Dietary caffeine intake and bone status of postmenopausal women[1–3]

Tom Lloyd, Nan Rollings, Douglas F Eggli, Kessey Kieselhorst, and Vernon M Chinchilli

ABSTRACT Dietary caffeine intake has been suggested as a risk factor for bone loss in postmenopausal women. We measured the bone density of both hips and the total body in 138 healthy, postmenopausal women aged 55–70 y who had either never used hormone replacement therapy (HRT) or had used HRT for < 1 y. In this cross-sectional study, participants were stratified according to their reported current and long-time caffeinated beverage use into one of three groups: low [0–2 cups (180 mL, or 6 oz per cup) caffeinated coffee per day], moderate (3–4 cups caffeinated coffee per day), or high (≥ 5 cups caffeinated coffee per day). Caffeine intake was measured from diet records and by gas chromatography of each subject's brewed, caffeinated beverages. No association between caffeine intake and any bone measurement was observed. The anthropometric and nutrient intakes of the three groups were similar. Compared with caffeine intake based on chemical analysis of brewed beverages, 3-d prospective food records and computer-assisted analysis overestimated caffeine intake by nearly two-thirds. In conclusion, the habitual dietary caffeine intake of this cohort of 138 postmenopausal women ranged from 0–1400 mg/d and was not associated with total body or hip bone mineral density measurements. This study does not support the notion that caffeine is a risk factor for bone loss in healthy postmenopausal women. *Am J Clin Nutr* 1997;65:1826–30.

KEY WORDS Caffeine intake, bone density, postmenopausal women, osteoporosis risk. physical activity

INTRODUCTION

Caffeine is the most widely consumed psychoactive substance in the world (1), with coffee supplying > 80% of the caffeine consumed by adults in the United States (2). In recent years, numerous studies have reported on caffeine as a possible risk factor for bone loss in adult women (3–13). These reports have given contradictory results. The majority reported no association between caffeine intake and fracture frequency or changes in bone density (3, 6, 7, 9, 11, 12). However, several studies with large cohorts have reported small but significant increases in either fracture frequency or bone loss associated with increased caffeine intake (4, 5, 8, 13). In many of these studies, variables known to affect bone loss, eg, smoking, body weight, physical activity, calcium intake, overall nutrient intake, and hormone replacement therapy (HRT) have not been or could not be adequately controlled for.

Further, comparison of past studies has been confounded by the variations in study design, age of subjects studied, techniques for bone measurements, and techniques for estimation of caffeine intake. For estimation of bone change in major studies reported since 1990, five used hip fracture (4, 5, 9, 12, 13), five used multisite bone mineral density measurement by dual-energy X-ray absorptiometry (DXA) (6, 8, 10, 11, 17), two used bone mineral density measurements of the calcaneus (3, 7), and three used calcium excretion or calcium balance studies (14–16). Methods for estimating caffeine intake have been equally varied: four of the studies used food-frequency questionnaires (5, 9, 10, 13), three used prospective food diaries (6, 11, 17), three used recall questionnaires (3, 7, 8), and two used interview techniques (4, 12). The caffeine content of brewed beverages can vary tremendously; for coffee, from 60 to 180 mg/180-mL (6 oz) cup and for tea, from 20 to 100 mg/180-mL cup (1, 2). Thus, isolating the contribution of caffeine intake to bone status in older women in random study populations has been confounded by many covariates.

The purpose of the present study was to determine the effect of long-term habitual dietary caffeine intake on bone status in healthy postmenopausal women. To reduce confounding variables, healthy postmenopausal women aged 55–70 y who had minimal or no exposure to HRT were studied. The study cohort contained approximately equal numbers of low-, moderate-, and high-caffeine consumers. Total body and bilateral hip bone measurements were made by DXA. Caffeine intake was measured in two ways: *1*) by computer-assisted analysis of 3-d prospective food diaries and *2*) by gas chromatography of each subject's caffeine-containing brewed beverages.

SUBJECTS AND METHODS

All procedures involving human subjects were reviewed and approved by the institutional review board for clinical research studies of the Pennsylvania State University, College of Medicine and University Hospital. We used a cross-sectional study

[1] From the Departments of Obstetrics and Gynecology, Radiology, and Clinical Nutrition, The Center for Biostatistics and Epidemiology, College of Medicine and University Hospital, Pennsylvania State University, The Milton S Hershey Medical Center, Hershey.

[2] Supported by a grant from the International Life Sciences Institute and The National Coffee Association.

[3] Address reprint requests to T Lloyd, Department of Obstetrics and Gynecology, The Milton S Hershey Medical Center, Hershey, PA 17033. E-mail: tal3@psu.edu.

Received August 27, 1996.

Accepted for publication December 20, 1996.

of healthy white women aged 55–70 y who were stratified according to their reported current and life-long caffeine use into one of three groups: *1*) low—equivalent to 0–2 cups caffeinated coffee per day, *2*) moderate—equivalent to 3–4 cups caffeinated coffee per day, and 3)high—equivalent to ≥ 5 cups caffeinated coffee per day.

Subject recruitment

Volunteers for this study were recruited by local newspaper advertisements from a pool of > 75 000 women in this age group within 25 miles (42 km) of the Hershey Medical Center. Only individuals responsible for their own care and food preparation were studied. Initial screening of potential applicants was done by telephone interview.

Subject population: inclusion and exclusion criteria

Postmenopausal white women between the ages of 55 and 70 y and between 70% and 130% of ideal body weight for height were studied. Approximately equal numbers of volunteers in the low-, moderate-, and high-caffeine groups were recruited. To control for the potentially confounding effect of exercise, we recruited within each of the three caffeine use groups subgroups with low, (no regular exercise program), moderate (1–3 times per week for ≥ 30 min per time), and high (> 3 times per week for ≥ 30 min per time) exercise patterns. There were 13 women in the low-activity (self-reported), low-caffeine group; 18 in the low-activity, moderate-caffeine group, 23 in the low-activity, high-caffeine group; 24 in the moderate-activity, low-caffeine group; 22 in the moderate-activity, moderate-caffeine group; 13 in the moderate-activity, high-caffeine group;8 in the high-activity, low-caffeine group; 12 in the high-activity, moderate-caffeine group; and 5 in the high-activity, high caffeine group. Applicants were excluded during the telephone interview with the project coordinator for any of the following reasons: 1) use of postmenopausal HRT for ≥ 1 y; *2*) clinically expressed osteoporosis (any previously detected osteoporosis-related fracture, eg, hip, wrist, or spine), use of bisphosphonates, calcitonin, or sodium fluoride; *3*) use of thiazides or other diuretics; *4*) any use of corticosteroids for > 3 mo; *5*) current smoking or a history of > 5 pack-y; *6*) chemotherapy for cancer; *7*) rheumatoid arthritis; *8*) history of any endocrine disease known to affect mineral metabolism (of the parathyroid, thyroid, adrenal gland, or ovary); *9*) current or past consumption of more than one alcoholic drink per day; and *10*) current participation in a resistive or load-bearing aerobic exercise program for more than 5 h/week. Subjects with any of the following may have been included: heart disease being treated with nitroglycerin, calcium channel blockers, or β blockers; diabetes being treated with insulin; arthritis of < 5 y duration being treated with nonsteroidal antiinflammatory drugs; depression being treated with antidepressant or psychotropic drugs for < 5 y.

For the purposes of sample size projection, a sample size large enough to detect a 4% difference in mean bone density between the low- and moderate-caffeine intake groups and a 4% difference between the moderate- and high-caffeine intake groups was calculated. This difference was chosen because epidemiologic data suggest that such a difference represents an ≈50% change in fracture risk (19). To attain 95% statistical power with a one-sided 5% significance level test, 44 women per group were needed. Our target sample size was set at 150 women. After telephone interviews with the applicants for this project, 146 individuals were invited to a clinic visit. Of these, eight were excluded from the data set at the time of the clinic visit because of the discovery of one of the following exclusion criteria: three had previously unannounced artificial joints, three were not yet truly postmenopausal, and two were long-term users of drugs known to alter bone mineral metabolism. A final study population of 138 qualified individuals formed the data set. This met our recruitment goal of 132 individuals that would provide 80% statistical power with a 0.05 significance level test for detecting a 4% difference in total body bone mineral density between low- and high-caffeine intakes. Of the 138 in the cohort, 21 had used some form of HRT previously. The mean length of usage was 4.8 ± 0.7 mo with a range of 1 wk to 11.5 mo.

Clinic visits and data collection

Each subject was scheduled for a single clinic visit, at which time the following data were collected: a medical history, anthropometric measurements, and occupational and recreational activity assessments. Before completing a 3-d diet diary, all subjects received comprehensive instructions on its use. The diet diary was reviewed for completeness and then analyzed by the Nutritionist III (version 7.0) software program (18). In addition to obtaining data on caffeine intake from major sources, namely caffeinated beverages (coffee, tea, and soft drinks), this program collects caffeine content data from minor sources such as chocolate-containing products. Nutrient intake was expressed per day.

Bone measurements were made on the Hologic QDR 2000 dual-energy X-ray absorptiometer (Hologic, Waltham, MA). Total body bone mineral content (TBBMC), area (TBBMA), and density (TBBMD) as well as femoral neck bone mineral content (FNBMC), area (FNBMA), and density (FNBMD) measurements were made. This method accurately measures integral bone density and is accurate throughout the normal bone density range. Details of this method and its reproducibility in our institution were reported previously (19).

Quantitation of caffeine in beverages

Each subject who routinely made a caffeinated beverage for herself provided a 30-mL sample of each regularly made beverage. The caffeine contents of the samples of each subject's brewed beverages were determined by gas chromotography by National Medical Services, Willow Grove, PA. In brief, to 0.5-mL aliquots of each subject's beverage sample, an internal standard (10, 11, dihydrodibenz (b,f) (1, 4) oxazepen-11-one) (Lederle, Pearl River, NY), 0.5 mL saturated NH_4Cl and 1.0 mL toluene were added. The samples were mixed, centrifuged for 5 min at room temperature and 1400 × *g*, and aliquots of the toluene layer were analyzed by gas chromatography. Calibrators and control samples were run along with study subject samples. The gas chromatography configuration included a Hewlett-Packard model 5890 with flame-ionization detector, model 7673 liquid autosampler (both Hewlett-Packard Co, Wilmington, DE) and a 15 m × 0.32 mm internal diameter capillary column using DB-1 liquid phase (J & W Scientific, Folson, CA) and helium as the carrier gas at 70 kPa. Column oven temperature was programmed from 100 °C to 300 °C at

20 °C/min. The detector and injection ports were maintained at 220 °C and 300 °C, respectively. The extracted beverage samples and spiked controls had CVs of 6.0% and 9.1% at 280 μg and 54 μg caffeine per milliliter original sample.

Statistical analysis

Descriptive statistics in the form of means and SDs were calculated for the three subgroups of self-reported caffeine intake. To compare these three subgroups, a one-way analysis of variance (ANOVA) was applied to the anthropometric, bone, and nutrient measurements. Linear regressions were applied to the bone measurement variables with the following regressors: actual caffeine intake, age, weight, calcium intake, vitamin C intake, vitamin D intake, and self-reported activity level. The SAS PROC GLM (SAS Institute, Cary, NC) statistical package was used for these procedures.

RESULTS

The descriptive statistics of the three study subgroups including physical description, exercise levels, bone measurements, daily nutrient intakes, and daily caffeine intake from food record analyses and from gas chromatography analyses are given in **Table 1**. Of the 23 variables presented in Table 1, the three study subgroups differed with respect to caffeine intake, body weight, and meat and dietary fat consumption. The high-caffeine use group ate slightly more meat and fat and was slightly heavier. The actual caffeine intake of the three subgroups approximated their self-reported usage. In the self-described low-caffeine intake group, 6 of the 45 were actually moderate caffeine users according to their diet records; in the self-described moderate-caffeine intake group, 12 of the 52 were slightly over the moderate intake of up to four cups coffee per day. In the self-described high-caffeine intake group, 16 of the 41 were below the high intake of five cups caffeinated coffee per day. Thus, 34 of the 138 volunteers (25%) misclassified themselves with respect to caffeinated beverage use.

By using 80 mg caffeine per 180-mL cup as a standard, analysis of the food records showed the low-caffeine group consumed just > one cup per day, the moderate-caffeine group about four cups per day, and the high-caffeine group about seven cups per day. However, the mean caffeine intake values for each of the three study groups derived from food record analyses were ≈1.8 times greater than the values obtained by adding the values from gas chromatography of the subjects' brewed beverages plus known values of commercial beverages

TABLE 1
Descriptive statistics of the study participants grouped according to self-reported caffeine consumption[1]

Variable	Caffeine consumption		
	Low (n = 45)	Moderate (n = 52)	High (n = 41)
Age (y)	62.5 ± 4.7[2]	62 ± 4.3	63.1 ± 4.8
Height (cm)	160.3 ± 6.8	158.5 ± 5.0	160.3 ± 5.25
Weight (kg)	63.4 ± 8.7	63.4 ± 8.7	68.6 ± 9.1[3]
Bone mineral measurements			
TBBMC (g)	1907 ± 307 (1816, 1999)[4]	1871 ± 251 (1801, 1941)	1912 ± 315 (1812, 2011)
TBBMA (cm^2)	1937 ± 186 (1882, 1993)	1909 ± 156 (1865, 1953)	1990 ± 158 (1940, 2039)
TBBMD (g/cm^2)	0.98 ± 0.09 (0.95, 1.01)	0.98 ± 0.08 (0.96, 1.00)	0.96 ± 0.10 (0.92, 0.99)
LFBMD (g/cm^2)	0.75 ± 0.10 (0.72, 0.78)	0.77 ± 0.09 (0.74, 0.79)	0.75 ± 0.11 (0.72, 0.79)
RFBMD (g/cm^2)	0.74 ± 0.10 (0.71, 0.77)	0.77 ± 0.09 (0.74, 0.79)	0.76 ± 0.11 (0.72, 0.79)
Nutrient intake			
Caffeine (mg/d)			
By gas chromatography	49.9 ± 55.3	179.6 ± 100.8	322.6 ± 167.8[5]
By Nutritionist III	95.1 ± 93.5	324.8 ± 131.7	575.1 ± 266[5]
Energy (kJ)	7101 ± 1463	7106 ± 1403	7440 ± 2188
Carbohydrates (g)	235.5 ± 56.3	239.5 ± 56.4	228.5 ± 66
Protein (g)	70.9 ± 15	68.8 ± 14.3	73.3 ± 18.6
Fat (g)	55.6 ± 18.3	54.2 ± 17.6	65.4 ± 27.1[6]
Calcium (mg)	766.9 ± 308.2	790 ± 246.7	781.9 ± 319.2
Magnesium (mg)	263.7 ± 98.1	281.7 ± 73.9	297.9 ± 96.6
Zinc (mg)	10.1 ± 7	10.8 ± 9.3	13.8 ± 11.5
Iron (mg)	15.1 ± 8.2	15.7 ± 8.3	18.8 ± 10.9
Food group intake (servings/d)			
Milk	1.2 ± 1.1	1.1 ± 0.8	1 ± 1
Fruit	3.3 ± 1.8	3.5 ± 2.1	2.8 ± 1.9
Vegetable	2.1 ± 1.2	1.8 ± 1	1.5 ± 1.1
Bread	8.8 ± 2.9	9.1 ± 2.7	8.7 ± 3.4
Meat	5 ± 1.4	4.6 ± 1.5	5.5 ± 1.9[7]

[1] TBBMC, total-body bone mineral content; TBBMA, total-body bone mineral area; TBBMD, total-body bone mineral density; LFBMD, left femoral bone mineral density; RFBMD, right femoral bone mineral density.

[2] $\bar{x}$ ± SD.

[3,6,7] Significantly different from low and moderate groups: [3] $P = 0.01$, [6] $P = 0.63$, [7] $P = 0.2$.

[4] $\bar{x}$ ± SD; 95% CIs in parentheses.

[5] All groups significantly different from each other, $P < 0.0001$.

and contributions from caffeine-containing food products other than coffee, tea, or soft drinks. This discrepancy between the two methods for obtaining caffeine intake was inspected by examining the geometric means for the entire study cohort (n = 138) and was found to be 1.85.

Greater detail of the relations between bone measurements and caffeine intake is provided in **Figure 1**. TBBMD for each study subject is shown on the horizonal axis (lower panel). Her caffeine intake according to chemical analysis of her caffeinated beverages plus contributions from food products is plotted on the vertical axis. The upper panel is arranged in the same fashion with each individual's caffeine measurement plotted according to her average FNBMD. There was no association between total body or average FNBMD and caffeine use as indicated by ANOVA or regression analysis, nor were any significant correlations found between dietary calcium intake and any of the bone measurements or between vitamin D or vitamin C intake and any of the bone measurements. The mean daily calcium intake for the entire cohort was 767 mg. To explore the possibility that caffeine might have an effect on individuals with lower daily calcium intakes, we separated the cohort into tertiles and compared the lowest calcium intake tertile (n = 46; mean daily intake 503 ± 92 mg Ca) with the 98 individuals in the second and third tertile. No differences in any of the bone measurements or partial correlations with caffeine or any of the other variables examined were found.

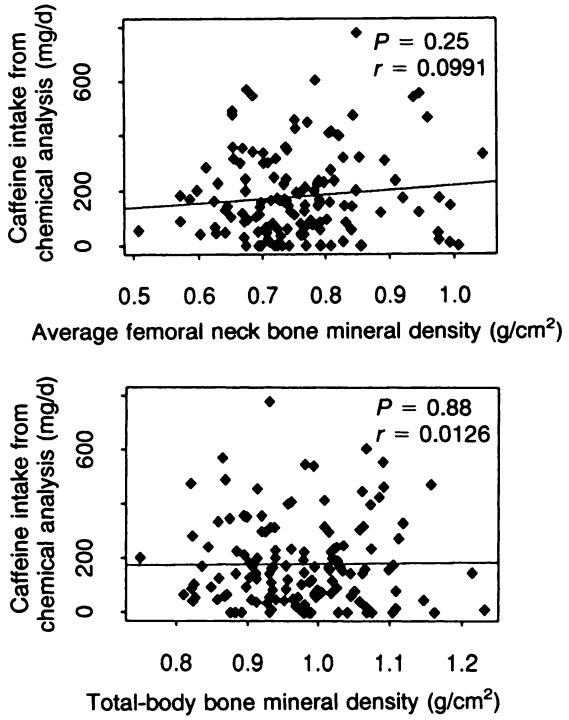

FIGURE 1. The relation of each subject's total body bone mineral density to her daily caffeine intake as determined by chemical analysis (gas chromatography) of her brewed beverages (for r = 0.0991, P = 0.25) and the relation of each subject's average femoral neck bone mineral density to her daily caffeine intake as determined by chemical analysis (gas chromatography) of her brewed beverages (for r = 0.0126, P = 0.88). Average femoral neck bone mineral density is the arithmetic mean of left and right femoral neck measurements.

Even when we adjusted for age, weight, and activity level, caffeine intake was not associated with any bone mineral measurements. Bone measurements of this study group were positively associated with weight and negatively associated with age. For example, for TBBMD, r^2 = 0.023 (P = 0.06) for weight and r^2 = 0.057 (P = 0.003) for age. When the 21 previous HRT users (mean usage: 4.8 mo) were compared with the 117 non-HRT users, no differences between the groups were found in any bone measurements or partial correlations with any of the above variables. The caffeine measurements by chemical analysis and from diet records for each study subject are compared in **Figure 2**. The two methods correlated well (r = 0.78) but they are nonconcordant.

DISCUSSION

High caffeine consumption has been proposed (4, 5, 13) and denied (3, 6, 7, 9) as a risk factor for decreased bone density and for increased likelihood of osteoporotic fractures in postmenopausal women. Comparison of recent major studies is complicated by the variety of bone-related measurements made, including hip fracture frequencies (4, 5, 9, 12, 13), bone mineral density measurements of the heel (3, 7), the spine (6, 8, 10, 11), total body (10, 11), and the hip (6, 8), and changes in urinary excretion of calcium (14–16). Comparison of caffeine intake among these studies is complicated by the variety of assessment techniques used, including food-frequency questionnaires (3, 5, 7–10, 13), interviews (4, 12), and 3- or 7-d diet records (6, 11, 17). The majority of these studies showed no effect of caffeine (3, 6–11) and two proposed a negative effect only for women whose dietary intake of calcium was < 800 mg/d (8, 10). In the largest recent epidemiologic study of risk factors for hip fracture in white women, the relative risk (RR) for caffeine intake of 1.2 was smaller than the risk for having a resting pulse > 80 beats/min (RR = 1.7) or for being in the lowest quantile for distant depth perception (RR = 1.4) (13). The medical importance of RR values of 1.2–1.5 as reported in four (5, 6, 12, 13) of the 13 cited studies may have been overemphasized by the media (20).

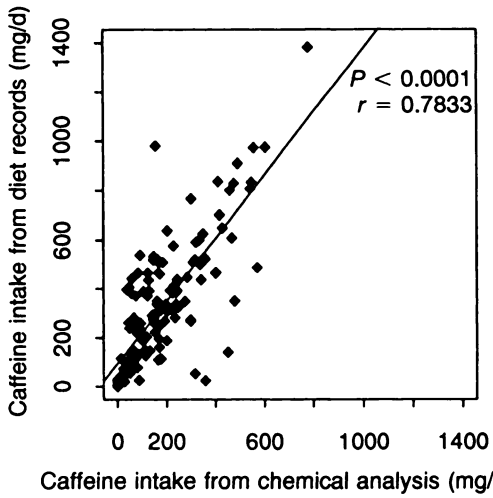

FIGURE 2. The relation for each subject between her daily caffeine intake as determined by chemical analysis and daily caffeine intake as measured from diet records (for r = 0.7833, P = < 0.0001).

The present study was designed to minimize confounding variables. Healthy postmenopausal white women who had used tobacco or HRT little or never were studied. They were recruited to obtain equal distributions among habitual low-, moderate-, and high-caffeine users. They were also recruited to represent low-, moderate-, and high-exercise patterns. As has been reported before (21), bone mineral density of the total body and of the hip was lower with greater age and higher with greater weight. However, caffeine intake, from zero to eight or more cups of caffeinated coffee per day, was not associated with any bone changes in this study population. When the cohort was separated by users of HRT (n = 21, mean usage: 4.8 ± 0.7 mo) or by the lowest tertile of dietary calcium intake (503 ± 92 mg/d) and compared with the respective remainder cohort, no differences either in absolute bone measurements or in partial correlations with any of the tested variables were observed.

More than 90% of the caffeine consumed by our cohort came from brewed coffee and tea. The computer-assisted estimate of dietary caffeine intake from prospective 3-d dietary records routinely produced values that were 1.8-fold higher than values obtained from gas chromatography of each subject's brewed beverage samples.

REFERENCES

1. Gilbert RM. Caffeine consumption. In: Spiller GA, ed. The methylxanthine beverages and foods: chemistry, consumption and health effects. New York: Alan R Liss, Inc, 1984;185–213.
2. Barone JJ, Grice HC. Seventh International Caffeine Workshop. Food Chem Toxicol 1994;32:65–77.
3. McCulloch RG, Bailey DA, Houston S, Dodd BL. Effects of physical activity, dietary calcium intake and selected lifestyle factors on bone density in young women. Can Med Assoc J 1990;142:221–7.
4. Kiel DP, Felson DT, Hannan MT, Anderson JJ, Wilson PWF. Caffeine and the risk of hip fracture. The Framingham Study. Am J Epidemiol 1990;132:675–84.
5. Hernandez-Avila M, Colditz GA, Stampfer MJ, Rosner B, Speizer FE, Willett WC. Caffeine, moderate alcohol intake, and risk of fractures of the hip and forearm in middle-aged women. Am J Clin Nutr 1991;54:157–63.
6. Cooper C, Atkinson EJ, Wahner HW, et al. Is caffeine consumption a risk factor for osteoporosis? J Bone Miner Res 1992;7:465–71.
7. Johansson C, Mellstrom D, Lerner U, Osterberg T. Coffee drinking: a minor risk factor for bone loss and fractures. Age Ageing 1992;21:20–6.
8. Barrett-Connor E, Chang JC, Edelstein SL. Coffee-associated osteoporosis offset by daily milk consumption. The Rancho Bernardo Study. JAMA 1994;271:280–3.
9. Cumming RG, Klineberg RJ. Case-control study of risk factors for hip fractures in the elderly. Am J Epidemiol 1994;139:493–503.
10. Harris SS, Dawson-Hughes B. Caffeine and bone loss in healthy postmenopausal women. Am J Clin Nutr 1994;60:573–8.
11. Reid IR, Ames RW, Evans MC, Sharpe SJ, Gamble GD. Determinants of the rate of bone loss in normal postmenopausal women. J Clin Endocrinol Metab 1994;79:950–4.
12. Tavani A, Negri E, LaVecchia C. Coffee intake and risk of hip fracture in Northern Italy. Prev Med 1995;24:396–400.
13. Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. N Engl J Med 1995;332:767–73.
14. Massey LK, Wise KJ. Impact of gender and age on urinary water and mineral excretion responses to acute caffeine doses. Nutr Res 1992;12:605–12.
15. Massey LK, Bergman EA, Wise KJ, Sherrard OJ. Interactions between caffeine and calcium on calcium and bone metabolism in older women. J Am Coll Nutr 1994;13:592–6.
16. Barger-Lax MJ, Heaney RP. Caffeine and the calcium economy revisited. Osteoporos Int 1995;5:97–102.
17. Lloyd T, Schaeffer JM, Walker MA, Demers LM. Urinary hormonal concentrations and spinal bone densities of premenopausal vegetarian and non-vegetarian women. Am J Clin Nutr 1991;54:1005–10.
18. N-Squared Computing. Nutritionist III. Version 7.0. Salem, OR: N-Squared Computing Analytical Software, 1991
19. Lloyd T, Eggli DF. Measurement of bone mineral content and bone density in healthy twelve year old, white females. J Nucl Med 1992;33:1143–54.
20. Tankes G. Epidemiology faces its limits. Science 1995;269:164–9.
21. Dawson-Hughes B, Shipp C, Sadowski L, Dallal G. Bone density of the radius, spine and hip in relation to percent of ideal body weight in postmenopausal women. Calcif Tissue Int 1987;40:310–4.