

# Chocolate consumption and bone density in older women<sup>1-3</sup>

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## ABSTRACT

**Background:** Nutrition is important for the development and maintenance of bone structure and for the prevention of osteoporosis and fracture. The relation of chocolate intake with bone has yet to be investigated.

**Objective:** We investigated the relation of chocolate consumption with measurements of whole-body and regional bone density and strength.

**Design:** Randomly selected women aged 70–85 y ( $n = 1460$ ) were recruited from the general population to a randomized controlled trial of calcium supplementation and fracture risk. We present here a cross-sectional analysis of 1001 of these women. Bone density and strength were measured with the use of dual-energy X-ray absorptiometry, peripheral quantitative computed tomography, and quantitative ultrasonography. Frequency of chocolate intake was assessed with the use of a questionnaire and condensed into 3 categories: <1 time/wk, 1–6 times/wk,  $\geq 1$  time/d.

**Results:** Higher frequency of chocolate consumption was linearly related to lower bone density and strength ( $P < 0.05$ ). Daily ( $\geq 1$  times/d) consumption of chocolate, in comparison to <1 time/wk, was associated with a 3.1% lower whole-body bone density; with similarly lower bone density of the total hip, femoral neck, tibia, and heel; and with lower bone strength in the tibia and the heel ( $P < 0.05$ , for all). Adjustment for covariates did not influence interpretation of the results.

**Conclusions:** Older women who consume chocolate daily had lower bone density and strength. Additional cross-sectional and longitudinal studies are needed to confirm these observations. Confirmation of these findings could have important implications for prevention of osteoporotic fracture. *Am J Clin Nutr* 2008;87:175–80.

**KEY WORDS** Chocolate, bone mineral density, older women, cross-sectional study, flavonoids, oxalate, fracture risk

## INTRODUCTION

Fractures are a leading cause of morbidity in older women. Lower bone density, mass, and strength are the principal risk factors for fractures in this population (1, 2). Nutrition is an important modifiable factor involved in the development and maintenance of bone and for the prevention of osteoporosis and fracture. It is acknowledged that a variety of dietary factors such as calcium, sodium, and protein can influence bone and the risk of fracture (3–7). It is also likely that a variety of other dietary factors have the potential to affect bone and the risk of fracture. Evidence suggests that flavonoid-rich foods and beverages may benefit bone health (8, 9). Chocolate can be an important source

of flavonoids in the diet in persons who regularly consume chocolate or chocolate-containing beverages (10). However, chocolate can also be an important source of oxalate (11, 12), which is an inhibitor of calcium absorption (13), and sugar, which may increase calcium excretion (14, 15). These components may adversely influence calcium balance.

The health effects of chocolate have been studied principally in relation to cardiovascular disease. Cocoa and chocolate have been promoted as having a range of beneficial cardiovascular properties (16). The effect of chocolate intake on other organ systems has not been studied. In light of our interest in the effect of nutrition on the aging skeleton, we have taken the opportunity to examine the relation between chocolate intake and bone structure. The objective of the present study was to investigate the relation of chocolate intake with measures of whole-body and regional bone density and strength in a random sample of older women at risk of osteoporosis as a result of the effects of an estrogen-deficient state (17).

## SUBJECTS AND METHODS

### Participants and study design

The participants involved in this study were recruited to a 5-y prospective, randomized, controlled trial of oral calcium supplements to prevent osteoporotic fractures. Women were recruited from the Western Australian general population of women aged > 70 y by mail with the use of the electoral roll. A random selection of 24 800 women on the electoral roll ( $n = 33\ 366$ ) was sent a letter to invite participation; 5586 women (22.5%) responded, and 1510 women were willing and eligible. A summary of progress through the phases of recruitment to 5 y is presented in **Figure 1** (7). Participants were ambulant and did not have any medical conditions likely to influence 5-y survival. At baseline,

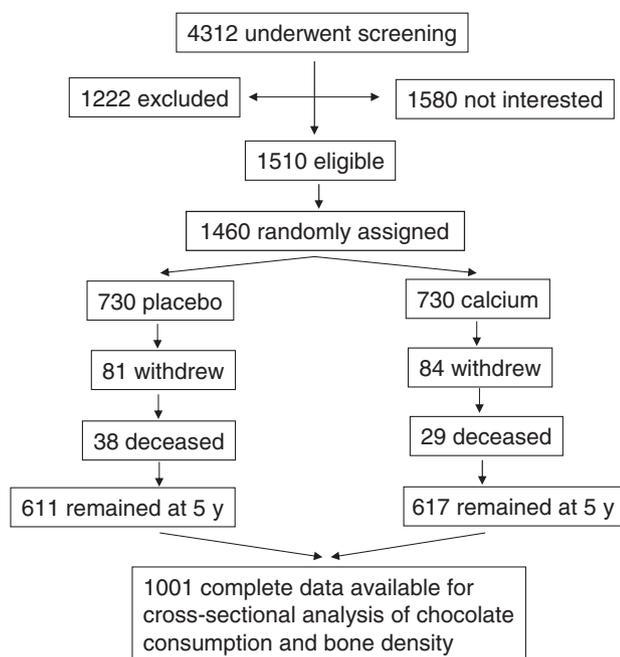
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**FIGURE 1.** Summary of progress through the phases of recruitment.

women were excluded if they had a significant current illness or if they were receiving any bone-active agent, including hormone replacement therapy. As previously reported, participants were similar in terms of disease burden and pharmaceutical consumption to whole populations of this age, but they were more likely to be from higher socioeconomic groups (6, 7). In the trial participants received 1.2 g of elemental calcium as calcium carbonate daily or a matched placebo. The current cross-sectional analysis uses data on the intake of chocolate and bone measurements collected at the 5-y time point and includes 1001 of these women for whom complete dietary and bone data were available. The primary reason that the current analysis included 1001 women of the 1228 women who completed the study to 5 y is that bone measurements were only performed on the women who were ambulant at 5 y. Informed consent was obtained, and the Human Rights Committee of the University of Western Australia approved the study.

### Demographics, clinical measurements, and physical activity

Participants completed a questionnaire that collected information about age, smoking history, years since menopause, physical activity, and residential postcode during a clinic visit (6, 7). Weight and height were measured at the 5-y assessment with participants wearing light clothes and no shoes, and the body mass index (BMI; in  $\text{kg}/\text{m}^2$ ) was calculated. Smoking status was coded into nonsmoker, ex-smoker, and current smoker. For physical activity, the women filled in a questionnaire that allowed estimation of energy used during exercise in kJ/d with the use of published energy costs of specific activities. The women were asked whether they participated in any sports, recreation, or regular physical activity. Women who answered “no” to this question scored zero, and women who answered “yes” were asked to list up to 4 sports, recreational activities, or forms of regular physical activity, including walking, that were undertaken in the past 3 mo. Energy expenditure (in kJ/d) for these activities was calculated

with the use of published energy costs. This measure was shown to be associated with bone density (6). Socioeconomic status was assessed with the use of relative social advantage related to residential postcodes according to the Australian Bureau of Statistics method (18). This variable was divided into 3 categories: lower, medium, and higher advantage.

### Assessment of diet and chocolate intake

As a result of our interest in the effects of beverages, in particular tea, on the skeleton (19), at year 5 of this longitudinal study we asked the participants to complete a questionnaire on beverage intake as well as a food-frequency questionnaire that contained specific reference to solid-chocolate intake.

#### Beverage intake

Participants completed a questionnaire that quantified usual beverage consumption. Participants were asked on average during the past 12 mo how many cups or glasses per week of chocolate (cocoa)-containing drinks they usually consumed. Responses ranged from 0 to 28. Responses were collapsed into 3 categories [ $<1$  cup/wk (rarely), 1–6 cups/wk (moderate),  $\geq 1$  cup/d (daily)].

#### Food-frequency questionnaire

A validated self-administered food-frequency questionnaire was administered from which the daily consumption of energy and nutrient intakes were estimated according to frequency of consumption and an overall estimate of usual portion size (20, 21). The frequency of solid chocolate intake was ranked from 1 to 10: 1) never, 2)  $<1$  time/mo, 3) 1–3 times/mo, 4) 1 time/wk, 5) 2 times/wk, 6) 3–4 times/wk, 7) 5–6 times/wk, 8) 1 time/d, 9) 2 times/d, 10)  $\geq 3$  times/d. Again, responses were collapsed into 3 categories:  $<1$  time/wk (rarely), 1–6 times/wk (moderate),  $\geq 1$  time/d (daily). The same food-frequency questionnaire was also administered at 1 y, which allowed for the assessment of the persistence of solid-chocolate intake over time. For solid-chocolate intake, 2.8% of women in rarely category at the 1-y assessment were in the daily category at the 5-y assessment, and 19% of women in the daily category at 1 y were in the rarely category at 5 y.

### Bone measurements

At the 5-y time point, bone density and strength were estimated with the use of 3 widely used and validated techniques (7). A fan-beam dual-energy X-ray absorptiometry (DXA; Hologic Acclaim 4500A; Hologic Corp, Waltham, MA) was used to measure the fat-free mass, fat mass, and bone density of the whole body (without the head) and the hip, including total hip, femoral neck, trochanter, and intertrochanter. Peripheral quantitative computed tomography (pQCT) was used to measure bone density and strength [polar stress-strain index (SSI)] in the tibia (XCT-2000; StraTec Medizintechnik GmbH, Pforzheim, Germany). The length of the tibia was measured from the inside medial malleolus to the anterior tibial tuberosity, and the measurement was taken at a site 4% of the length of the tibia proximal to the ankle joint. The voxel size was set at 150  $\mu\text{m}$  in the  $x$  and  $y$  directions and 1000  $\mu\text{m}$  in the  $z$  direction. The SSI was calculated as the product of the section modulus and cortical density normalized to the maximal physiologic cortical density of human bones (1200  $\text{mg}/\text{cm}^3$ ) for the polar moment. Calcaneal quantitative ultrasonography (QUS) was used to measure bone density (broadband ultrasound

attenuation and speed of sound) and strength (stiffness) in the heel (Lunar Achilles; GE Lunar Corp, Madison, WI).

### Statistical analysis

Statistical analyses were performed with the use of SPSS 11.5 software (SPSS Inc, Chicago, IL). The characteristics of the populations are presented as mean  $\pm$  SD for normally distributed variables and median (interquartile range) for skewed variables.  $P < 0.05$  was the level of significance in 2-tailed testing. The differences between groups according to frequency of chocolate consumption were assessed with the use of analysis of variance for normally distributed variables, Kruskal-Wallis  $H$  for skewed variables, and the chi-square test for categorical variables. The significance of the trend across frequency of chocolate consumption was calculated with the use of linear regression with bone measurements as the dependent variable and frequency of chocolate consumption as the independent variable. Between-group differences in bone measurements were analyzed with the use of general linear models with bone measurements as the dependent variable and frequency of chocolate consumption as the fixed factor. Bonferroni adjustment was used for post hoc comparisons

of bone measures between the  $\geq 1$  time/d and the  $< 1$  time/wk frequencies of chocolate consumption. For linear regression and general linear models, potential confounding factors, including age, BMI, smoking status, physical activity, socioeconomic status, years since menopause, calcium treatment status, and dietary variables, were included as covariates in multivariate models. Because of the potential that multicollinearity, particularly between dietary variables, could influence adjusted models, stepwise linear regression analysis was also performed.

### RESULTS

Subset analyses of the differences in demographics and bone structure according to chocolate intake that was determined from the beverage questionnaire or the food-frequency questionnaire showed similar trends. In the interests of clarity the summary statistic of total chocolate intake, including solid chocolate and chocolate-containing beverages, was used [ $< 1$  time/wk (rarely), 1–6 times/wk (moderate),  $\geq 1$  time/d (daily)].

The characteristics of participants according to frequency of chocolate consumption are reported in **Table 1**. In comparison to

**TABLE 1**

Characteristics of the 1001 women included in the cross-sectional analysis according to frequency of chocolate consumption<sup>1</sup>

	Rarely, $< 1$ time/wk ( $n = 482$ )	Moderate, 1–6 times/wk ( $n = 368$ )	Daily, $\geq 1$ time/d ( $n = 151$ )	<i>P</i>
<b>Demographics</b>				
Percentage of women (%)	48	37	15	
Age (y)	80.1 $\pm$ 2.7 <sup>2</sup>	79.8 $\pm$ 2.6	80.0 $\pm$ 2.6	0.38
Calcium treatment group (% of physically active)	49	52	50	0.75
Ever smoked (%)	37	35	34	0.76
Physical activity (kJ/d)	477 (162, 854) <sup>3</sup>	492 (171, 872)	493 (181, 831)	0.99
Socioeconomic status (% of higher advantage)	48 <sup>a</sup>	55 <sup>b</sup>	56 <sup>b</sup>	0.01
Time since menopause (y)	31 (27, 36)	31 (28, 35)	31 (28, 35)	0.81
<b>Body composition</b>				
Height (m)	1.57 $\pm$ 5.8	1.58 $\pm$ 5.7	1.57 $\pm$ 6.0	0.51
Body weight (kg)	68.1 $\pm$ 12.9 <sup>a</sup>	67.5 $\pm$ 11.3 <sup>a,b</sup>	64.8 $\pm$ 11.4 <sup>b</sup>	0.01
Fat mass (kg)	24.6 $\pm$ 7.7	24.1 $\pm$ 6.9	23.2 $\pm$ 6.7	0.13
Lean mass (kg)	41.2 $\pm$ 5.3	41.3 $\pm$ 4.8	40.3 $\pm$ 4.9	0.14
BMI (kg/m <sup>2</sup> )	27.4 $\pm$ 4.4 <sup>a</sup>	27.2 $\pm$ 4.2 <sup>a,b</sup>	26.2 $\pm$ 4.1 <sup>b</sup>	0.02
<b>Nutrient intake</b>				
Energy (MJ/d)	6.3 $\pm$ 1.9 <sup>a</sup>	7.0 $\pm$ 2.0 <sup>b</sup>	7.6 $\pm$ 2.3 <sup>c</sup>	$< 0.001$
Protein (g/d) <sup>d</sup>	79.0 $\pm$ 12.9 <sup>a</sup>	74.7 $\pm$ 13.4 <sup>b</sup>	72.6 $\pm$ 13.5 <sup>b</sup>	$< 0.001$
Total fat (g/d) <sup>d</sup>	60.4 $\pm$ 9.1 <sup>a</sup>	62.0 $\pm$ 9.0 <sup>b</sup>	61.4 $\pm$ 9.1 <sup>a,b</sup>	$< 0.03$
Saturated fat (g/d) <sup>d</sup>	23.1 $\pm$ 6.2 <sup>a</sup>	25.3 $\pm$ 6.1 <sup>b</sup>	25.4 $\pm$ 6.1 <sup>b</sup>	$< 0.001$
Total carbohydrate (g/d) <sup>d</sup>	178.6 $\pm$ 23.3 <sup>a</sup>	179.1 $\pm$ 23.2 <sup>a</sup>	185.6 $\pm$ 23.4 <sup>b</sup>	0.005
Starch (g/d) <sup>d</sup>	90.3 $\pm$ 17.9	87.7 $\pm$ 17.7	86.7 $\pm$ 17.9	0.03
Sugar (g/d) <sup>d</sup>	86.9 $\pm$ 20.1 <sup>a</sup>	90.2 $\pm$ 20.5 <sup>a</sup>	97.8 $\pm$ 20.8 <sup>b</sup>	$< 0.001$
Fiber (g/d) <sup>d</sup>	22.2 $\pm$ 5.2 <sup>a</sup>	20.7 $\pm$ 5.1 <sup>b</sup>	20.6 $\pm$ 5.2 <sup>b</sup>	$< 0.001$
Calcium (mg/d) <sup>d</sup>	898 $\pm$ 147	881 $\pm$ 261	934 $\pm$ 263	0.11
Potassium (g/d) <sup>d</sup>	2.87 $\pm$ 0.68 <sup>a</sup>	2.75 $\pm$ 0.77 <sup>b</sup>	2.75 $\pm$ 1.22 <sup>b</sup>	$< 0.001$
Magnesium (mg/d) <sup>d</sup>	288 $\pm$ 73 <sup>a</sup>	277 $\pm$ 82 <sup>b</sup>	287 $\pm$ 133 <sup>a,b</sup>	0.006
Alcohol users (%)	74 <sup>a</sup>	81 <sup>b</sup>	72 <sup>a</sup>	0.02
Alcohol intake in users (g/d)	4.1 (0.8, 12.5)	4.5 (0.8, 12.8)	3.7 (0.7, 12.7)	0.70
Fresh fruit (pieces/d)	2.23 $\pm$ 1.01	2.18 $\pm$ 1.02	2.24 $\pm$ 1.09	0.76
Vegetables (no. of different vegetables/d)	3.81 $\pm$ 1.10	3.72 $\pm$ 1.17	3.69 $\pm$ 1.29	0.35

<sup>1</sup> Normally distributed variable results are  $\bar{x} \pm$  SD, analyzed using ANOVA with Bonferroni adjustment for multiple comparisons; skewed variable results are medians with 25th and 75th percentiles in parentheses, analyzed with Kruskal-Wallis test. Categorical variable results are percentages, analyzed with the chi-square test. Values in the same row with different superscript letters are significantly different,  $P < 0.05$ .

<sup>2</sup>  $\bar{x} \pm$  SD (all such values).

<sup>3</sup> Median; 25th, 75th percentiles in parentheses (all such values).

<sup>4</sup> Adjusted for energy intake with ANOVA.

participants who rarely consumed chocolate (<1 time/wk), participants who consumed chocolate daily ( $\geq 1$  time/d) had a significantly lower body weight and BMI and a higher socioeconomic status and total energy intake. Estimated fat mass and lean mass were not significantly different between the groups. After adjustment for energy intake, daily chocolate consumers had significantly higher intakes of total and saturated fat, total carbohydrate, and sugar and lower intakes of protein, starch, fiber, and potassium. Fresh fruit and vegetable intakes were not different between the groups.

A higher chocolate consumption was associated with lower bone density and strength, assessed with the use of DXA (Table 2), pQCT, and QUS (Table 3). In univariate models a significant trend was observed for lower bone density and strength with more frequent chocolate consumption. A slight attenuation of these relations was observed after adjustment for potential confounders, primarily because of inclusion of BMI in the model. However, most trends remained significant.

In univariate analysis, for measurements of bone density, daily ( $\geq 1$  time/d) consumption of chocolate, in comparison to <1 time/wk, was associated with significantly lower estimates of bone density of the whole body ( $\bar{x}$ :  $-26$  mg/cm<sup>2</sup>; 95% CI:  $-43$ ,  $-9$  mg/cm<sup>2</sup>), total hip ( $\bar{x}$ :  $-42$  mg/cm<sup>2</sup>; 95% CI:  $-66$ ,  $-18$  mg/cm<sup>2</sup>), femoral neck ( $\bar{x}$ :  $-35$  mg/cm<sup>2</sup>; 95% CI:  $-55$ ,  $-16$  mg/cm<sup>2</sup>), trochanter ( $\bar{x}$ :  $-32$  mg/cm<sup>2</sup>; 95% CI:  $-53$ ,  $-11$  mg/cm<sup>2</sup>), and intertrochanter ( $\bar{x}$ :  $-53$  mg/cm<sup>2</sup>; 95% CI:  $-83$ ,  $-23$  mg/cm<sup>2</sup>;  $P < 0.01$  for all). pQCT volumetric bone density of the tibia ( $\bar{x}$ :  $-15$  mg/cm<sup>3</sup>; 95% CI:  $-23$ ,  $-7$  mg/cm<sup>3</sup>;  $P < 0.01$ ) and heel broadband ultrasound attenuation assessed by QUS ( $\bar{x}$ :  $-15$  mg/cm<sup>3</sup>; 95% CI:  $-23$ ,  $-7$  mg/cm<sup>3</sup>;  $P < 0.05$ ) showed a similar effect. For measurements of bone strength, daily chocolate consumption, in comparison to <1 time/wk, was associated with lower pQCT assessed by polar SSI of the tibia ( $\bar{x}$ :  $-73$  mm<sup>3</sup>; 95%

CI:  $-117$ ,  $-29$  mm<sup>3</sup>;  $P < 0.01$ ), and QUS measured heel stiffness ( $\bar{x}$ :  $-2.6\%$ ; 95% CI:  $-4.9\%$ ,  $-0.2\%$ ;  $P < 0.05$ ). The magnitude of the estimated differences in bone density and bone strength was slightly attenuated after adjustment for potential confounding factors, primarily because of the inclusion of BMI in the models. However, most differences remained significant (Table 2 and Table 3). Multivariate analysis with the use of stepwise linear regression did not alter the interpretation of the models (results not presented).

## DISCUSSION

Older women who consumed chocolate on a daily basis had lower bone density and strength in comparison to women who rarely consumed chocolate. Bone density and strength were assessed with the use of DXA, pQCT, and QUS, which assess bone structure in different ways and at different skeletal sites. These techniques have been evaluated extensively as predictors of fracture propensity and have been found to be effective (22, 23).

The principal risk of a lower bone density and strength appeared to be restricted to the 15% of women consuming chocolate  $\geq 1$  times/d. The estimated effect size is of a similar or greater magnitude compared with other dietary factors thought to be determinants of bone markers, such as calcium (6, 7), sodium (24), protein (25), and tea (9, 19).

Because the relations persisted after adjustment for covariates suggests that the effect may be associated with a constituent of chocolate rather than an associated lifestyle or dietary factor. If the observed relation between chocolate intake and bone measurements is causal, the components of chocolate responsible are uncertain. Chocolate can be a source of calcium (26) and flavonoids (10, 16), which are thought to have beneficial effects on bone (6–9). Chocolate is usually also rich in sugar and contains

**TABLE 2**

Mean bone density assessed with dual-energy X-ray absorptiometry according to frequency of chocolate consumption in a cross-sectional study of older postmenopausal women<sup>1</sup>

	Rarely, <1 time/wk (n = 482)	Moderate, 1–6 times/wk (n = 368)	Daily $\geq 1$ time/d (n = 151)	P for trend <sup>2</sup>
Whole body (mg/cm <sup>2</sup> )				
Univariate	848 (839, 856) <sup>a,3</sup>	851 (841, 861) <sup>a</sup>	822 (808, 836) <sup>b</sup>	0.024
Fully adjusted model <sup>4</sup>	849 (841, 857) <sup>a</sup>	849 (840, 858) <sup>a</sup>	822 (807, 836) <sup>b</sup>	0.012
Total hip (mg/cm <sup>2</sup> )				
Univariate	811 (799, 823) <sup>a</sup>	805 (792, 818) <sup>a</sup>	769 (749, 789) <sup>b</sup>	0.002
Fully adjusted model <sup>4</sup>	810 (799, 821) <sup>a</sup>	803 (791, 815) <sup>a</sup>	774 (755, 794) <sup>b</sup>	0.007
Femoral neck (mg/cm <sup>2</sup> )				
Univariate	687 (677, 697) <sup>a</sup>	677 (668, 688) <sup>a</sup>	652 (636, 668) <sup>b</sup>	0.001
Fully adjusted model <sup>4</sup>	687 (678, 696) <sup>a</sup>	676 (666, 686) <sup>a</sup>	655 (638, 672) <sup>b</sup>	0.002
Trochanter (mg/cm <sup>2</sup> )				
Univariate	632 (621, 645) <sup>a</sup>	629 (618, 640) <sup>a</sup>	599 (582, 617) <sup>b</sup>	0.011
Fully adjusted model <sup>4</sup>	632 (622, 642) <sup>a</sup>	627 (616, 638) <sup>a</sup>	601 (583, 620) <sup>b</sup>	0.016
Intertrochanter (mg/cm <sup>2</sup> )				
Univariate	958 (943, 973) <sup>a</sup>	949 (933, 965) <sup>a</sup>	905 (878, 931) <sup>b</sup>	0.002
Fully adjusted model <sup>4</sup>	955 (942, 969) <sup>a</sup>	946 (932, 961) <sup>a</sup>	914 (889, 938) <sup>b</sup>	0.012

<sup>1</sup> Values in the same row with different superscript letters are significantly different.  $P < 0.05$ .

<sup>2</sup> Analyzed with linear regression.

<sup>3</sup>  $\bar{x}$ ; 95% CI in parentheses (all such values).

<sup>4</sup> Included age, BMI, smoking status, physical activity (in kJ/d), socioeconomic status (higher, medium, or lower advantage), years since menopause, calcium treatment status (placebo or calcium), and total intakes of energy, protein, saturated fat, sugar, fiber, calcium, alcohol, potassium, magnesium, fruit, and vegetables.

TABLE 3

Mean bone density and strength in the tibia assessed with peripheral quantitative computed tomography (pQCT) and in the heel assessed with calcaneal quantitative ultrasonography (QUS) according to frequency of chocolate consumption in a cross-sectional study of older women<sup>1</sup>

	Rarely, <1 time/wk (n = 482)	Moderate, 1–6 time/wk (n = 368)	Daily, >1 time/d (n = 151)	P for trend <sup>2</sup>
pQCT				
Bone density (mg/cm <sup>3</sup> )				
Univariate	239 (235, 243) <sup>a,3</sup>	238 (233, 242) <sup>a</sup>	224 (218, 231) <sup>b</sup>	0.002
Fully adjusted model <sup>4</sup>	238 (234, 242) <sup>a</sup>	237 (233, 241) <sup>a</sup>	227 (220, 234) <sup>b</sup>	0.019
Polar SSI (mm <sup>3</sup> )				
Univariate	553 (530, 576) <sup>a</sup>	558 (534, 582) <sup>a</sup>	480 (445, 515) <sup>b</sup>	0.011
Fully adjusted model <sup>4</sup>	551 (531, 572) <sup>a</sup>	556 (533, 579) <sup>a</sup>	493 (456, 531) <sup>b</sup>	0.048
QUS				
BUA <sup>4</sup> (dB/MHz)				
Univariate	102 (101, 103) <sup>a</sup>	101 (100, 102) <sup>a,b</sup>	99 (98, 100) <sup>b</sup>	0.016
Fully adjusted model <sup>4</sup>	102 (101, 103) <sup>a</sup>	101 (100, 102) <sup>a,b</sup>	100 (99, 101) <sup>b</sup>	0.074
SOS <sup>5</sup> (m/s)				
Univariate	1517 (1515, 1519)	1516 (1513, 1518)	1513 (1508, 1517)	0.098
Fully adjusted model <sup>4</sup>	1516 (1514, 1518)	1516 (1513, 1519)	1514 (1509, 1518)	0.333
Stiffness (%)				
Univariate	72.6 (71.4, 73.7) <sup>a</sup>	71.8 (70.5, 73.1) <sup>a,b</sup>	69.9 (67.9, 72.0) <sup>b</sup>	0.038
Fully adjusted model <sup>4</sup>	72.2 (71.1, 73.4)	71.9 (70.6, 73.2)	70.6 (68.6, 72.7)	0.161

<sup>1</sup> SSI, stress-strain index; BUA, broadband ultrasound attenuation; SOS, speed of sound. Values in the same row with different superscript letters are significantly different,  $P < 0.05$ .

<sup>2</sup> Analyzed with linear regression.

<sup>3</sup>  $\bar{x}$ ; 95% CI in parentheses (all such values).

<sup>4</sup> Included age, BMI, smoking status, physical activity (in kJ/d), socioeconomic status (higher, medium, or lower advantage), years since menopause, calcium treatment status (placebo or calcium), and total intakes of energy, protein, saturated fat, sugar, fiber, calcium, alcohol, potassium, magnesium, fruit, and vegetables.

the methylxanthines, theobromine and caffeine (27), and oxalate (11, 12). Moderate caffeine intake would appear to have little effect on bone (28), and any effect of theobromine remains uncertain.

Oxalate is a potent inhibitor of calcium absorption (13). Furthermore, a single 100-g dose of dark chocolate was found to increase calcium excretion by 147% (14). The basis for this is not clear, but it is likely to include an effect of sugar to increase urinary calcium excretion (14, 15), dependent in part on an increase in plasma insulin that itself stimulates calciuria (29). Cocoa was also shown to have a separate effect to stimulate insulin, independent of the refined carbohydrate load (30). These effects are consistent with the observation of higher urinary calcium excretion after consuming chocolate in comparison to a matching dose of sugar (14). Furthermore, once creatinine clearance falls < 60 mL/min, a frequent occurrence in elderly women, plasma insulin increases more under the influence of a refined carbohydrate load, which is an effect related to the magnitude of the calciuria (29, 31). Whether these effects are sufficient to account for our observation is uncertain. Tea, another important dietary source of flavonoids (9), oxalate (20), and caffeine (9), is thought to be protective against loss of bone density (9, 19). However, data to support the importance of calcium nutrition and positive calcium balance on bone structure are strong (32). Thus, a high intake of chocolate may be another example of a nutrient that impairs this balance.

The current study has certain weaknesses. It is a cross-sectional association analysis restricted to elderly women that has not uncovered a potential mechanism for the suggested deleterious effect of chocolate on the skeleton. Although we

adjusted for a variety of potential confounding factors, it remains possible that chocolate is a surrogate for some other factor and is not responsible for the observed relation. Diet, lifestyle, or environmental factors that were either not measured or inadequately measured may account for the relation. The trend for lower BMI with higher frequency of chocolate consumption in this population is of interest. Yet adjustment for BMI only had a small effect to attenuate the observed differences. There is also potential for misclassification of frequency of chocolate consumption. However, classification of chocolate consumption by 2 independent instruments, the beverage questionnaire and the food-frequency questionnaire, resulted in similar relations. In addition, we believe that misclassification between frequencies of  $\geq 1$  times/d and <1 time/wk would be low.

This is the first study to investigate the relation between chocolate intake and bone structural measurements and raises concerns that frequent chocolate consumption may increase the risk of osteoporosis and fracture. Further cross-sectional and longitudinal studies are needed to confirm these observations. Confirmation could have important implications for prevention of osteoporosis and fracture.

The author's responsibilities were as follows—JM: data analysis, preparation of manuscript; had full access to all the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis; AD: Study design, participant recruitment, data acquisition, and preparation of manuscript; VB: data analysis and preparation of manuscript; IMD: study design and preparation of manuscript; RLP: Study design, participant recruitment, data acquisition, and preparation of manuscript. None of the authors had a personal or financial conflict of interest.

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