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Pavičić Žeželj, Sandra; Cvijanović, Olga; Mičović, Vladimir; Bobinac, Dragica; Crnčević-Orlić, Željka; Malatestinić, Giulia

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Effect of Menopause, Anthropometry, Nutrition and Lifestyle on Bone Status of Women in the Northern Mediterranean

S Pavicic Zezelj[1], O Cvijanovic[2], V Micovic[1], D Bobinac[2], Z Crncevic-Orlic[3], G Malatestinic[1]

ABSTRACT

Objective: *The purpose of this study was to explore the influence of age, menopause, anthropometry, nutrition and lifestyle on bone status of women of the Northern Mediterranean Region of Croatia, which is considered the Adriatic Coast of Southeast Europe.*

Methods: *Quantitative ultrasound measurement was performed on the women's right heel and the values of the primary parameters (the Broad Ultrasonic Attenuation and the Speed of Sound [BUA and SOS]) were obtained. Dietary data were assessed with specially designed semi-quantitative food frequency questionnaire. Multiple regression analysis was employed to examine the influence of age and anthropometry, as well as hormonal and nutritional factors on BUA and SOS.*

Results: *In all female subjects, both primary parameters were predicted by menopause. Among nutrition and lifestyle factors, carbohydrates were significant predictors for BUA ($\beta = -0.151$, $p < 0.05$), and smoking is significant predictor for SOS ($\beta = -0.113$, $p < 0.05$). In premenopausal women, BUA is significantly predicted by body height ($\beta = 0.71$, $p < 0.05$) and body mass index ($\beta = 1.44$, $p < 0.05$). In postmenopausal women, both primary parameters are strongly predicted by age and anthropometric parameters. Besides, SOS is significantly predicted by smoking ($\beta = -0.18$, $p < 0.01$) and alcohol ($\beta = -0.13$, $p < 0.05$). Besides, SOS is significantly predicted by smoking ($\beta = -0.18$, $p < 0.01$) and alcohol ($\beta = -0.13$, $p < 0.05$).*

Conclusion: *Bone quality in women from the Croatian Mediterranean Region mostly depends on their hormonal status. When the effect of menopause is controlled, bone status becomes dependent on age and anthropometry.*

Keywords: Anthropometry, lifestyle, menopause, nutrition, quantitative ultrasound

Efecto de la Menopausia, la Antropometría, la Nutrición y el Estilo de Vida Sobre la Condición Ósea de las Mujeres en el Mediterráneo Norte

S Pavicic Zezelj[1], O Cvijanovic[2], V Micovic[1], D Bobinac[2], Z Crncevic-Orlic[3], G Malatestinic[1]

RESUMEN

Objetivo: *El propósito de este estudio fue explorar la influencia de la edad, la menopausia, la antropometría, la nutrición y el estilo de vida en la condición ósea de las mujeres de la región mediterránea norteña de Croacia, que es considerada la Costa Adriática del Sudeste de Europa.*

Métodos: *Se realizó una medicación cuantitativa mediante ultrasonido en el talón derecho de las mujeres y se obtuvieron los valores de los parámetros primarios (la atenuación del ultrasonido de banda ancha y la velocidad del sonido [AUB y VDS]). Los datos dietéticos fueron evaluados mediante un cuestionario semi-cuantitativo de frecuencia de alimentos, especialmente diseñado. Se empleo el análisis de regresión múltiple para examinar la influencia de la edad y la antropometría, así como los factores hormonales y nutritivos sobre AUB y VDS.*

Resultados: *En todas las sujetos hembras, ambos parámetros primarios fueron predichos por la menopausia. Entre los factores nutrición y estilo de vida, los carbohidratos fueron predictores*

From: [1]Teaching Institute of Public Health Mountain-Littoral County, Rijeka, Croatia, [2]Department of Anatomy, Rijeka School of Medicine, Rijeka, Croatia and [3]Department of Internal Medicine, Rijeka University Hospital, Rijeka, Croatia

Correspondence: Dr O Cvijanovic, [2]Department of Anatomy, University of Rijeka, Brace Branchetta 20, 51 000, Rijeka, Croatia. E-mail: olgac@medri.hr

significativas de para AUB (β = -0.151, p < *0.05), y fumar es el predictor significativo para VDS (β = -0.113,* p < *0.05). En las mujeres premenopáusicas, la AUB es predicha significativamente por la altura del cuerpo (β = 0.71, < 0.05) y el índice de masa corporal (β = 1.44,* p < *0.05). En las mujeres postmenopáusicas, ambos parámetros primarios son fuertemente predichos por la edad y los parámetros antropométricos. Además, la VDS es predicha significativamente por el hábito de fumar (β = -0.18,* p < *0.01) y el alcohol (β = -0.13,* p < *0.05).*

Conclusión: *La calidad ósea de las mujeres de la región mediterránea croata depende principalmente de su estado hormonal. Cuando el efecto de la menopausia se controla, la condición ósea se hace dependiente de la edad y la antropometría.*

Palabras claves: Antropometría, estilo de vida, menopausia, nutrición, ultrasonido cuantitativo

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INTRODUCTION

The dynamics of bone remodelling is predominantly dependent on age and hormones, although there is evidence of other risk factors: decreased body mass index, unbalanced nutrition and physical inactivity, which could compromise physiological processes in osseous tissue (1−3). The strength of bone is determined by structural and material properties of the osseous tissue. Distribution of the osseous tissue in the spaces represents bone structural properties, while constituents of the bone matrix (collagen fibers and crystals of calcium phosphate) represent bone material properties (4). Balanced dietary calcium intake and good calcium absorption in the intestine, mediated by biologically active vitamin D, are important predictors for maintaining stiff and strong bones throughout life. Calcium is an almost ubiquitous mineral while vitamin D is a rare food nutrient. Deficiencies of both calcium and vitamin D are associated with low-bone quality conditions such as osteomalacia and osteoporosis (5). During life, dietary carbohydrates and proteins are needed to maintain body mass. Recent research has documented that elevated calcium urine excretion is secondary to animal protein consumption and if the diet is not supplemented by calcium, there could be a decrease in bone mineral density (6). Also, physiological changes in serum glucose concentration can stimulate bone resorption activity in a diurnal cycle (7). Except for dietary factors, certain lifestyle habits such as high caffeine and alcohol intake, as well as smoking could also compromise bone health (8−10). For instance, high caffeine intake is related to bone loss in menopausal women with low dietary calcium intake (8).

Nowadays, quantitative ultrasound (QUS) is widely used in the assessment of skeletal status. SAHARA Clinical Bone Sonometer measures the speed of sound (SOS, in m/s) and the Broad Ultrasonic Attenuation (BUA in dB/MHz) of the ultrasound beam passed through the heel. It has been proposed that BUA and SOS are related not only to bone density but also to structural properties of the bone. Thereby BUA reflects the number of spatial orientation of bone trabeculae, while SOS mostly reflects elastic properties of bone (11−13).

The aim of this study was to explore the influence of menopause, age, diet, anthropometry and lifestyle on BUA and SOS of female study participants. This inves-tigation was conducted in the Northern Mediterranean Region of Croatia.

SUBJECTS AND METHODS

Women with sedentary occupations were randomly chosen by primary care physicians in down town Rijeka. Under supervision of a family doctor, 800 women received the health questionnaire and 693 women completed it correctly. Exclusion criteria for further participation in the survey were: chronic diseases (n = 42) and hormone replacement therapy (n = 30). Women who took medicines that could alter bone metabolism, including food supplements with added calcium if taken longer than six months, were also excluded from further investigation (n = 86).

Depending on hormonal status, 535 women in the age range 30 to 85 years were divided into two subgroups: women with regular periods (premenopausal women, n = 263) and women in menopause (postmenopausal women, n = 272). A criterion for menopause was one year of amenorrhoea. Informed consent was obtained from all study participants. The local ethics committee approved the study and it was carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983.

This survey was based on person-to-person interview by trained interviewers. Dietary and non-dietary data were assessed with baseline questionnaires. The questionnaire consisted of two parts: the first part was a validated questionnaire that was used to obtain average daily calcium intake of Croatian women and in the second part were questions related to lifestyle, that were self-reported. The women were asked to give their average consumption of offered food items over the last month, providing a frequency range from once per month, to one or few times per day. Portion size was declared as small, medium and large and women noted their intake with the help of trained interviewers, demonstrating typical dishes (plates, cups and glasses) and food models or pictures in natural size. The

nutritive and energy values of each food noted were calculated using the composition tables of raw and cooked food [Kaic-Rak, 1990] (14). Attained dietary intake was compared to recommended daily allowance (RDA) and dietary reference intake [DRI] (15, 16).

The lifestyle questionnaire included questions on age and health-related habits (alcohol and coffee consumption, smoking and physical activity). The women declared their alcohol and café consumption, which was expressed as number of drinks per day, from which gram of ethanol/day and milligram of caffeine/day was calculated. Information on current and past smoking habits was collected by asking women about the number of cigarettes they currently smoked or used to smoke per day. Smoking habits were graded as smokers and non-smokers. A non-smoker was a woman who had never smoked or occasionally smoked less than one cigarette a day. A smoker was a woman who had smoked every day, one or more cigarette per day.

To assess physical activity, women were asked to record how many hours per week they spent in organized physical activities (recreation, gymnastics and physical training). According to physical activity results, women were divided into two groups: active women (more than two hours per week) and non-active women [less than two hours per week] (17). Body height was measured on a portable stadiometer, to the nearest 0.5 cm. Bodyweight was measured on an electronic scale (Tanita Corp., Tokyo, Japan) with accuracy of ± 0.1 kg. Measurements of bodyweight and body height were performed by trained mentors, with participants wearing no shoes and only light underwear. Body mass index was calculated as bodyweight divided by body height squared. Information on lifestyle habits (smoking, physical activity, alcohol and coffee consumption) were obtained by a self-administered questionnaire.

Quantitative ultrasound measurement was performed on the right heel, using Clinical Bone Sonometer Sahara Hologic (Hologic Inc, Bedford, Massachusetts, USA). Trained physicians performed the measurements. Primary parameters: attenuation of sound waves (BUA in dB/MHz) and speed of sound (SOS in m/s) were measured.

Descriptive parameters of measurements used were determined as well as bivariant correlations of all measurements in order to avoid multicolinearity in regression analysis. Multiple regression analysis was employed to determine the effects of menopause, age, anthropometry, nutrition and lifestyle on BUA and SOS (Model 2) and the effects of menopause, age and anthropometry on BUA and SOS, when effects of nutrition and lifestyle on the same dependent variables were controlled (Model 1). The same multiple regression analysis with exclusion of predictive variable "menopause" was applied to two subgroups of women (premenopausal women and postmenopausal women). Statistica 7.1 (Stat Soft Inc, Tulsa, United States) was used in the statistical analysis.

RESULTS

Age, anthropometric, dietary and lifestyle characteristics of the study participants are presented at the Table 1.

Table 1: Characteristics of study subjects divided by menopausal status

Characteristics	Premenopausal (n = 263)	Postmenopausal (n = 272)	*p* value
Age (years)	45.6 ± 6	58.79 ± 8.2	< 0.001
Bodyweight (kg)	67.9 ± 10.7	70.1 ± 10.4	< 0.05
Body height (cm)	164.7 ± 5.8	162.66 ± 5.8	< 0.001
Body mass index (kg/m^2)	25 ± 3.9	26.5 ± 3.9	< 0.001
Calcium (mg/day)	967.1± 421.7	893.2 ± 354.8	< 0.05
Calcium (% DRI)	93.6 ± 42.2	76.6 ± 31.4	< 0.001
Phosphorous (mg/day)	1357.5 ± 440.6	1292.2 ± 385.8	0.07
Phosphorous (% DRI)	193.9 ± 62.9	184. 6 ± 55.1	0.07
Protein (g/day)	74.9 ± 22.8	71.8 ± 19.6	0.08
Protein (g/kg BM)	1.1 ± 0.4	1 ± 0.3	< 0.05
Carbohydrate (g/day)	182.4 ± 68.3	175.8 ± 58.6	0.23
Vitamin D (µg/day)	1.7 ± 0.6	1.7 ± 0.6	0.97
Vitamin D (% RDA)	34 ± 12.9	34 ± 12.3	0.97
BUA (dB/MHz)	73.3 ± 14.7	66.3 ± 14.5	< 0.001
SOS (m/s)	1548.2 ± 25	1534.7 ± 25.7	< 0.001
Smoking	0.3 ± 0.5	0.2 ± 0.4	< 0.001
Organized activity	0.3 ± 0.5	0.3 ± 0.4	0.05
Caffeine (mg/day)	224.4 ± 116.9	202 ± 109.4	< 0.05
Alcohol (g/day)	3 ± 4.6	2 ± 4.6	< 0.05

Both regression models significantly predicted BUA and SOS in all women (Tables 3 and 4) and in postmenopausal women (Tables 6 and 7). In premenopausal women, only BUA was significantly predicted by both regression models (Table 5).

Both primary bone parameters were predicted by menopause while only SOS was predicted by age, whether by the regression model 1 or 2. Among nutrition and lifestyle factors, carbohydrate is the strongest predictor for BUA and smoking is the strongest predictor for SOS, with a trend for smoking to be predictive for BUA (Tables 3 and 4). In premenopausal women, body height and BMI are the strongest predictors for BUA, with a trend for body weight being predictive for BUA, in regression model 2 (Table 5). In postmenopausal women, BUA and SOS are strongly predicted by age and anthropometric parameters whether regression model 1 or 2. Besides, SOS is strongly predicted by smoking and alcohol, whereas there is a trend for smoking to be predictive for BUA (Tables 6 and 7).

Generally, BUA and SOS are 30% better predicted by regression model 2 than by regression model 1.

DISCUSSION

The results of this study revealed that menopause is the strongest predictor for BUA and SOS in the study population of women from the Croatian Mediterranean Region. Among nutrition and lifestyle factors, carbohydrates are the strongest predictor for BUA and smoking is the strongest predictor for SOS. Dependent on hormonal subdivision of the women, postmenopausal subjects were strongly predicted by age and

Table 2: Bivariant correlations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Age (years)	1.00																		
2. Bodyweight (kg)	0.16	1.00																	
3. Body height (cm)	-0.21	0.24	1.00																
4. Body mass index (kg/m^2)	0.27	0.88	-0.24	1.00															
5. Calcium (mg/day)	-0.12	0.00	0.07	-0.04	1.00														
6. Calcium (% DRI)	-0.26	-0.03	0.09	0.08	0.98	1.00													
7. Phosphorous (mg/day)	-0.02	0.02	0.03	-0.00	0.04	0.04	1.00												
8. Phosphorous (% DRI)	-0.02	0.02	0.03	-0.00	0.04	0.04	1.00	1.00											
9. Protein (g/day)	-0.09	0.05	0.14	-0.01	0.76	0.75	0.06	0.06	1.00										
10. Protein (g/kg BM)	-0.15	-0.40	0.02	-0.40	0.70	0.71	0.04	0.04	0.88	1.00									
11. Carbohydrate (g/day)	-0.04	0.02	0.13	-0.04	0.36	0.37	0.05	0.05	0.74	0.65	1.00								
12. Vitamin D (µg/day)	0.16	0.01	-0.06	0.04	0.03	0.00	-0.02	-0.02	-0.01	-0.01	0.00	1.00							
13. Vitamin D (% RDA)	0.16	0.01	-0.06	0.04	0.03	0.00	-0.02	-0.02	-0.01	-0.01	0.00	1.00	1.00						
14. BUA (dB/MHz)	-0.20	0.11	0.08	0.07	0.09	0.11	0.04	0.04	0.04	-0.02	-0.05	0.01	0.01	1.00					
15. SOS (m/s)	-0.26	-0.07	0.04	-0.09	0.05	0.08	0.03	0.03	-0.02	0.00	-0.12	0.02	0.02	0.84	1.00				
16. Smoking	-0.22	-0.12	0.05	-0.14	0.01	0.04	0.01	0.01	-0.02	0.04	0.00	0.05	0.05	-0.04	-0.04	1.00			
17. Organized activity	-0.12	-0.09	0.07	-0.12	0.09	0.11	0.03	0.03	0.04	0.09	-0.01	0.01	0.01	0.04	0.07	0.04	1.00		
18. Caffeine (mg/day)	-0.14	0.00	0.06	-0.02	0.00	0.03	0.08	0.08	0.03	0.01	0.01	-0.02	-0.02	0.03	0.03	0.29	0.07	1.00	
19. Alcohol (g/day)	0.04	0.01	0.06	-0.02	0.03	0.03	-0.02	-0.02	0.09	0.08	0.08	0.09	0.09	-0.03	-0.05	0.03	0.05	0.00	1.00

All correlations bigger than **0.085** are significant at levels $p < 0.05$. With Bonferroni correction for number of different parameters which are concurrently assessed (N(N-1)/2 = 300) all correlations greater than **0.162** are significant at the levels $p < 0.05$.

Table 3: Multiple regression analysis of BUA criterion on "Menopause, Age and Anthropometry" and "Menopause, Age, Anthropometry, Nutrition and Lifestyle".

Model	R	R^2	Model variance by the levels of hierarchical regression				
			Variance R^2	Variance F	df1	df2	*p*
1	0.277 (a)	0.077	0.068	8.819	5	529	0.000
2	0.318 (b)	0.101	0.077	4.170	14	385	0.000

a Predictors: (Constant), Subgroup 2, BMI, body height, bodyweight, age

b Predictors: (Constant), Subgroup 2, BMI, body height, bodyweight, age, phosphorous (mg/day), carbohydrate (g/day), organized activity, Caffeine (mg/day), vitamin D (µg/day), alcohol (g/day), smoking, calcium (mg/day), protein (g/day)

Model		Coefficients			t	*p*	Tolerance
		B	Standard error	β			
1	(Constant)	93.632	107.480		0.871	0.384	
	Age (years)	-0.147	0.089	-0.100	-1.658	0.097	0.522
	Bodyweight (kg)	0.362	0.752	0.257	0.481	0.631	0.006
	Body height (cm)	-0.166	0.654	-0.065	-0.253	0.800	0.026
	Body mass index (kg/m^2)	-0.433	2.022	-0.114	-1.078	0.831	0.006
	Subgroup 2	-5.581	1.694	-0.187	-3.300	0.001	0.545
2	(Constant)	56.954	108.365		0.526	0.599	
	Age (years)	-0.144	0.094	-0.094	-1.538	0.125	0.466
	Bodyweight (kg)	0.120	0.758	0.085	0.158	0.874	0.006
	Body height (cm)	0.053	0.666	0.021	0.081	0.935	0.025
	Body mass index (kg/m^2)	0.174	2.035	0.046	0.085	0.931	0.006
	Subgroup 2	-5.826	1.743	-0.195	-3.342	0.001	0.509
	Protein (g/day)	0.063	0.071	0.096	0.893	0.374	0.170
	Carbohydrate (g/day)	-0.035	0.016	-0.151	-2.173	0.030	0.362
	Calcium (mg/day)	0.002	0.003	0.046	0.623	0.533	0.323

Table 3: (Cont'd) Multiple regression analysis of BUA criterion on "Menopause, Age and Anthropometry" and "Menopause, Age, Anthropometry, Nutrition and Lifestyle".

Model		Coefficients			t	*p*	Tolerance
		B	Standard error	β			
	Phosphorous (mg/day)	0.001	0.001	0.022	0.514	0.607	0.323
	Vitamin D (µg/day)	0.662	1.024	0.028	0.647	0.518	0.936
	Organized activity	0.696	1.390	0.021	0.501	0.617	0.961
	Smoking	-2.886	1.544	-0.081	-1.811	0.071	0.861
	Caffeine (mg/day)	0.002	0.006	0.021	0.480	0.631	0.895
	Alcohol (g/day)	-0.145	0.138	-0.045	-1.052	0.295	0.935

a Dependent variable BUA
b Subgroup 2 = postmenopausal women

Table 4: Multiple regression analysis of SOS criterion on "Menopause, Age and Anthropometry" and "Menopause, Age, Anthropometry, Nutrition and Lifestyle".

Model	R	R^2					
			Model variance by the levels of hierarchical regression				
			Variance R^2	Variance F	df1	df2	*p*
1	0.282 (a)	0.079	0.071	9.137	5	529	0.000
2	0.337 (b)	0.114	0.145	4.763	14	520	0.000

a Predictors: (Constant), Subgroup 2, BMI, body height, bodyweight, age
b Predictors: (Constant), Subgroup 2, BMI, body height, bodyweight, age, phosphorous (mg/day), carbohydrate (g/day), organized activity, caffeine (mg/day), vitamin D (µg/day), alcohol (g/day), smoking, calcium (mg/day), protein (g/day)

Model		Coefficients			t	*p*	Tolerance
		B	Standard error	β			
1	(Constant)	1661.037	188.162		8.828	0.000	
	Age (years)	-0.399	0.156	-0.148	-2.563	0.011	0.522
	Bodyweight (kg)	0.539	1.316	0.219	0.416	0.682	0.006
	Body height (cm)	-0.552	1.145	-0.124	-0.482	0.636	0.026
	Body mass index (kg/m^2)	-1.606	3.539	-0.242	-0.454	0.650	0.006
	Subgroup 2	-8.195	2.965	-0.156	-2.764	0.006	0.545
2	(Constant)	1579.474	188.626		8.374	0.000	
	Age (years)	-0.433	0.163	-0.160	-2.654	0.008	0.466
	Bodyweight (kg)	-0.009	1.319	-0.004	-0.007	0.995	0.006
	Body height (cm)	-0.025	1.148	-0.006	-0.022	0.982	0.025
	Body mass index (kg/m^2)	-0.211	3.543	-0.032	-0.063	0.952	0.006
	Subgroup 2	-8.647	3.034	-0.165	-2.850	0.005	0.509
	Protein (g/day)	0.065	0.123	0.053	0.529	0.597	0.170
	Carbohydrate (g/day)	-0.065	0.028	-0.157	-2.279	0.023	0.359
	Calcium (mg/day)	0.002	0.005	0.029	0.401	0.688	0.323
	Phosphorous (mg/day)	0.001	0.003	0.012	0.284	0.776	0.968
	Vitamin D (µg/day)	2.553	1.782	0.061	1.432	0.153	0.936
	Organized activity	1.808	2.420	0.031	0.747	0.455	0.961
	Smoking	-6.826	2.687	-0.113	-2.541	0.011	0.861
	Caffeine (mg/day)	0.004	0.010	0.016	0.369	0.712	0.895
	Alcohol (g/day)	-0.333	0.240	-0.059	-0.395	0.165	0.935

a Dependent variable SOS
b Subgroup 2 = postmenopausal women

Table 5: Multiple regression analysis of BUA criterion on "Age and Anthropometry" and "Age, Anthropometry, Nutrition and Lifestyle" in premenopausal women

Model	R	R^2	Model variance by the levels of hierarchical regression				
			Variance R^2	Variance F	df1	df2	*p*
1	0.276 (a)	0.076	0.062	5.304	4	258	0.000
2	0.322 (b)	0.103	0.057	2.211	13	249	0.018

a Predictors: (Constant), BMI, Body height , Bodyweight, Age

b Predictors: (Constant), BMI, body height, bodyweight, age, phosphorous (mg/day), carbohydrate (g/day), organized activity, caffeine (mg/day), vitamin D (µg/day), alcohol (g/day), smoking, calcium (mg/day), protein (g/day)

Model		Coefficients			t	*p*	Tolerance
		B	Standard error	β			
1	(Constant)	-202.266	137.669		-1.469	0.143	
	Age (years)	0.106	0.153	0.043	0.689	0.491	0.913
	Bodyweight (kg)	-1.374	0.974	-1.007	-1.410	0.160	0.007
	Body height (cm)	1.508	0.830	0.597	1.817	0.070	0.033
	Body mass index (kg/m^2)	4.615	2.642	1.226	1.747	0.082	0.007
2	(Constant)	-247.056	140.695		-1.756	0.080	
	Age (years)	0.117	0.161	0.048	0.724	0.470	0.829
	Bodyweight (kg)	-1.669	0.993	-1.223	-1.681	0.094	0.007
	Body height (cm)	1.791	0.848	0.709	2.113	0.036	0.032
	Body mass index (kg/m^2)	5.420	2.693	1.441	2.013	0.045	0.007
	Protein (g/day)	-0.012	0.097	-0.018	-0.122	0.903	0.158
	Carbohydrate (g/day)	-0.027	0.022	-0.126	-1.240	0.216	0.348
	Calcium (mg/day)	0.002	0.004	0.056	0.515	0.607	0.301
	Phosphorous (mg/day)	0.000	0.002	0.005	0.083	0.938	0.934
	Vitamin D (µg/day)	0.232	1.438	0.010	0.161	0.872	0.902
	Organized activity	1.041	1.946	0.034	0.545	0.593	0.917
	Smoking	-2.691	2.021	-0.086	-1.332	0.184	0.867
	Caffeine (mg/day)	0.007	0.008	0.059	0.924	0.356	0.883
	Alcohol (g/day)	-0.090	0.197	-0.029	-0.465	0.646	0.924

a Dependent variable BUA

anthropometric parameters, while premenopausal subjects were strongly predicted by body height and body mass index. In menopause, bone loss is determined by oestrogen withdrawal which could obscure the influence of age and other risk factors such as anthropometry, nutrition and lifestyle on bone health. The strong influence of the menopausal state on bone health in the present study population is consistent with Japanese and Arabic studies (1, 18). When the effect of menopause is controlled, like in the subgroups of premenopausal and postmenopausal women, the strongest predictors for BUA and SOS were anthropometric parameters. In postmenopausal women, BUA and SOS were positively predicted by body weight, but negatively predicted by BMI, which is only partially consistent with other studies, which generally found a positive effect of BMI on QUS parameters (1, 19, 20). Low BMI values are defined as one of the risk factors for developing osteoporosis. According to BMI values, postmenopausal women were overweight, and as far as bone structural properties were considered, such increase in BMI values was not desirable in maintaining good bone quality.

In the Greek study where mean age of the adult study population was 48.7 years it was found that carbohydrates had a negative effect on BUA (R= - 0.077, $p < 0.05$) which is consistent with our result on the influence of carbohydrates on BUA (β = - 0.151, $p < 0.05$) [Table 3] (19). An experimental study on chicken osteoclasts has indicated that changes in glucose concentration in the physiological range may directly increase the rate of bone resorption in a diurnal cycle (7). Our result of a negative influence of carbohydrates on BUA suggests that carbohydrates are unfavourable for bone struc-ture and bone health in general.

Table 6: Multiple regression analysis of BUA criterion on "Age and Anthropometry " and "Age, Anthropometry, Nutrition and Lifestyle" in postmenopausal women

Model	R	R^2	Model variance by the levels of hierarchical regression				
			Variance R^2	Variance F	df1	df2	*p*
1	0.198(a)	0.039	0.025	2.712	4	267	0.031
2	0.297(b)	0.088	0.042	1.924	13	258	0.028

a Predictors: (Constant), BMI, body height , bodyweight, age
b Predictors: (Constant), BMI, body height, bodyweight, age, phosphorous (mg/day), carbohydrate (g/day), organized activity, caffeine (mg/day), vitamin D (μg/day), alcohol (g/day), smoking, calcium (mg/day), protein (g/day)

Model		Coefficients			t	*p*	Tolerance
		B	Standard error	β			
1	(Constant)	448.159	175.808		2.549	0.011	
	Age (years)	-0.254	0.108	-0.144	-2.362	0.019	0.966
	Bodyweight (kg)	2.514	1.218	1.813	2.065	0.042	0.005
	Body height (cm)	-2.268	1.079	-0.917	-2.102	0.036	0.019
	Body mass index (kg/m^2)	-6.570	3.243	-1.760	-2.026	0.044	0.005
2	(Constant)	474.071	177.887		2.665	0.008	
	Age (years)	-0.245	0.119	-0.139	-2.061	0.040	0.777
	Bodyweight (kg)	2.694	1.233	1.942	2.186	0.032	0.004
	Body height (cm)	-2.449	1.095	-0.990	-2.239	0.026	0.018
	Body mass index (kg/m^2)	-7.092	3.282	-1.900	-2.161	0.032	0.005
	Protein (g/day)	0.056	0.105	0.076	0.534	0.594	0.173
	Carbohydrate (g/day)	-0.022	0.025	-0.087	-0.864	0.399	0.346
	Calcium (mg/day)	0.005	0.004	0.118	1.146	0.253	0.332
	Phosphorous (mg/day)	-0.000	0.002	-0.004	-0.079	0.944	0.930
	Vitamin D (μg/day)	0.543	1.457	0.023	0.373	0.719	0.926
	Organized activity	1.289	2.008	0.039	0.642	0.521	0.954
	Smoking	-4.182	2.452	-0.112	-1.706	0.089	0.815
	Caffeine (mg/day)	-0.000	0.008	-0.003	-0.047	0.962	0.888
	Alcohol (g/day)	-0.268	0.195	-0.086	-1.376	0.178	0.908

a Dependent variable BUA

An average dietary calcium intake for Croatian women amounted to 929 50 mg daily, which is insufficient, when compared to standard Dietary Reference Intakes (DRI). Dietary Reference Intakes recommends 1000 mg of calcium daily for women younger than 50 years and 1200 mg of calcium daily for women older than 50 years (15). The Croatian dietary standard of 800 mg/day is not adjusted for age dependent needs. Although calcium and phosphorous were positively related to ultrasound parameters in some other studies (6, 19, 20), neither one of these two minerals significantly predicted BUA and SOS in the present study.

EPIC – Potsdam Cohort revealed that German women took somewhat less dietary calcium but those women took 53% more dietary vitamin D than Croatian women. Moreover, their BUA values (110.4 dB/MHz) were 36% higher than BUA values in Croatian women [69.75 dB/MHz] (6). Calcium absorption in the intestine is mediated by biologically active vitamin D and if deficient, less calcium is deposited in the skeleton, which therefore becomes insufficiently mineralized. Insufficient dietary vitamin D intake has contributed to low BUA in Croatian women. When compared to other studies, the BUA values in the present study were less than in Italian women (113 dB/MHz) but similar to Greek women (69.7 dB/MHz) while SOS values were similar whether Italian (1544 m/s), Greek (1.548 m/s) or Croatian women (1541.36 m/s) were compared (19, 20).

Previous investigations have established that smoking can change bone physiology by accelerating menopause onset, reducing calcium absorption in the intestine, indirectly changing oestrogen metabolism and by reducing body mass

Table 7: Multiple regression analysis of SOS criterion on "Age and Anthropometry " and "Age, Anthropometry, Nutrition and Lifestyle" in postmenopausal women

Model	R	R^2	Model variance by the levels of hierarchical regression				
			Variance R^2	Variance F	df1	df2	*p*
1	0.249(a)	0.062	0.048	4.395	4	267	.002
2	0.347(b)	0.120	0.076	2.709	13	258	.001

a Predictors: (Constant), BMI, body height, bodyweight, age

b Predictors: (Constant), BMI, body height, bodyweight, age, phosphorous (mg/day), carbohydrate (g/day), organized activity, caffeine (mg/day), vitamin D (µg/day), alcohol (g/day), smoking, calcium (mg/day), protein (g/day)

Model		Coefficients			t	*p*	Tolerance
		B	Standard error	β			
1	(Constant)	2432.098	310.116		7.685	0.000	
	Age (years)	-0.508	0.190	-0.161	-2.673	0.008	0.966
	Bodyweight (kg)	5.211	2.148	2.105	2.426	0.016	0.005
	Body height (cm)	-4.935	1.903	-1.117	-2.594	0.010	0.019
	Body mass index (kg/m^2)	-14.368	5.721	-2.157	-2.512	0.013	0.005
2	(Constant)	2432.449	311.988		7.797	0.000	
	Age (years)	-0.542	0.209	-0.172	-2.598	0.011	0.777
	Bodyweight (kg)	5.474	2.162	2.211	2.532	0.012	0.004
	Body height (cm)	-5.160	1.918	-1.168	-2.695	0.008	0.018
	Body mass index (kg/m^2)	-15.262	5.756	-2.291	-2.651	0.009	0.005
	Protein (g/day)	0.018	0.185	0.014	0.098	0.922	0.173
	Carbohydrate (g/day)	-0.032	0.044	-0.074	-0.740	0.464	0.346
	Calcium (mg/day)	0.007	0.007	0.091	0.900	0.369	0.332
	Phosphorous (mg/day)	-0.005	0.004	-0.087	-1.316	0.189	0.930
	Vitamin D (µg/day)	1.318	2.555	0.031	0.516	0.607	0.926
	Organized activity	1.707	3.521	0.038	0.485	0.628	0.954
	Smoking	-11.626	4.301	-0.175	-2.703	0.007	0.815
	Caffeine (mg/day)	0.004	0.015	0.015	0.241	0.811	0.888
	Alcohol (g/day)	-0.717	0.341	-0.129	-2.103	0.036	0.908

a Dependent variable SOS

(19, 21). We found that smoking is a negative predictor for SOS ($\beta = - 0.113$, $p < 0.05$) which is similar to another study where there was a negative association between number of cigarettes and bone stiffness [$r = - 0.030$, $p = 0.05$] (19). The finding of a negative influence of alcohol on SOS values in postmenopausal women is difficult to interprete since we lack comparative data. The Italian and Greek studies did not find any significant influence of alcohol on the study population. Certain studies have indicated that alcohol consumption is favourable for bone health in postmenopausal women (22) while others have held the opposite opinion (23, 24). Therefore, further investigations are needed to resolve what quantity and what kind of alcohol is favourable for bone and which quantity of alcohol is harmful for bone health.

In conclusion, the results of this study support previously published data on the negative influence of menopause on bone health. The finding on carbohydrates contributes to a better understanding of their possible negative influence on bone status. Anyway, this matter ought to be further investigated. Very low dietary vitamin D intake in Croatian women should alert Public Health Officials to promote controlled sun exposure and to ensure better intake of vitamin D in daily diet. This means that the benefits of 2100 solar hours per year and easy accessible Mediterranean food rich with vitamin D should be promoted not only in the Croatian population but in other countries in the Mediterranean region.

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REFERENCES

1. Saadi HF, Reed RL, Carter AO, Duun EV, Qazaq HS, Al-Suhaili AR. Quantitative ultrasound of the calcaneus in Arabian women: relation to anthropometric and lifestyle factors. Maturitas 2003; **44:** 215−23.
2. Devine A, Dhaliwal SS, Dick IM, Bollerslev J, Prince RL. Physical Activity and Calcium Consumption Are Important Determinants OF lower Limb Bone Mass in Older Women. J Bone Miner Res 2004; **19:** 1634−9.
3. Korperlainen R, Korperlainen J, Heikkinen J, Väänänen K, Keinänen-Kiukaanniemi S. Lifestyle factors are associated with osteoporosis in lean women but not in normal and overweight women: a population – based cohort study of 1222 women. Osteoporos Int 2003; **14:** 34−43.
4. Seeman E, Delmas PD. Bone quality – the material and structural basis of bone strength and fragility. N Engl J Med 2006; **354:** 2250−61.
5. Holick MF. Vitamin D deficiency. N Engl J Med 2007; **357:** 266−81.
6. Weikert C, Dietmar W, Hoffman K, Kroke A, Bergmann MM, Boeing H. The Relation between Dietary Protein, Calcium and Bone Health in Women: Results from the EPIC-Postdam Cohort. Ann Nutr Metab 2005; **49:** 312−8.
7. Williams JP, Blair HC, McDonald JM, McKenna MA, Jordan SE, Williford J et al. Regulation of osteoclastic bone resorption by glucose. Biochem Biophys Res Commun 1997; **235:** 646−51.
8. Ilich JZ, Kerstetter JE. Nutrition in Bone Health Revisited: A Story Beyond Calcium. J Am Coll Nutr 2000; **19:** 715−37.
9. Ganry O, Baudoin C, Pardelone P. Effect of alcohol intake on bone mineral density in elderly women. The EPIDOS study. Am J Epidemiol 2000; **151:** 773−80.
10. Rapuri PB, Gallagher JC, Balhorn KE, Ryschon KL. Smoking and bone metabolism in elderly women. Bone 2000; **27:** 429−36.
11. Wetter AC, Economos CD. Relationship between quantitative ultrasound, anropometry and sports participation in college aged adults. Osteoporos Int 2004; **15:** 799−06.
12. Faulkner KG, Glüer CC, Majumdar S, Lang P, Engelke K, Genant HK. Noninvasive measurements of bone mass, structure, and strenght: Current Methods and Experimental Techniques. AJR Am J Roentgenol 1991; **157:** 1229−37.
13. Gonnelli S, Cepollaro C. The use of ultrasound in the assessment of bone status. J Endocrinol Invest 2002; **25:** 389−97.
14. Kaic-Rak A, Antonic K. Nutritive tables (Prehrambene tablice), Zagreb: Institute of Public Health Zagreb, 1990.
15. Bryant RJ, Cadogan J, Weaver CM. The New Dietary Reference Intakes for Calcium: Implications for Osteoporosis. J Am Coll Nutr 1999; **18:** 406S–412S.
16. The National Academy of Science. Recommended dietary allowance, Washington, DC: National Academy Press,1989.
17. Montomoli M, Gonnelli S, Giacchi M, Mattei R, Cuda C, Rossi S et al. Validation of food frequency questionnaire for nutritional calcium intake assessment in Italian women. Eur J Clin Nutr 2002; **56:** 21−30.
18. Yamaguchi J, Truman G, Cameron ID. Lifestyle factors affecting bone ultrasonometry of the calcaneus in Japanese women. Calcif Tissue Int 2000; **66:** 43−6.
19. Babaroutsi E, Magkos F, Manios Y, Sidossis LS. Lifestyle factors affecting heel ultrasound in Greek females across different life stages. Osteoporos Int 2005; **16:** 552−61.
20. Adami S, Giannini S, Giorgino R, Isaia GC, Maggi S, Sinigaglia L et al. Effect of Age, Weight and Lifestyle Factors on Calcenael Quantitative Ultrasound in Premenopausal Women: The ESOPO Study. Calcif Tissue Int 2004; **74:** 317−21.
21. Tanaka M, Itoh K, Abe S, Imai K, Masuda T, Koga R et al. Relationship between nutrient factors and osteo-sono assessment index in calcaneus of young Japanese women. Nutrition Research 2001; **21:** 1475−82.
22. Rapuri PB, Gallagher JC, Balhorn KE, Ryschon KL. Alcohol intake and bone metabolism in elderly women. Am J Clin Nutr 2000; **72:** 1206−13.
23. Klein RF, Fausti KA, Carlos AS. Ethanol inhibits human osteoblastic cell proliferation. Alcohol Clin Exp Res 1996; **20:** 572−8.
24. Bikle DD. Alcohol-induced bone disease. World Rev Nutr Diet 1993; **73:** 53−73.