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## Changes in bone metabolism markers and ultrasound parameters in postmenopausal women induced by soy isoflavones

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### Summary

**Introduction:** the results of the works published on the role of isoflavones in the prevention of postmenopausal osteoporosis are contradictory. The objective of our study is to evaluate the effects of nutritional intervention with a milk product enriched with soy isoflavones on bone metabolism in Spanish postmenopausal women.

**Subjects and methods:** a randomised controlled double blind trial was carried out in 99 postmenopausal women who were allocated to two groups: group S (n=48), with a consumption of a milk product enriched with soy isoflavones (50mg/day), and group C (n=51), with a consumption of a control milk product over 12 months. Hormone parameters and markers for bone metabolism were assessed at the baseline and at one year. Ultrasound of the calcaneum (QUS, Hologic Sahara®, North Carolina, US.) was used as the evaluation tool for bone mass.

**Results:** at 12 months, a decrease in blood levels of tartrate-resistant acid phosphatase and osteoprotegerin occurred ( $2.18 \pm 0.8$  vs  $1.76 \pm 0.54$  U/l,  $p < 0.001$ , and  $5.21 \pm 3.36$  vs  $3.89 \pm 1.47$  pmol/L,  $p = 0.007$ , respectively), as well as an increase in 25-OH-vitamin D ( $24.48 \pm 9.85$  vs  $28.18 \pm 10.45$  ng/ml,  $p < 0.001$ ) with no differences between the groups. There were no significant changes in hormone parameters and the rest of the bone markers. In terms of the QUS, in the total sample there was an increase in the sound velocity [SOS] ( $1517.86 \pm 38.13$  vs  $1525.11 \pm 35.6$  m/s,  $p = 0.036$ ), QUI ( $76.37 \pm 19.87$  vs  $80.82 \pm 18.26$ ,  $p = 0.012$ ), estimated bone mineral density [Est. BMD] ( $0.408 \pm 0.13$  vs  $0.435 \pm 0.12$  g/cm<sup>2</sup>,  $p = 0.013$ ) and T-score ( $-1.55 \pm 1.12$  vs  $-1.31 \pm 1.03$ ,  $p = 0.019$ ). In group S, positive changes occurred in QUI ( $74.37 \pm 18.87$  vs  $78.83 \pm 13.68$ ,  $p = 0.032$ ) and Est. BMD ( $0.397 \pm 0.12$  vs  $0.423 \pm 0.09$  g/cm<sup>2</sup>,  $p = 0.04$ ), whilst in group C there were no significant differences.

**Conclusions:** the daily consumption of these milk products increases levels of 25-OH-vitamin D and results in a decrease in markers for bone metabolism. A diet rich in soy isoflavones may be an option as a preventative measure against the effects of the menopause on bone.

**Key words:** soy isoflavones, bone metabolism, postmenopausal.

## Introduction

The post- and peri-menopausal periods are a physiological state characterised by the cessation of ovarian hormonal secretion, leading to significant physiological and psychosocial changes in the lives of women<sup>1</sup>.

In the light of the adverse effects of hormone replacement therapy, there has been increased interest in alternatives to improve menopausal symptoms and their long-term complications. The phytoestrogens are non-steroidal compounds which are structurally and/or functionally related to the placental or ovarian estrogens, and which may have antagonistic, agonistic or partial effects on the estrogen receptor. The isoflavones are the most active phytoestrogens, the most notable being those found in soya.

Due to this similarity with estradiol, the action of the phytoestrogens is mediated by the estrogen receptors (ER)  $\alpha$  and  $\beta$ . Their tissue distribution is different, the action of their natural or synthetic ligands having specific effects in each tissue. The isoflavones have greater affinity for ER $\beta$ . This finding has been put forward to explain the low incidence of clinical effects associated with the menopause in countries with a high consumption of phytoestrogens. Also, lower stimulatory effects are obtained in the breast and endometrium compared with 17 $\beta$ -estradiol, which triggers the transcriptional pathway of ER $\alpha$ <sup>2</sup>.

Taking into account these data, foods enriched with soya isoflavones could be considered as "functional foods" – those which include a component which provides a specific beneficial physiological, in addition to a purely nutritional, effect, and which results in an improvement in the state of health and contribute to the risk of developing diseases<sup>3</sup>.

The aim of our study is to evaluate the effects of nutritional intervention with a milk product enriched with soya isoflavones on the bone metabolism of Spanish postmenopausal women.

## Subjects and methods

This nutritional study was carried out with a randomised, controlled double blind design. The participants were recruited from the Endocrinology Clinic at the Centre for Specialisation in the University Hospital of San Cecilio, Granada. They all gave their signed informed consent to be included. The study was carried out with the approval of the Ethics Committee of the hospital, and was adjusted to meet the relevant directives for research in humans.

99 postmenopausal women between 45 and 65 years of age with physiological amenorrhea of at least one year's development, were selected. The study excluded patients with: serious cardiorespiratory, renal, hepatic or gastrointestinal disease; any hormonal drug treatment or any treatment affecting bone mass or vitamin D metabolism, including calcium and vitamin D supplements. The participants were distributed by random sampling into two groups: Group S, with 48 women,

who consumed the milk product enriched with isoflavones, and Group C, of 51 women, who consumed a control milk product. The daily amount of both products consumed was 500 ml over 12 months. In Group S, the daily quantity of isoflavones administered was 50 mg (Table 1).

At the start of the study epidemiological data was collected regarding age, time of development of the menopause, smoking habits and consumption of alcohol, and a basic physical examination was carried out to determine the body mass index (BMI) and the systolic (SPL) and diastolic (DPL) pressure levels.

Measurements were taken at the baseline and at 12 months for hormones, biochemistry and markers for remodelled bone. The hormonal data analysed were: follicle-stimulating hormone (FSH), leutinising hormone (LH) and 17 $\beta$ -estradiol. In addition, blood levels of calcium, phosphorus, parathormone, 25-OH-vitamin D and osteoprotegerin (OPG, ELISA BI-20402, BIO-MEDICA-GRUPPE, Wien, Austria) were measured. The markers for remodelled bone for formation measured were osteocalcin (OC, electrochemiluminescence immunoassay, analyser Elecsys, Roche Diagnostics, IN) and bone alkaline phosphatase (FAO, ELISA, Tandem-R Ostase TM, Hybritech Europe, Liege, Belgium). The markers for resorption included were tartrate-resistant acid phosphatase 5 $\beta$  (TRAP5 $\beta$ , colourimetry, Hitachi 704 Boehringer Mannheim GmbH) and carboxy-terminal telopeptide of type I collagen (CTX, enzymatic immunoassay, analyser Elecsys CrossLaps, Roche Diagnostics SL, Barcelona, Spain).

At the start of the study and at 12 months bone mass was estimated using ultrasound of the calcaneum (QUS, Hologic® Sahara® Waltham, NC, USA). The parameters provided were: speed of sound (SOS), attenuation coefficient (BUA, broadband ultrasound attenuation), QUI [QUI = 0.41(SOS) + 0.41(BUA) – 571], and estimated bone mineral density [Est. BMD = 0.002592  $\times$  (BUA+SOS) – 3.687 g/cm<sup>3</sup>]. The measurements were carried out in the dominant foot in the manufacturers' standard conditions<sup>4,5</sup>.

The statistical programme used was SPSS version 15.0. The quantitative variables were expressed as averages and standard deviations (SD) and the dichotomous variables as a percentage. The normality of the variables was analysed using the Kolmogorov-Smirnov test. A value of  $p < 0.05$  was considered to be statistically significant. For the comparison of the qualitative variables the chi-squared test was used. In the quantitative variables the t-student average comparison test for independent samples (intergroup differences) and paired samples (intragroup differences) was used.

## Results

### Epidemiological characteristics

The average age was 55.8 years (SD=6.9) with an average menopausal development time of 3.9 years (SD=4.1). 76.8% did not consume alcohol and 79.8% did not smoke. The average BMI was

28.35 kg/m<sup>2</sup> (SD=4.67); the average SPL, was 126 mmHg (SD=18) and DPL, 79 mmHg (SD=11). Statistically significant differences were found in the two groups (Group C compared with Group S) in the period of development of the menopause: 5.8 years (SD=3.7) as opposed to 7.9 years (SD=4.2),  $p=0.008$ .

#### Development of markers for bone metabolism

Table 2 specifies the markers for bone metabolism in the population studied during the follow up period.

In the total sample there was an increase in blood concentration of 25-OH-vitamin D ( $p<0.001$ ). In addition, the OPG ( $p=0.007$ ) and the TRAP ( $p<0.001$ ) diminished. Notable in Group C was the increase in the blood concentration of 25-OH-vitamin D ( $p=0.023$ ). There was a decrease in OPG ( $p=0.05$ ) and TRAP ( $p=0.001$ ). In Group S there was also an increase in blood concentration of 25-OH-vitamin D ( $p=0.001$ ) and a decrease in OPG ( $p=0.037$ ) and TRAP ( $p<0.001$ ). No statistically significant differences were found between the two groups in the rest of the measurements.

#### Development of bone mass estimated by ultrasound of the calcaneum

The parameters measured by QUS are shown in Table 3 and Figure 1.

In the total sample there was a significant increase in SOS ( $p=0.036$ ), QUI ( $p=0.012$ ), and estimated BMD ( $p=0.013$ ) and T-score ( $p=0.019$ ) between the start and after 12 months of the study. In Group C these changes were not significant, while in Group S there were favourable changes in QUI ( $p=0.032$ ) and estimated BMD ( $p=0.04$ ). There were no statistically significant differences found between the two groups.

#### Discussion

One of the central problems in relation to functional foods is to establish a scientific basis on which to support the beneficial properties which are attributed to their components. The epidemiological evidence suggests that the consumption of soya products is beneficial in relation to problems associated with the menopause. In this context we proposed to evaluate the effects of nutritional intervention with a milk product enriched with soya isoflavones on the bone metabolism in a group of Spanish postmenopausal women. In our study, the consumption of soya isoflavones resulted in favourable changes in bone mass.

Postmenopausal osteoporosis translates clinically into an increase in the risk of fracture and is a public health problem<sup>6</sup>. The observation that women from southeast Asia show a lower incidence of osteoporosis led to the hypotheses that the phytoestrogens from soya could be an alternative for the prevention of loss of bone mass associated with the menopause.

The role of the estrogens *in vitro* is to inhibit the development of the osteoclasts, favouring their apoptosis by stimulating the production of growth

Table 1. Nutritional content of milk products used in the study

Composition 500 ml	Group C	Group S
Calorific value (Kcal)	232	266
Proteins (g)	15.4	19.7
Carbohydrates (G)	23.6	29
Fats (g)	8.6	8
Vitamin A (UI)	3,000	3,000
Vitamin D (UI)	152	148.8
Vitamin B12 (µg)	1.9	2.1
Calcium (ng)	600	800
Phosphorus (ng)	600	630
Soya isoflavones (mg)	---	50

transformation factor beta (TGF- $\beta$ ) by the osteoblasts, in addition to inhibiting the production of interleukin 6 (IL-6), the principal stimulant for resorption. They also prevent osteoblast apoptosis. Deficiency estrogen also increases the apoptosis of the osteocytes, which alters the mechano-sensory function of the canalicular system for repairing microdamage, contributing to bone fragility<sup>7</sup>. The action mechanism by which the isoflavones protect against bone loss is not completely known, it being suggested that they modulate the receptor activator osteoprotegerin/ligand system for nuclear factor  $\kappa$ B (OPG/RANKL). With estrogen deficiency the production of OPG reduces and there is a strong response by the osteoclast precursors to RANKL<sup>8</sup>. The isoflavones, and specifically the genisteins, stimulate the activity of the osteoprotegerin. Moderate activity is sufficient to stimulate bone formation<sup>9,10</sup>.

The clinical studies carried out are highly variable in terms of their design, taking into account the duration of the supplementation, the dose prescribed and taken, the source of soya used, or the epidemiological characteristics of the population. A meta-analysis which reviewed ten clinical trials concluded that nutritional intervention with isoflavones could attenuate bone loss in the spines of postmenopausal women<sup>11</sup>, coinciding with the findings of Marini et al. who confirmed how treatment over two years with genistein had positive effects in the BMD of postmenopausal women with osteopenia<sup>12</sup>. A study of the effect on ultrasound of the calcaneum obtained similar results<sup>13</sup>.

Table 2. Change in markers for bone metabolism

		0 months average (SD)	12 months average (SD)	p
Calcium (mg/dl)	Total	9.25 (0.33)	9.17 (0.33)	0.388
	Group C	9.22 (0.32)	9.14 (0.35)	0.095
	Group S	9.29 (0.34)	9.37 (0.43)	0.336
Phosphorus (mg/dl)	Total	3.37 (0.45)	3.60 (0.43)	0.219
	Group C	3.4 (0.39)	3.6 (0.44)	0.776
	Group S	3.35 (0.5)	3.61 (0.97)	0.098
PTH intact (pg/ml)	Total	47.22 (16.84)	45.91 (16.51)	0.16
	Group C	47.83 (15.98)	47.27 (15.71)	0.582
	Group S	46.58 (17.86)	44.45 (17.39)	0.118
25-OH-vitamin D (ng/ml)	Total	24.48 (9.85)	28.18 (10.45)	<0.001*
	Group C	23.56 (10.16)	26.48 (10.69)	0.023*
	Group S	25.46 (9.51)	29.91 (10.02)	0.001*
OPG (pmol/L)	Total	5.21 (3.36)	3.89 (1.47)	0.007*
	Group C	5.68 (4.05)	4.1 (1.83)	0.05*
	Group S	4.72 (2.35)	3.69 (0.95)	0.037*
OC (ng/ml)	Total	15.46 (7.1)	17.13 (7.36)	0.096
	Group C	14.46 (7.15)	16.21 (6.84)	0.803
	Group S	16.31 (7.02)	18.1 (7.82)	0.083
FAO ( $\mu$ g/ml)	Total	15.47 (9.25)	16.03 (6.43)	0.068
	Group C	15.52 (11.63)	15.51 (7.01)	0.946
	Group S	15.42 (5.86)	16.59 (5.76)	0.092
TRAP5 $\beta$ (U/l)	Total	2.18 (0.8)	1.76 (0.54)	<0.001*
	Group C	2.15 (0.81)	1.74 (0.5)	0.001*
	Group S	2.21 (0.79)	1.78 (0.59)	<0.001*
CTX (ng/ml)	Total	0.47 (0.21)	0.42 (0.2)	0.064
	Group C	0.44 (0.19)	0.41 (0.19)	0.122
	Group S	0.52 (0.22)	0.42 (0.23)	0.335

PTH intact: parathormone intact; OPG: osteoprotegerin; OC: osteocalcin; FAO: bone alkaline phosphatase; TRAP5 $\beta$ : tartrate-resistant acid phosphatase 5 $\beta$ ; CTX: carboxy-terminal telopeptide of type I collagen.

\*p: statistically significant intragroup differences (p<0,05)

Table 3. Changes in bone mass estimated by QUS

		0 months average (DE)	12 months average (DE)	P
SOS (m/s)	Total	1517.86 (38.13)	1525.11 (35.6)	0.036*
	Group C	1520.2 (40.9)	1527.72 (42.51)	0.161
	Group S	1515.66 (35.59)	1522.66 (27.85)	0.120
BUA (dB/MHZ)	Total	61.6 (15.71)	64.38 (14.99)	0.057
	Group C	63.29 (15.73)	67.21 (16.89)	0.180
	Group S	60.18 (15.68)	61.72 (12.58)	0.182
QUI	Total	76.37 (19.87)	80.82 (18.26)	0.012*
	Group C	78.5 (20.87)	82.94 (22.1)	0.143
	Group S	74.37 (18.87)	78.84 (13.68)	0.032*
DMO (g/cm <sup>2</sup> )	Total	0.407 (0.13)	0.435 (0.12)	0.013*
	Group C	0.419 (0.13)	0.449 (0.14)	0.135
	Group S	0.397 (0.12)	0.423 (0.09)	0.040*
T-score	Total	-1.55 (1.12)	-1.31 (1.03)	0.019*
	Group C	-1.44 (1.17)	-1.19 (1.25)	0.144
	Group S	-1.64 (1.07)	-1.43 (0.79)	0.056

QUS: quantitative ultrasound in the calcaneum; SOS: speed of sound; BUA: broadband ultrasound attenuation, coefficient of attenuation; QUI= 0.41 (SOS) + 0.41 (BUA) – 571; BMD: estimated bone mineral density [Est. BMD=0.002592 × (BUA+SOS)-3.687, g/cm<sup>3</sup>].

\* p: statistically significant intragroup differences (p<0.05)

However, in spite of these favourable results there are also works which do not give evidence of change<sup>14</sup>. A recent intervention study in premenopausal women which evaluated the status of various ions, markers for bone metabolism and thyroid function found no differences in these parameters after the incorporation in the diet over ten weeks of soya isoflavones<sup>15</sup>.

It can be said that, although there is some experimental evidence which suggests a relationship between the consumption of isoflavones and an improvement in bone condition, these are not considered to be conclusive<sup>16</sup>.

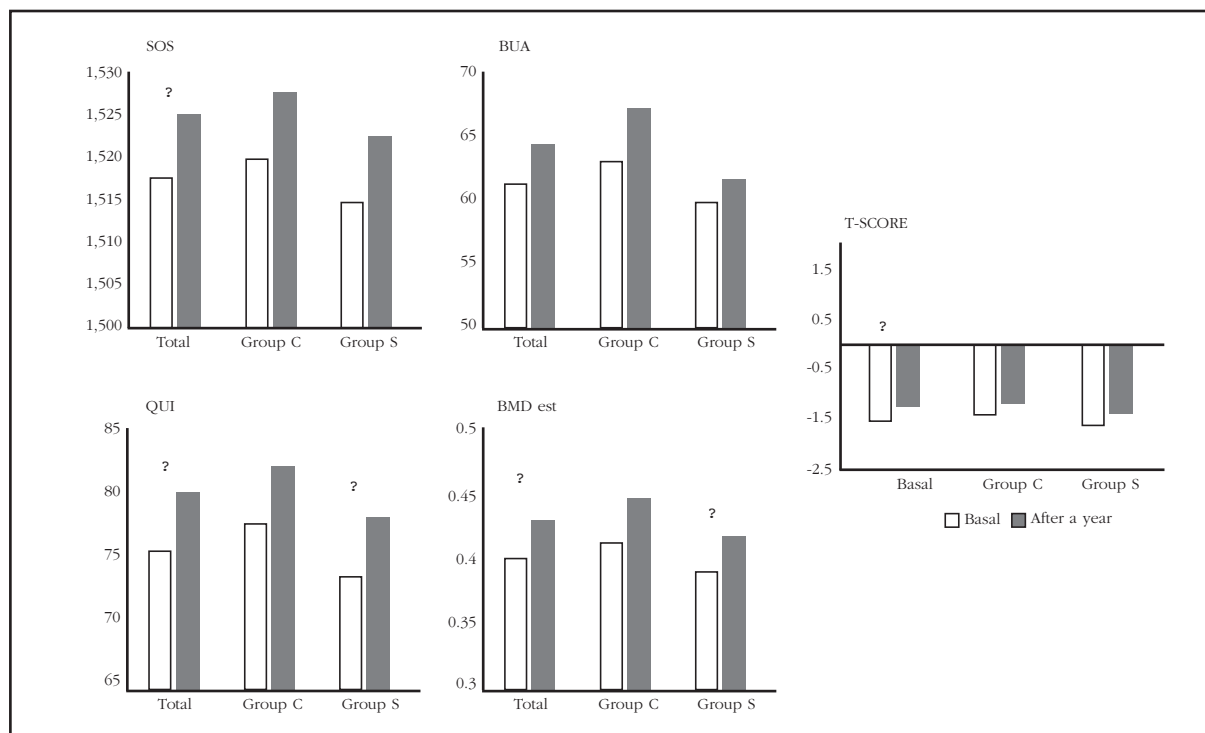
In relation to our results, there was a decrease in blood concentrations of TRAP and OPG and an increase in levels of vitamin D without differences between groups, which may be explained by the calcium and 25-OH-vitamin D contained in the milk preparations used. With respect to the evaluation of bone mass through ultrasound in the calca-

neum, a global increase was observed in all the parameters after a year of follow up, although the changes in QUI and estimated BMD in the group which consumed soya isoflavones were significant.

Our work suffers from some methodological limitations which do not make it possible to be certain whether the differences encountered were solely due to the supplementation with soya isoflavones. One way, hypothesis contrast models used are valid as a statistical method for comparison between groups. In conclusion, the daily consumption of these milk products increases levels of 25-OH-vitamin D and results in a decrease in markers for bone remodelling. A diet rich in soya isoflavones may be an option as a preventative measure against the effects of the menopause on the bone.

**Conflict of interest:** JFC is a member of the Research Department of Puleva Biotech, Granada, Spain.

Figure 1. Changes in the parameters of ultrasound in the calcaneum (QUS)



QUS: quantitative ultrasound in the calcaneum; SOS: speed of sound; BUA: broadband ultrasound attenuation, coefficient of attenuation; QUI=  $0.41 \text{ (SOS)} + 0.41 \text{ (BUA)} - 571$ ; BMD: estimated bone mineral density [Est.  $\text{BMD} = 0.002592 \times (\text{BUA} + \text{SOS}) - 3.687$ ,  $\text{g/cm}^2$ ].

\* p: statistically significant intragroup differences ( $p < 0.05$ )

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