



## Trabajo Original

Valoración nutricional

### Dietary antioxidant quality score (DAQs) is associated with bone mass assessed by calcaneal quantitative ultrasound in young women

*El índice de calidad antioxidante de la dieta (DAQS) está asociado con la masa ósea evaluada mediante ultrasonido cuantitativo en el calcáneo en mujeres jóvenes*

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#### Abstract

**Introduction:** Evidence suggests that intake of antioxidants could positively influence bone mass by preventing bone metabolism against oxidative stress.

**Objective:** We aimed to investigate the possible influence of single antioxidant intakes and dietary antioxidant quality score (DAQs) on calcaneal quantitative ultrasound (QUS) in a population of young adults.

**Methods:** A total of 605 young Spanish adults participated in this study (median age  $20.38 \pm 2.67$ ). Bone mass was measured by calcaneal QUS to determine broadband ultrasound attenuation (BUA, dB/MHz) parameter. Body composition was assessed by bioelectrical impedance analysis and dietary intakes were determined using a 72-hour diet recall interview. DAQs was applied to calculate antioxidant nutrients intake. Linear regression analyses were performed to investigate the possible influence of DAQs on calcaneal QUS.

**Results:** Most of young adults showed a low-quality antioxidant intake (only 17.6% of women and 20.3% of men had a score of 4 or 5 in DAQs). A positive correlation between DAQs and BUA was observed in women ( $r = 0.117$ ;  $p = 0.024$ ). Linear regression analysis revealed that DAQs was significantly associated with BUA parameter in women after adjusting by body weight, height, calcium intake and physical activity (PA) ( $p = 0.035$ ). No significant associations between single antioxidant and calcaneus QUS measurement were found.

**Conclusion:** Our findings suggest that high-quality antioxidant intakes could influence bone health in young women. Future studies should further investigate the protective role of antioxidant nutrients against osteoporosis.

#### Key words:

Antioxidants.  
Calcaneus  
quantitative  
ultrasound. Nutrition.  
Young adults.

#### Resumen

**Introducción:** la evidencia sugiere que la ingesta de antioxidantes podría influir positivamente en la masa ósea mediante la prevención contra el estrés oxidativo del metabolismo óseo.

**Objetivo:** el objetivo fue investigar la posible influencia del consumo de antioxidantes y del índice de calidad antioxidante de la dieta (DAQs) en la masa ósea, evaluada mediante ultrasonido cuantitativo (QUS) en el calcáneo en una población de adultos jóvenes.

**Métodos:** un total de 605 adultos jóvenes españoles participaron en este estudio (mediana  $20,38 \pm 2,67$  años). La masa ósea se evaluó mediante QUS en el calcáneo para determinar el parámetro de atenuación de ultrasonido de banda ancha (BUA, dB/MHz). La composición corporal se determinó mediante bioimpedancia eléctrica y la ingesta dietética se determinó a través del recordatorio de 72 horas. El DAQs se aplicó para calcular la ingesta total de nutrientes antioxidantes. Se realizaron análisis de regresión lineal para investigar la posible influencia del DAQs en QUS en el calcáneo.

**Resultados:** la mayoría de los adultos jóvenes mostraron una ingesta de antioxidantes de baja calidad (solo el 17,6% de las mujeres y el 20,3% de los hombres presentaron una puntuación de 4 o 5 en DAQs). Se observó una correlación positiva entre el DAQs y el BUA en las mujeres ( $r = 0,117$ ;  $p = 0,024$ ). El análisis de regresión lineal reveló que el DAQs se asociaba significativamente con el parámetro BUA en las mujeres después de ajustar por peso corporal, altura, ingesta de calcio y actividad física ( $p = 0,035$ ). No se encontraron asociaciones significativas entre la ingesta de antioxidantes individuales y QUS en el calcáneo.

**Conclusión:** nuestros resultados sugieren que una ingesta de antioxidantes de alta calidad podría influir en la salud ósea en mujeres jóvenes. Futuros estudios deben profundizar en el papel protector de los nutrientes antioxidantes contra la osteoporosis.

#### Palabras clave:

Antioxidantes.  
Ultrasonido  
cuantitativo de  
calcáneo. Nutrición.  
Adultos jóvenes.

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## INTRODUCTION

Osteoporosis is considered to be a public health problem characterized by low bone density and reduced bone quality through the deterioration of bone microarchitecture (1). As a consequence, sufferers have an increased susceptibility to osteoporotic fractures (2). Osteoporosis is a multifactorial and complex disease determined by both genetic and environmental factors (3).

Oxidative stress and low serum levels of antioxidants have been proposed to be contributors to osteoporosis. *In vitro* and animal studies have shown that oxidative stress could induce bone loss by modulating osteoclast activation and osteoblast suppression (4-7). In this line, a number of epidemiologic studies have reported positive associations between oxidative stress and bone mineral density (BMD) (8,9).

Evidence suggests that intake of antioxidants could positively influence bone mass by preventing bone metabolism against oxidative stress. Previous studies have investigated a relationship between antioxidants intake and BMD, fracture risk and osteoporosis reporting contradictory results (10-14). Most studies generally analyzed the association between single antioxidant intake and bone status. Little is yet known regarding diet quality indexes of antioxidant intakes and their potential relation with bone status. However, people consume foods with complex combinations of antioxidant nutrients (15) and therefore, this traditional approach misses information regarding interactions between different antioxidant contained in food.

Quantitative ultrasound (QUS) has been proposed as an alternative method to assess bone mass and provides parameters of bone structure (microstructure, elasticity and connectivity) (16). The QUS has been valued for its high correlation with BMD measured by DXA (17). Its portability, non-invasiveness, radiation-free and low cost nature make it a useful method for assessing bone status (18). Until now, no studies have examined the relationship between antioxidant intakes and bone mass assessed by QUS. Therefore, the aim of the current study was to investigate the influence of single antioxidant intakes and dietary antioxidant quality score (DAQs) on calcaneal QUS in young adults. We hypothesized that a high-quality antioxidant intake would be associated with a greater calcaneal QUS parameter.

## METHODS

### SUBJECTS

Six hundred and five individuals aged 18 to 25 (69.3% females and 30.7% males) agreed to participate in this study and were recruited from different academic centers of Granada (Spain). All participants were evaluated by means of a detailed medical history. Subjects with any of the following criteria were excluded from the study: history of bone disease, metabolic or endocrine diseases, hormone-replacement therapy or current treatments that could affect bone mass. Written informed consent was obtained from each participant and the study was approved by local ethics

committees and conducted in accordance with the Declaration of Helsinki.

### ANTHROPOMETRIC MEASUREMENTS

Body weight (kg) and fat mass (%) were measured twice (without shoes and in light clothes) to the nearest 0.11 kg by bioelectrical impedance analysis (TANITA BC-418MA®). A Harpenden stadiometer (Holtain 602VR®) was used for height measurements. Height was measured twice without shoes to the nearest 0.5 cm. The averages of the two values for each measurement were used in the analysis. Anthropometric measurements were performed in the morning after a 12-h fast and 24-h abstention from exercise. BMI was calculated as weight over height squared (kg/m<sup>2</sup>).

### CALCANEAL QUS

Bone mass status was measured by ultrasonography at the right calcaneus (BUA, dB/MHz) using the CUBA clinical ultrasound bone densitometer (McCue Ultrasonics Limited, Compton, Winchester, UK). The calcaneus is used for QUS assessment because it contains a high percentage of trabecular bone and it is easily accessible (19). Daily calibrations were made with physical phantom to control the long-term stability of the apparatus.

### DAILY NUTRIENT INTAKE

Daily nutrient intake was assessed by a 72-hour diet recall interview considering intakes on Thursday, Friday and Saturday to capture weekly variations in weekdays and weekend. In a face-to-face interview with well-trained investigators, individuals were asked to recall all food consumed in the preceding 72 hours, including foods eaten outside the home, nutrition supplements and beverages. In order to improve the accuracy of the descriptions of meals, pictorial food models were employed. A computerized food analysis program (Nutriber 1.1.5) was used to assess completed food records (20). The food composition table reported by Mataix et al. was used for conversion of food into nutrients (21).

### PHYSICAL ACTIVITY

Physical activity was assessed using a self-administered questionnaire (International Physical Activity Questionnaire-IPAQ). The questionnaire has proven to be a valid instrument for measuring PA in the European adult population (22). It was used to calculate the total hours of vigorous PA, moderate PA and walking over the last seven days. A MET-h was derived by multiplying the respective total hours by the metabolic equivalent of task (MET) value for vigorous PA (MET = 8.0), moderate PA (4.0) and walking (3.3), and then adding all three (22).

## ANTIOXIDANT NUTRIENT INTAKE

A DAQs was used to calculate antioxidant nutrients (23). The test score assessed the consumption of vitamin C, vitamin E, vitamin A, selenium and zinc. Daily nutrient intake was compared to that of the daily recommended intake for the Spanish population (RDI) (24). When the nutrient intake was below 2/3 of the RDI, a value of 0 was obtained, and when the intake was above 2/3 of the RDI, a value of 1 was obtained. DAQs is scored with a final score range from 0 (very poor quality) to 5 (high quality).

## STATISTICAL ANALYSIS

SPSS Statistic version 21.0 (SPSS, Chicago, IL, USA) was used for all the analyses. Mean and standard deviation (SD) where normally distributed and as median (interquartile range) where skewed are given as descriptive statistics. Sex-specific differences were assessed by independent t-test. Spearman's correlation coefficient ( $r$ ) was used to test the correlation between antioxidant nutrients, DAQs and calcaneus QUS adjusted by age, body weight, height, calcium intake and physical activity. To analyze the associations between single antioxidants intake, DAQs and calcaneus QUS, multiple regression analysis was performed after adjusting by age, body weight, height, calcium intake and physical activity. Results are reported as standardized  $\beta$ -coefficient,  $R$ ,  $R^2$ , adjusted  $R^2$ ,  $t$  and  $p$  value.  $p$ -values  $< 0.05$  were considered to be statistically significant.

## RESULTS

The basic characteristics were summarized separately for men and women in table I. The mean age for the study population was  $20.4 \pm 2.7$ , and the mean BMI was  $22.6 \pm 3.7$  kg/m<sup>2</sup>. Significant differences between men and women were observed in height, weight, BMI, fat mass, physical activity and BUA values. Men had a significantly higher body height, weight, and BMI than women ( $p < 0.001$ ), whereas women had a significantly higher fat mass than men ( $p < 0.001$ ). The reported energy intake was higher in men than in women, but there was no evidence of any significant differences. Average calcium intake was below the recommended intake level (RDA) in both genders. The mean calcaneus BUA for the sample was  $86.7 \pm 17.6$  (dB/MHz) and males had a significantly higher BUA than females ( $p < 0.001$ ). Regarding the intake of antioxidant nutrients, the average intakes of vitamin E, vitamin A and zinc were lower than the recommended in both sexes. By contrast, the intake of vitamin C and selenium reached the dietary goals. Considering gender, a significant difference has been observed concerning the intake of vitamin C ( $p = 0.036$ ).

The percentages of young adults that are below 2/3 of the RDI for antioxidant nutrients are shown in table II. Regarding vitamin E, a higher percentage of women and men had inadequate antioxidant intake (defined as intakes below 2/3 of the DRI). As can be observed, most women were low antioxidant consumers (only

**Table I.** Basic characteristics of the study population (n = 605)

	Females	Males
N (%)	419 (69.3)	186 (30.7)
Age	$20.4 \pm 2.7$	$20.5 \pm 2.6$
Height (m)	$1.6 \pm 0.1^{**}$	$1.7 \pm 0.1$
Weight (kg)	$59.4 \pm 10.1^{**}$	$73.1 \pm 13.0$
BMI (kg/mg <sup>2</sup> )	$22.1 \pm 3.6^{**}$	$23.6 \pm 3.7$
Fat mass (%)	$24.4 \pm 7.3^{**}$	$15.1 \pm 5.4$
Intake		
Daily energy intake (kcal/day)	$1,990 \pm 1,275^{**}$	$2,139 \pm 716$
Calcium intake (mg/day)	$799.7 \pm 346.4$	$855.2 \pm 377.1$
Vitamin C (mg/day)	$79.6 \pm 65.5^*$	$93.2 \pm 76.9$
Vitamin E (mg/day)	$5.4 \pm 3.4$	$5.9 \pm 3.9$
Vitamin A (ug/day)	$595.2 \pm 489.8$	$627.7 \pm 447.8$
Zn (mg/day)	$8.9 \pm 3.9$	$9.9 \pm 4.3$
Se (ug/day)	$132.8 \pm 89.0$	$143.9 \pm 79.3$
Total physical activity (MET-hrs) <sup>a</sup>	$38.7 (0-246.2)^{**}$	$61.7 (0- 243.5)$
BUA (dB/MHz)	$83.3 \pm 15.8^{**}$	$96.31 \pm 16.8$

Data are shown as mean  $\pm$  SD. \* $p < 0.05$  between females and males; \*\* $p < 0.001$  between females and males. All the nutrients were adjusted for energy intake. BMI: Body mass index mineral; MET: Metabolic equivalent of a task, representing energy expenditure per day. <sup>a</sup>MET-hrs are expressed as mean and range.

**Table II.** Daily intake of the antioxidant nutrients in the study population

	Females	Males
	% sample 2/3 RDI	% sample 2/3 RDI
Vitamin C (mg/day)	34.2	32.3
Vitamin E (mg/day)	84.8	78.9
Vitamin A (ug/day)	57.7	66.7
Zn (mg/day)	67.1	61.3
Se (ug/day)	12.5	6.4

17.6% of women had a score of 4 or 5 in DAQs). In this line, only 20.3% of men showed a high-quality antioxidant intake (DAQs 4 or 5).

Spearman's correlation revealed a positive relationship between DAQs and calcaneus BUA in women ( $r = 0.117$ ;  $p = 0.024$ ) (Table III). In order to analyze the influence of DAQs and each antioxidants intake on calcaneal QUS, multiple regression models were applied after adjusting by body weight, height, calcium intake and physical activity (Table IV). Interestingly, the multiple regression analysis revealed that DAQs was significantly associated with BUA parameter in women ( $p = 0.035$ ). No significant

**Table III. Spearman correlation coefficients (r) between antioxidant nutrients, DAQs and calcaneal QUS**

	Calcaneal BUA			
	Females		Males	
	r	p-value	r	p-value
Vitamin C (mg/day)	0.083	0.107	0.089	0.251
Vitamin E (mg/day)	0.047	0.343	0.047	0.547
Vitamin A (ug/day)	0.036	0.487	0.036	0.487
Zn (mg/day)	0.041	0.431	0.111	0.151
Se (ug/day)	0.079	0.128	-0.048	0.533
DAQs	0.117	0.024	0.049	0.531

DAQs: Dietary antioxidant quality score. Adjusted by age, weight, height, calcium intake and physical activity.

associations between single antioxidant nutrient and calcaneus QUS measurement were found.

**DISCUSSION**

The present study explores the associations between DAQs and single antioxidant intakes on calcaneal QUS measurement in a sample of 605 young adults. Our findings provide evidence for the influence of DAQs on calcaneal BUA parameter in young women, supporting the hypothesis that a high-quality antioxidant intake could positively influence bone mass in young women. To our knowledge, there has been no previous study investigating the association of DAQs on bone mass assessed by calcaneal QUS measurement.

To date, only two studies have investigated the association between DAQs and bone status (11, 12). In agreement with our findings, Rivas et al. reported a significant positive association between DAQs and BMD among 280 healthy women aged 18 to > 45 (p = 0.021) (12). On the other hand, in the study of De França et al., 150 postmenopausal women over 45 years old with osteoporosis were included (11). In contrast to Rivas et al. and with our findings, any relationship was found between DAQs and BMD in any skeletal sites. One possible reason for this discrepancy may be attributed to the limited sample size or to the sample consisting of osteoporotic women. DAQs could not be suitable for assessing the association of antioxidant dietary intakes and bone mass in osteoporotic subjects since the antioxidant considered in this score could have a minimum effect on low BMD values. In addition, in this study they applied an adaption of the original DAQs since they used estimated averages requirements (EAR) instead of RDI. It should be noted that both previous studies used DXA for measurements of bone mass, none of them used calcaneal ultrasound, and hence, we could not compare our effect sizes for BUA.

This study is the first one to explore the association of DAQs with bone mass in a population of men. Although our study report-

**Table IV. Association between antioxidant nutrients and DAQs on calcaneal QUS measurement (dB/MHz)**

Variables	Females						Males							
	r	r <sup>2</sup>	Adjusted r <sup>2</sup>	SE	Coefficient	t	p	r	r <sup>2</sup>	Adjusted r <sup>2</sup>	SE	Coefficient	t	p
Vitamin C (mg)	0.335	0.112	0.100	14.988	0.080	1.618	0.106	0.259	0.067	0.040	16.485	0.090	1.194	0.234
Vitamin E (mg)	0.329	0.108	0.096	15.003	0.045	0.912	0.362	0.240	0.058	0.029	16.630	0.057	0.743	0.458
Vitamin A (ug)	0.327	0.107	0.095	15.011	0.033	0.683	0.495	0.251	0.063	0.035	16.648	0.064	0.838	0.403
Zn (mg)	0.328	0.107	0.095	15.009	0.036	0.733	0.464	0.276	0.076	0.049	16.525	0.136	1.813	0.072
Se (ug)	0.334	0.112	0.100	14.973	0.075	1.531	0.127	0.246	0.060	0.033	16.626	-0.040	-0.535	0.593
DAQs	0.341	0.117	0.105	14.951	0.104	2.114	0.035	0.248	0.062	0.033	16.556	0.087	1.123	0.263

DAQs: Dietary antioxidant quality score. Adjusted by age, weight, height, calcium intake and physical activity.



ed a lack of association, we cannot completely discard an association of DAQs with bone mass in men due to the relatively small sample size compared to that of women. Further studies including larger samples are required to assess the relationship between DAQs and bone mass in men.

In this study, when antioxidant nutrient intakes were analyzed separately, any association between single antioxidants and calcaneus QUS was observed. Previous studies have assessed the association between select dietary antioxidants, vitamin C (10,14,25-28), vitamin E (14,29), vitamin A (14,30-32), zinc (33) and selenium (14,29,34) and bone mass, revealing inconsistent findings. One possible cause of inconclusive results could be differences in sample sizes and characteristics, study designs and dietary assessments among studies. It must be highlighted that these studies have focused on the effects of single antioxidants on bone health. By using this approach, potential interactions among different antioxidant dietary intakes have been ignored because people consume food with a complex combination of antioxidants, rather than single antioxidants. Consequently, in order to analyze the effect of overall diet and detect possible interactions, recent studies are using diet quality indexes as an alternative method (10,35,36).

The current study has a larger sample size than previous studies exploring the association of DAQs and bone mass. Moreover, this study provides the first investigation of DAQs and bone health in both men and women. Furthermore, all analyses were adjusted for relevant covariates known to affect bone mass. One limitation of our study was its cross-sectional design, from which causality could not be inferred. Another limitation is inherent to the assessment of dietary intake using a self-administered questionnaire. The literature supports the use of 72-hour recall as a pertinent method for assessing nutrient intake since it collects better data on the typical or average diet (37). However, evidence of under-reporting of food intake in self-administered questionnaires has been reported previously (37,38). In our study the 72-hour recall was interviewer-driven. Additionally, well-trained investigators asked study subjects to recall all food intakes and, in order to improve the accuracy of the descriptions of meals, pictorial food models were employed. Another potential limitation is the lack of data regarding the culinary treatments that might influence on the reported intake of antioxidants. Finally, the effect of other antioxidants such as flavonoids was not considered.

In summary, our findings of significant associations between DAQs and calcaneal ultrasound in young women reflect the protective role of high-quality antioxidant intakes as an environmental factor contributing to bone health. Future studies should further explore the potential influence of antioxidant nutrients against osteoporosis.

## HUMAN AND ANIMAL RIGHTS AND INFORMED CONSENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the insti-

tutional and/or national research committee and with the Declaration of Helsinki of 1964 and its later amendments or comparable ethical standards. Written informed consent was obtained for all participants.

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