precision caliper measured the distance between the probes. The probes were positioned on the mediolateral phalangeal surfaces, using the phalanx head as a reference point. Positioning and repositioning this instrument is easy because the prominences of the lower phalangeal epiphysis are used as reference points. Instrument precision was determined from three measurements in eight subjects at intervals not exceed-

TABLE II.

CAFFEINE CONTENT PER SERVING, DEPENDING ON COFFEE TYPE, TEA, SOFT DRINK, OR CHOCOLATE*

Food/beverage	Type	Caffeine/serving (mg)	Serving size
Coffee	Ground roasted	42	75-mL cup
	Instant	39	75-mL cup
	Decaffeinated	1.5	75-mL cup
Tea	Looseleaf or bag	30	150-mL cup
	Instant	20	150-mL cup
Soft drink		33	330-mL can
with caffeine		20	200-mL bottle
Hot cocoa		4	150-mL cup
Chocolate milk		4	150-mL cup
Chocolate candy		1.5–6	1 oz

* Modified from Barone and Roberts.[9]

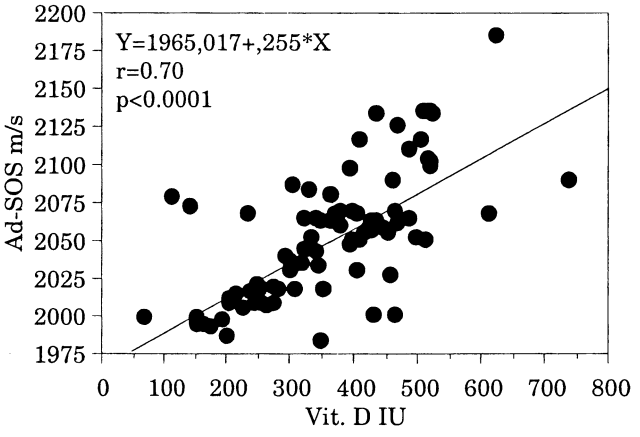

FIG. 1. Correlation, by simple regression, of Ad-SOS (m/s) with daily vitamin D intake. Ad-SOS, amplitude-dependent speed of sound.

ing 21 d. The coefficient of variation was 0.77%, and the interobserver coefficient of variation was 1.1%.

Statistical Analysis

All the values were expressed as mean ± standard deviation. The normal distribution of data were confirmed by calculating skewness and kurtosis before applying standard tests. The group differences were compared with analysis of variance. $P < 0.05$ was considered statistically significant. Regression and correlation analyses were used when appropriate to examine relations between continuous variables. Also, partial correlation, adjusted for important confounding variables such as age, weight, and years since menopause, was used when appropriate. These analyses were carried out with StatView 5.0.1 (SAS Institute, Cary, NC, USA) on a Macintosh computer.

RESULTS

Data in relation to age, years since menopause, anthropometric characteristics, Ad-SOS, total caffeine intake, and intake of other nutrients (minerals, folate, vitamins E and D, and macronutrients) are shown in Table I. Seventy-three women (78.5%) consumed less than 100 mg/d of caffeine, 20.4% consumed more than 800 mg/d of Ca, 9.6% had a daily Ca:P (mg/mg) intake ratio greater than or equal to 1, and 10.7% had Ca:protein (mg/g) intake ratio greater than or equal to 20. Only two women (2.1%) consumed more than 8 mg/d of vitamin E; 44% consumed at least 400 IU/d of vitamin D and 92% consumed at least 40 mg/d of protein. Overall, the greater protein intake and lower vitamin E intake in relation to the Spanish and European Union RDAs are clearly significant. The other nutrients studied were within normal RDA ranges. Fluoride intake in food seemed low compared with the RDA, but the fluoride content of drinking water was not considered in this study.

Ad-SOS did not differ between groups with regard to caffeine or calcium intake, Ca:P and Ca:protein ratios, or caffeine plus Ca, Ca:P, or Ca:protein intake (analysis of variance with Fisher's PLSD post hoc test). There was a significant difference with regard to vitamin D intake (≤400 IU/d), with Ad-SOS being greater in the group that consumed more than 400 IU/d (2086 ± 43 m/s versus 2037 ± 32 m/s; $P < 0.0001$).

With regard to caffeine intake, the only difference for the nutrients studied was carbohydrate intake, which was significantly higher in the group with high caffeine intake (398 ± 125 mg/d versus 245 ± 85 mg/d; $P < 0.0001$). Total energy intake also was greater in this group (12 125 ± 4173 kJ versus 9069 ± 2993 kJ; $P < 0.001$).

Ad-SOS correlated negatively with age ($r = -0.48$, $P < 0.0001$; 95% confidence intervals [CI] = −0.63 to −0.31) and years since menopause ($r = -0.46$, $P < 0.0001$; CI = −0.61 to −0.27). With regard to nutrient intake, simple regression studies showed only one significant relation (positive) between Ad-SOS and vitamin D intake ($r = 0.70$, $P < 0.0001$; CI = 0.20–0.31; Fig. 1). Multiple regression analysis of all the nutrients studied showed that the only significant relation was between Ad-SOS and vitamin D intake ($\beta = 0.26$, $P < 0.0001$; CI = 0.19–0.32). Also, multiple regression analysis with Ad-SOS as the dependent variable and weight, height, and energy intake as independent variables did not show a statistically significant association. In the biochemical study (data not shown), not alterations were observed.

TABLE III.

RELATIONS ACCORDING TO FOOD TYPE AND PERCENTAGE OF INTAKE IN THE TOTAL DIET

Food type	%Diet*
Fats	7.9 ± 2.0
Cereals	14.1 ± 5.4
Eggs	2.0 ± 1.2
Fish	6.6 ± 4.7
Fruit	15.8 ± 9.4
Greens	13.6 ± 10.1
Meats	11.6 ± 8.5
Milky	17.3 ± 12.5
Sugars	11.0 ± 6.2

* Data are presented as mean ± standard deviation.

DISCUSSION

Quantitative ultrasound identifies a cohort of at-risk individuals and bone properties different from those identified with DXA.[4] The assessment of bone densitometry using quantitative phalangeal ultrasound to measure Ad-SOS is a precise and reproducible technique that discriminates between control and osteoporotic sub-

jects. Its diagnostic sensitivity is similar to that of DXA of the lumbar spine and femoral neck,[11,12] and Ad-SOS measurements correlate well with DXA measurements.[10] This makes this method appropriate for evaluating bone mass.

It is difficult to determine exact caffeine intake in a given population because it depends on a series of variables that can affect quantification. In fact, most studies evaluate mainly coffee, tea, and caffeine-containing soft drinks, without determining the amount corresponding to chocolate. In this study, we evaluated all sources of caffeine by using the values reported by Barone and Roberts[9]; values were corrected for serving sizes in Spain, where a cup of coffee is about 75 mL rather than approximately 150 mL as in the United States. Serving sizes, in milliliters, of tea, cocoa, and chocolate milk are about the same. Massey[13] suggested that these sources can be ignored in the practical assessment of total intake because chocolate food and beverages have low caffeine content. We evaluated these sources to estimate total caffeine intake as accurately as possible. By doubly quantifying on food intake with caffeine content and calculating the quantity (mg) of caffeine according to the values reported by Barone and Roberts,[9] we believe that gives a very approximate estimate of the caffeine intake. In our study population, 97% of the women drank coffee and 50% drank tea. In the United States, about 80% of adults older than 50 y drink coffee and about 40% drink tea.[13]

The low caffeine intake of our Spanish population (mean $\sim$ 60 mg) was interesting. Barone and Roberts[9] reported that average caffeine intake in the United States is approximately 240 mg/d (4 mg/kg); Massey[13] reported a similar intake of 227 mg/d. One the one hand, the fact that our Ad-SOS values were similar to those reported in the United States[14] suggests that caffeine does not influence phalangeal quantitative ultrasound. On the other hand, in our group phalangeal Ad-SOS values did not differ between women with low ($<$100 mg/d) and high ($\geq$100 mg/d) caffeine intake. Also, no correlation was observed between Ad-SOS values and daily caffeine intake. In contrast, Hasling et al.[8] reported that 112 mg of caffeine increases calcium loss.

It has been reported that caffeine affects bone only when calcium intake is low.[15–17] However, Lloyd et al.[1] found no association between current caffeine intake and bone density in postmenopausal women. Our results were comparable to those of Lloyd et al.,[1] with similar Ad-SOS values for women with calcium intakes below or above 800 mg/d. Ad-SOS did not differ between groups when caffeine and calcium intakes were considered together. With regard to Ca intake and bone mass, the ratios of Ca:protein and Ca:P (which should be 20 mg:1 g or more[18] and $>$1 mg/mg,[19] respectively) may be more important than calcium intake per se. In our study, no significant differences were observed in Ad-SOS in relation to adequate or low Ca:P or Ca:protein ratios. There were no differences in Ad-SOS values with regard to combined caffeine and Ca:P or Ca:protein levels.

Independent of the negative correlation of Ad-SOS with age and years since menopause, which has been amply documented,[20] simple and multiple regression analyses positively correlated Ad-SOS only with vitamin D intake in this study. No correlation was observed with the other nutrients studied. One of the main, if not the principal, effect of vitamin D on bone is to facilitate its mineralization. In an elegant study, Tavakoli and Evans[21] found that the speed of ultrasound propagation in bone depends on the degree of bone mineralization. In patients undergoing treatment with anticonvulsants, we recently observed[22] that Ad-SOS is directly related to serum levels of vitamin D (25-OH-D_3). After the ingestion of a large dose of 25-OH-D_3, Ad-SOS values at 30 d had risen. That finding and the results of the current study and those reported by Tavakoli and Evans[21] confirm the importance of mineralization and, ultimately, of vitamin D on the speed of transmission of ultrasound in bone. Those studies[21,22] demonstrated the importance of an adequate intake of vitamin D to maintain the bone mass as measured by quantitative ultrasound. It also showed a significant difference in Ad-SOS values, depending on whether vitamin D intake was lower than, equal to, or greater than 400 IU/d. We believe that the changes we observed in vitamin D levels were not related to the seasonal variations in available vitamin D. To control for this factor, our study was carried out during the 2 mo when the amount of vitamin D provided by the environment is relatively constant: solar and meteorologic conditions were stable, and there was little change in the dietary habits of the participants.[22]

Aside from postmenopausal estrogen deficiency, various factors have been suggested as risk factors for osteoporosis, including low calcium intake, low or high protein intake, and high phosphorus or caffeine intake.[23] Our study showed that a large percentage of women (79.6%) consume less than 800 mg/d of calcium and 92% have a protein intake higher than recommended. Similar circumstances are common in other populations.[24] Cooper et al.[25] found no relation between protein intake and bone mineral density in 218 postmenopausal women with a protein intake that was almost twice as high as recommended. The same can be said of the debated role of calcium or the Ca:protein ratio.[26] Therefore, our results cannot be compared with those of other studies because few studies have examined micronutrients in postmenopausal women selected according to the inclusion criteria that we established. For instance, we know of no other study that has evaluated folates and copper, although copper is an important mineral for bone development.[27]

Our study of postmenopausal women, selected according to strict criteria, showed that phalangeal bone Ad-SOS was not influenced by the intake of caffeine or other nutrients, with the exception of vitamin D, and emphasized the importance of an adequate intake of vitamin D.

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