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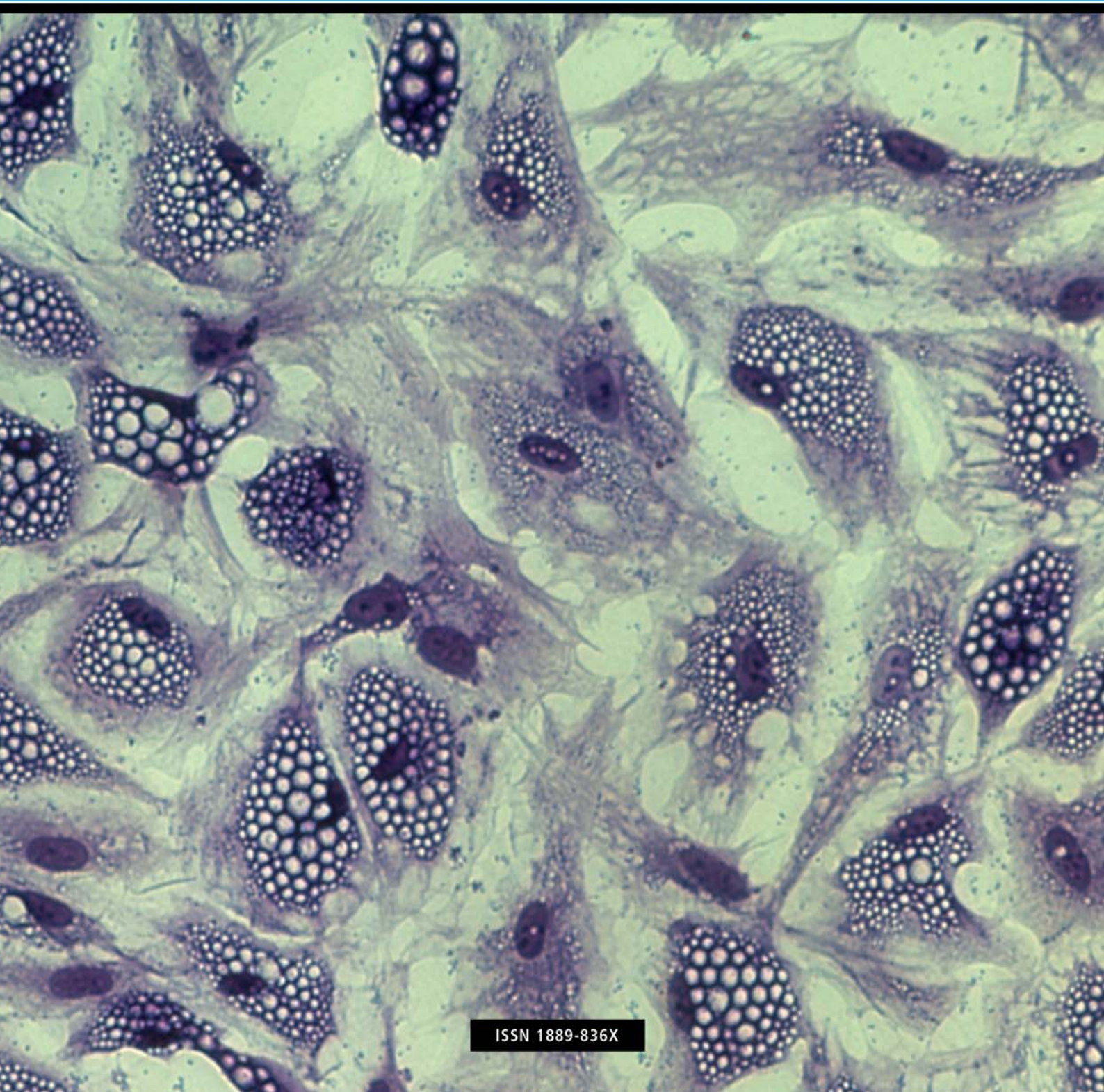

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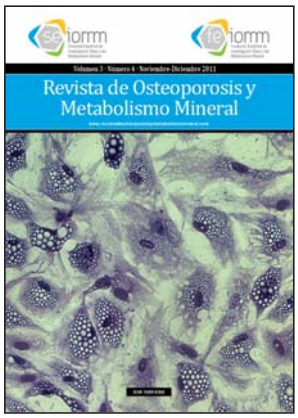

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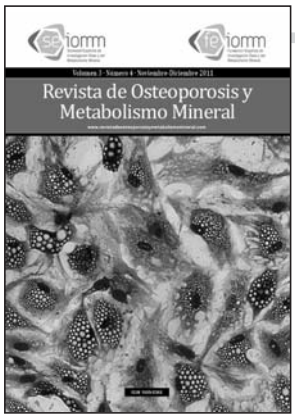

Our cover

Adipocyte differentiation from mesenchymal stem cells isolated from human bone marrow

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Raquel Santiago Mora,
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Committee of experts

Volume 3 reviewers (2011)

Isoflavones and bone

Cano A

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The appearance of scales for the prediction of the absolute risk of fragility fracture and the consequent definition of thresholds for pharmacological intervention has significantly limited the number of women eligible for treatment among those who are in their first years of the menopause. What is certain is the deterioration that many of them suffer in terms of bone metabolism as a consequence of rapid hypogonadism, but there are no defined strategies for the use of drugs to limit this phenomenon. In its day, hormone therapy solved this problem, but its limitations to use in women with symptoms sufficient to affect quality of life has left many users without an efficacious option. It is true that life style changes, especially diet and exercise, alleviate the problem, but they are not an entirely satisfactory solution. The advances which are being made in the action mechanism of plant extracts, both in the form of pure molecules prepared to the equal quality of medicines, or foods in which they are found in sufficient concentrations (functional foods), are raising new expectations. There has been significant progress in the knowledge of the molecular mechanisms of many of these substances, especially the isoflavones. Although there are differences between their components, we know that they are capable of activating estrogen receptors, particularly isoform β, and that this is followed by the activation of different signalling pathways in various experimental models, essentially cellular. The fundamental question, however, is what is their true clinical significance.

On this point the evidence is more limited and to date, still confusing. On the one hand is the unfinished business of the symptoms, where there are few clinical studies of quality, and those that there are present difficulties derived from their inclusion of groups with low numbers of participants or of other methodological drawbacks. On the other, there is the question of their eventual efficacy in limiting chronic diseases which more or less clearly have their roots in hypogonadism, such as cardiovascular disease or osteoporosis. The questions in relation to the former have recently been reviewed[1], and with respect to the latter, particularly welcome is the article by García-Martín et al. in this issue[2]. With a control group and with a randomised double blind design, the authors conclude that after a year of follow up the supplementation with 50 mg/day of isoflavones improves the bone parameters evaluated by ultrasound. There are favourable changes overall in some of the markers for bone metabolism evaluated, although without differences between the two groups. Perhaps the inclusion of a high number of cases would have revealed the suggested advantage of the isoflavones. It is curious that the intervention is associated with a decrease in osteoprotegerin (OPG). This finding is contrary to that published by other groups[3], and *a priori*, is in opposition to the protection seen in the ultrasound parameters. Therefore, what cannot be discounted is that this data has even greater value, given the highly varied provenance of the OPG, and of its growing value as biomarker for cardiovascular disease, as the same group has just well reviewed[4].

A literature review regarding the actions of the isoflavones in bone, however, shows that were are dealing with an area in which there are significant discrepancies. For example, a recent clinical trial

did not find a protective effect on the bone in women who took tablets containing 200 mg of isoflavones for two years[5], and a meta-analysis which examined the action on bone mineral density came to similar conclusions[6]. However, another meta-analysis found there to be protection, albeit reduced[7]. Also, with regard to biochemical markers for bone metabolism, a recent meta-analysis found a slight protective action in relation to resorption[8]. Finally, there is very little information on the effect on ultrasound parameters, and again, in this, the value of the García-Martín study should be highlighted.

How to cast some light on this apparently tricky matter? Evidently, more clinical research is required, but this does not seem to be a simple task due to a series of conditions particular to these types of preparations.

On the one hand, is the great variety of molecules and the differences between their effects, including the metabolic capacities of the individual, which is not the same between, for example, the isoflavones genistein and biochanin A. And in terms of individual metabolisation, it is also important to note that equol, a metabolite of daidzein, is generated by the action of intestinal flora, but only in certain individuals. There are no exact figures, but it is calculated that between 35% and 50% of individuals are capable of producing it. This adds an important factor to the variability of the results of therapeutic actions, given that equol is considered to be one of the most powerful isoflavones. In this area, it would have been useful if the García-Martín[2] study had included details of the mixture of isoflavones used.

But on the other hand, there is the response threshold. A meta-analysis which examined the action on vasomotor symptoms found a clear dose-dependent action in a period which reached up to 160 mg/day of isoflavones, with a threshold of acceptability of approximately 80 mg/day[9]. There are also differences between purified isoflavones and soya protein, at least in the matter of cardiovascular protection, as has been demonstrated in the analysis of the American Heart Association[10].

In conclusion, therefore, this is a promising field, but one in which order needs to be imposed. What needs to be clarified is what isoflavones should be used, purified or not, at what dose and, probably, what type of user will obtain, or not, some protective effect. Studies such as that of García Martín are particularly welcome, given their good design which contributes to the accumulation of more evidence.

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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 ± 0.8 vs 1.76 ± 0.54 U/l, p<0.001, and 5.21 ± 3.36 vs 3.89 ± 1.47 pmol/L, p=0.007, respectively), as well as an increase in 25-OH-vitamin D (24.48 ± 9.85 vs 28.18 ± 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 ± 38.13 vs 1525.11 ± 35.6 m/s, p=0.036), QUI (76.37 ±19.87 vs 80.82 ± 18.26, p=0.012), estimated bone mineral density [Est. BMD] (0.408 ± 0.13 vs 0.435 ± 0.12 g/cm², p=0.013) and T-score (-1.55 ± 1.12 vs -1.31 ± 1.03, p=0.019). In group S, positive changes occurred in QUI (74.37 ± 18.87 vs 78.83 ± 13.68, p=0.032) and Est. BMD (0.397 ± 0.12 vs 0.423 ± 0.09 g/cm², 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[1].

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 estrogenic receptors (ER) α and β. 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 ERβ. 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 17β-estradiol, which triggers the transcriptional pathway of ERα[2].

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[3].

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 17β-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 5β (TRAP5β, colourimetry, Hitachi 704 Boehringer Manheim 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 × (BUA+SOS) – 3.687 g/cm^2]. The measurements were carried out in the dominant foot in the manufacturers' standard conditions[4,5].

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/m² (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.

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

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[6]. 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 transformation factor beta (TGF-β) 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[7]. 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 κB (OPG/RANKL). With estrogen deficiency the production of OPG reduces and there is a strong response by the osteoclast precursors to RANKL[8]. The isoflavones, and specifically the genisteins, stimulate the activity of the osteoprotegerin. Moderate activity is sufficient to stimulate bone formation[9,10].

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[11], 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[12]. A study of the effect on ultrasound of the calcaneum obtained similar results[13].

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 (µg/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
TRAP5β (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; TRAP5β: tartrate-resistant acid phosphatase 5β; 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^2)	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^2].
* 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[14]. 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[15].

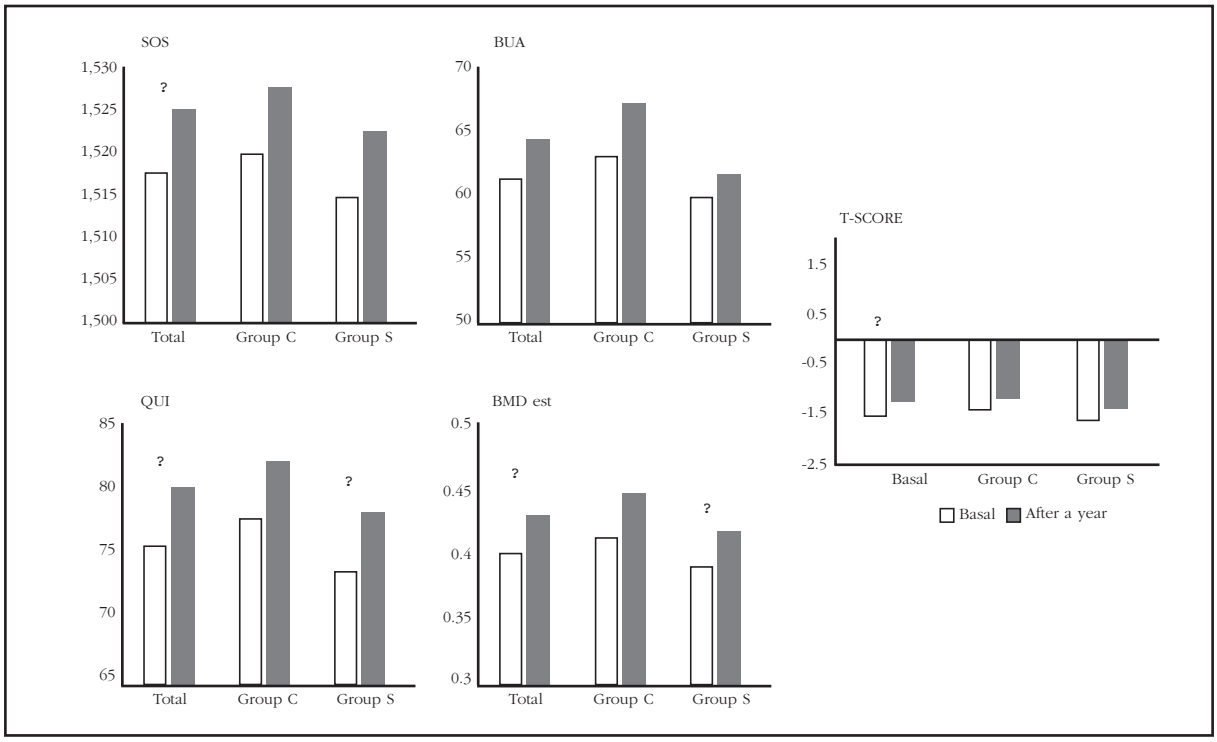
It can be said that, although there is some experimental evidence which suggests a relationship between the consumption of isoflavones and an improvement in bone condition, these are not considered to be conclusive[16].

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 calcaneum, 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 (SOS) + 0.41 (BUA) – 571); BMD: estimated bone mineral density [Est. BMD=0.002592 × (BUA+SOS)-3.687, g/cm^2] .
* p: statistically significant intragroup differences ($p<0.05$)

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Usefulness of FRAX® in the study of fractures in the alcoholic patient

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Summary

FRAX® index is a prognostic tool to assess the risk of osteoporotic fracture. Although ethanol ingestion, liver disease and body mass index are considered independent prognostic factors in the FRAX® score, we have observed that in chronic alcoholics there are several variables not included in the FRAX® index, which show a relation with prevalent fractures and/or low BMD. Therefore, in this study we compare the relation of FRAX® index with those of other variables, such as lean and fat mass, liver function parameters, and amount of ethanol consumed, with the presence or not of prevalent fractures in 57 chronic alcoholic men, older than 40 years, drinkers of more than 200 g ethanol/day during a long time. We found that FRAX® index was significantly higher among those with any fracture, but the same happened with BMI, total fat amount, and fat amount at arms, as well as total amount of ethanol. The FRAX® index did not show differences among those with or without vertebral fractures, or rib fractures. Patients with rib fractures showed differences in total fat amount and right arm fat amount when compared with patients without rib fractures. Therefore, these results suggest that in the alcoholic, other variables, such as amount of ethanol consumed and fat mass, should be considered, in addition to FRAX®, in the prediction of fractures.

Key words: *FRAX® index, alcoholism, bone alterations, fractures, osteopenia, body composition.*

Introduction

The alcoholic patient is exposed to a higher risk of fractures, due, essentially, to two factors: on the one hand, the reduction in bone mass, a multifactorial phenomenon, influenced by many mechanisms, such as the alcohol itself[1-5], the associated malnutrition[6,7], the eventual hepatopathy[8], the secondary hormonal alterations due both to the alcohol and the hepatopathy, and the possible effect of the pro-inflammatory cytokines; on the other hand, the kind of life the alcoholic has, which exposes these patients to falls and traumas which contribute to these fractures[9]. Today, we have clinical tools which allow us to predict the risk of fracture prospectively. One of these, currently in vogue, is FRAX®, an index which includes variables such as the body mass index (BMI), bone mineral density (BMD), age, history of fracture, family history of fracture, alcohol itself, conditions associated with osteoporosis such as hypogonadism (which also affects alcoholics), corticoids, hepatopathy, and others[10]. However, in previous studies we have seen that bone mass in alcoholics is related to lean mass and fat mass[6,11], and that various cytokines, by acting on the receptor activator for nuclear factor κ B (RANK), and its ligand RANKL[12], may also play a pathogenic role. In addition, other variables such as vitamin D[7], may have an influence on fractures, as well as certain social and personal aspects of the environment of the alcoholic, which impact on their life style and their risk of fracture and trauma. None of these parameters is directly included in FRAX®, which means that it is important to compare the value of this tool with those of the variables cited, and to analyse whether lean mass, fat mass, hepatic function, quantity of alcohol consumed, or FRAX® is associated most closely with the presence of fracture in the alcoholic patient, in a cross section of a population with a certain number previous fractures. This is the objective of this work, part of a wider prospective study designed to analyse the relative value of the aforementioned parameters in the diagnosis of fractures occurring in this group of alcoholics followed in the long term.

Patients and methods

57 male patients over 40 years of age, who had given their informed consent, and who had been consecutively admitted to the internal medicine service of our Centre due to organic problems related to the excessive consumption of alcohol, drinkers of great quantities of alcohol (210 ± 90 g/day) over 31 ± 9 years, were included, adapting the FRAX® criteria, designed for the evaluation of risk of fracture in individuals over the age of 40 years. The patients included in this study had sustained significant after effects as a result of their chronic consumption of alcohol: thirty three were cirrhotic, 8 had neoplasms, and 22 died within a period of 18 months (inter-quartile rate 11-56 months) from their inclusion in the study.

X-rays (Xr) of the post-anterior (PA) and lateral (L) thorax were carried out in order to evaluate the presence of rib fractures, while in the lateral Xr we were looking for dorsal vertebral fractures, applying morphometric criteria[13]. To this we added a detailed anamnesis, to see whether or not they had earlier fractures. In some cases it was not possible to correctly evaluate the Xr in the thorax. We also performed a densitometric study using double energy X-ray absorptiometry (DXA) with a LUNAR densitometer (GE HealthCare), to evaluate bone mass in different parts of the skeleton (bones of upper limbs, lower limbs, ribs, spine, pelvis and total), and the T-score in the spinal column and hip. Using these T-score values we grouped our patients as osteoporotic, osteopenic or normal, according to the criteria currently in use[14].

We carried out a nutrition assessment including, in addition to the aforementioned densitometric parameters, a previously validated subjective scale of nutritional assessment, which is based on the qualitative assessment of the lean mass and fat mass in the abdomen, upper and lower limbs, temporal muscle and Bichat's ball[15]. We calculated FRAX® in all the cases[10].

A routine analysis was carried out in all patients, which included albumen, prothrombin activity and blood bilirubin, as well as determining IGF-1 (chemoluminescence, DPC, Los Angeles, CA, USA), 1-25 dihydroxyvitamin D3 (radioimmunoanalysis, Nichols, San Juan de Capistrano, CA, USA), and parathyroid hormone (PTH, immunochemiluminescence, Siemens, Munich, Germany).

This study had the approval of the Ethics Committee of the University Hospital of the Canary Islands. It forms part of a wider prospective study designed to analyse the relative value of the aforementioned parameters in the diagnosis of fractures occurring in this group of alcoholics followed over the long term.

Statistical method

We calculated the difference existing between patients with and without existing fractures in relation to the FRAX® index, lean mass, fat mass, nutritional assessment, and analytical parameters related to hepatic function. Through the Kolmogorov-Smirnov test we determined whether the variables studied were adjusted or not to a parametric distribution. The tests used to compare differences between two groups were the student's T test, and the Mann-Whitney U test in the case of a non-parametric distribution of the variable analysed. To determine which variables were independently related to the FRAX® index we carried out multivariant analysis, introducing lean mass, fat mass, age, prothrombin, albumin, bilirubin, FRAX® index, BMI and subjective nutritional assessment.

Results

Thirty two of the 57 patients studied had had at least one fracture. In 4 cases this fracture was related to a serious trauma (in general, traffic accidents): 1 fracture of the tibia, another of the tibia and fibula, another of both hips, and the other of lumbar vertebrae and multiple ribs. In the thoracic Xr 24 old rib fractures were identified (as opposed

Table 1. Differences between patients with or without fractures (all types of fractures included)

	With fracture (n=32)	Without fracture (n=24)	T (Z); p
Age (years)	53.94 ± 8.81	54.21 ± 11.03	T=0.10; NS
Body mass index	24.79 ± 3.23	27.05 ± 4.29	T=2.04; p=0.047
FRAX® index	4.14 ± 2.27	2.30 ± 1.28	T=3.7; p<0.001
Daily alcohol consumption (g)	214 ± 88	203 ± 98	T=0.42; NS
Years of consumption	33.03 ± 8.51	28.30 ± 8.01	T=1.98; p=0.053
Vitamin D (pg/ml)	28.00 ± 16.87	31.85 ± 14.23	T=0.79; NS
IGF-1 (ng/ml)	99.7 ± 104.6 47.1 (27.9-183.60)	67.8 ± 44.85 48.3 (32.9-105.0)	Z=0.21; NS
PTH (pg/ml)	90.23 ± 132.01 51.40 (29.83-86.23)	60.62 ± 47.37 49.0 (26.25-82.40)	Z=0.56; NS
Prothrombin (%)	75.46 ± 22.13	68.98 ± 27.90	T=0.92; NS
Albumin (g/dl)	3.29 ± 0.57	3.29 ± 0.82	T=0.03; NS
Bilirubin (mg/dl)	3.61 ± 3.65 2.5 (1.1-6)	4.43 ± 4.60 2.35 (1.2-5)	Z=0.73; NS
Total BMD (g/cm^2)	1.07 ± 0.10	1.08 ± 0.095	T=0.59; NS
T-score total hip	-1.28 ± 1.09	-0.83 ± 1.10	T=1.52; NS
T-score L2-L4	-1.39 ± 1.15	-1.39 ± 1.16	T=0.38; NS
Total lean mass (g)	50.085 ± 5.145	53.052 ± 7.653	T=1.64: NS
Total fat mass (g)	17.704 ± 6.620	22.584 ± 9.656	T=2.12; p=0.039

The data are expressed as the mean ± standard deviation, and were compared using the student's T test (T). After the application of the Kolmogorov-Smirnov test it was observed that some variables did not adjust to a parametric distribution. In these cases, in addition to the mean and standard deviation the median and, in brackets, the interquartile range were also provided, and the two groups (with or without fractures) were compared using the Mann-Whitney U test (Z)

to 20 without fracture) and in the spinal Xr, 13 (as against 25). In Tables 1-3 the data from patients with or without fractures in the different locations analysed is summarised. As we see, the total fat mass was greater in those who did not have fractures (any fracture, not even of the rib), and the same for BMI, and marginally, those patients who had been drinkers for longer also had more fractures.

It is notable that in no case was the total BMD significantly different between patients with or without previous fractures. With the variables already mentioned we carried out a logistic regression study to see which factors could be independently related to fractures. We found that, although, with respect to any fracture, the factors to which they were independently related were, first the FRAX® index, then prothrombin activity and lastly the duration of (alcohol) intake in years (Table 4), in relation to rib fractures the first parameter to which it was independently related was total fat mass (Table 5). It is also worth highlighting the fact that none of the parameters chosen played an independent role in relation to the presence or absence of vertebral fractures.

In Figures 1 and 2 we show the ROC curves which illustrate the global capacity of the fat mass and the FRAX® index to diagnose any fracture (1a and 1b) and rib fracture (2a and 2b). As can be seen, FRAX® is useful in both cases, especially to diagnose any osteoporotic fracture, while in the fat area it is only the rib fracture which is diagnosed.

Discussion

The FRAX® index is a widely used index for the diagnosis of risk of fracture[10]. It is, therefore, a prognostic index, and it is as such that it should

Tabla 2. Patients with or without rib fractures

	With fracture (n=24)	Without fracture (n=20)	T (Z); p
Age (years)	52.96 ± 8.33	54.90 ± 12.15	T=0.63 ; NS
Body mass index	24.78 ± 3.36	27.04 ± 3.97	T=2.05; p=0.047
FRAX® index	3.76 ± 1.93	3.04 ± 2.30	T=1.14; NS
Daily alcohol consumption (g)	217 ± 94	198 ± 96	T=0.68; NS
Years of consumption	31.96 ± 6.57	32.15 ± 11.20	T=0.70; NS
Vitamin D (pg/ml)	26.86 ± 16.07	33.86 ± 15.97	T=1.36; NS
IGF-1 (ng/ml)	108.2 ± 112.5 47.1 (28.4-191.0)	80.6 ± 61.13 53.5 (32.9-118.2)	Z=0.04; NS
PTH (pg/ml)	58.37 ± 44.35 45.60 (28.7-85.4)	82.18 ± 80.93 52.8 (30.55-95.68)	Z=0.85 ; NS
Prothrombin (%)	77.69 ± 22.05	71.03 ± 27.44	T=0.79; NS
Albumin (g/dl)	3.35 ± 0.56	3.28 ± 0.73	T=0.38; NS
Bilirubin (mg/dl)	3.18 ± 2.42 2.25 (1.23-5)	4.33 ± 4.54 3.20 (1.1-5.6)	Z=0.73; NS
Total BMD (g/cm^2)	1.06 ± 0.11	1.07 ± 0.08	T=0.23; NS
T-score total hip	-1.33 ± 1.10	-0.88 ± 0.86	T=1.49; NS
T-score L2-L4	-1.38 ± 1.25	-1.54 ± 0.87	T=0.19; NS
Total lean mass (g)	50.321 ± 5.201	53.063 ± 8.136	T=1.38: NS
Total fat mass (g)	17.015 ± 6.250	21.671 ± 8.827	T=2.00; p=0.052

The data are expressed as the mean ± standard deviation, and were compared using the student's T test (T). After the application of the Kolmogorov-Smirnov test it was observed that some variables did not adjust to a parametric distribution. In these cases, in addition to the mean and standard deviation the median and, in brackets, the interquartile range were also provided, and the two groups were compared using the Mann-Whitney U test (Z)

be considered, although it is obvious that the same factors which allow one to predict a future fracture ought also to be capable of differentiating between patients with or without fractures at any given moment. In this work we have analysed the capacity of this index to detect these differences in alcoholic patients, since in this group there is a series of factors which may distort its value. There is no doubt as to the existence of osteopathy in the chronic alcoholic. Already observed by Saville in the 1960s[16], Oppenheim[9] subsequently applied the term "battered alcoholic syndrome" to those alcoholic patients with more than three fractures in different states of consolidation. Later, the classic works of Israel[1], Diamond[2] and others[17-19], to cite only a few, serve only to confirm that in alcoholics, independently of cirrhosis, there is a metabolic osteopathy characterised by osteopenia, in which malnutrition plays a significant role[6,20]. This is due, above all, to defective bone formation, although there being some controversy with respect to reabsorption, which expresses an imbalance between the formation and destruction of bone. But certain aspects, on which we comment below, make this different. Firstly, age: alcohol reduces life expectancy, and osteoporosis in the alcoholic, although increasingly serious with age, appears much earlier than when associated with the menopause, for example, or with senility. Secondly, the nutritional state. This is often clinically evaluated in a general way, through BMI, or subjectively, but without paying attention to the fat or lean areas of the body which may be altered selectively; it is common for some alcoholics to have a relative increase in fat mass accompanied by a parallel decrease in lean mass, with a

Tabla 3. Patients with or without dorsal fractures

	With fracture (n=13)	Without fracture (n=25)	T (Z); p
Age (years)	56.15 ± 9.67	54.48 ± 10.52	T=0.48 ; NS
Body mass index	27.39 ± 4.09	26.28 ± 3.86	T=0.74; NS
FRAX® index	4.17 ± 2.69	2.96 ± 1.67	T=1.71; NS
Daily alcohol consumption (g)	202 ± 130	223 ± 91	T=0.53; NS
Years of consumption	35.77 ± 10.64	29.33 ± 7.43	T=2.08; p=0.046
Vitamin D (pg/ml)	30.30 ± 18.70	34.14 ± 18.64	T=0.56; NS
IGF-1 (ng/ml)	89.1 ± 74.8 53.5 (33.8-152.1)	81.7 ± 99.0 46.9 (30.4-91.2)	Z=0.53; NS
PTH (pg/ml)	82.45 ± 93.32 55.10 (27.02-93.05)	99.99 ± 143.18 55.0 (42.25-92.20)	Z=0.62; NS
Prothrombin (%)	74.00 ± 24.47	70.91 ± 21.14	T=0.39; NS
Albumin (g/dl)	3.55 ± 0.77	3.17 ± 0.59	T=1.61; NS
Bilirubin (mg/dl)	3.63 ± 3.61 2.2 (1.0-5.3)	4.49 ± 5.09 2.75 (1.15-5.88)	Z=0.30; NS
Total BMD (g/cm^2)	1.07 ± 0.10	1.10 ± 0.10	T=0.69; NS
T-score total hip	-1.09 ± 1.25	-0.98 ± 1.12	T=0.28; NS
T-score L2-L4	-1.79 ± 1.18	-1.14 ± 1.34	T=1.48; NS
Total lean mass (g)	51.271 ± 7.673	51.947 ± 5.149	T=0.30: NS
Total fat mass (g)	23.778 ± 9.270	20.682 ± 7.301	T=1.09; NS

The data are expressed as the mean ± standard deviation, and were compared using the student's T test (T). After the application of the Kolmogorov-Smirnov test it was observed that some variables did not adjust to a parametric distribution. In these cases, in addition to the mean and standard deviation the median and, in brackets, the interquartile range were also provided, and the two groups (with or without fractures) were compared using the Mann-Whitney U test (Z)

normal or even raised BMI (malnourished obesity). This is important since although the decrease in lean mass reduces bone formation[21], the fat may exert opposing effects, since, although contributing to the weight, and thus increasing the bone mass, it may also be the source of cytokines which can cause bone lesions, such as tumour necrosis factor (TNF)[22]. It is also notable that the total fat mass replaces the FRAX® index in its capacity to diagnose existing fractures at any given moment. As we have just indicated, the fat mass, which may be elevated in the alcoholic, contributes significantly to total weight. It is this weight which is opposed to gravity, and which our skeleton has to support, which exerts a stimulating effect on osteoformation. But it is also worth noting that we did not see a relationship between fracture and lean mass. Lean mass determined by densitometry may be misleading in the alcoholic since the presence of ascites or oedemas may falsify the results[23]. In this study we cannot discount the influence of hydrosaline retention, although generally the densitometry was carried out when the patient was ready to be discharged, or, at least, a few days after treatment.

A third factor to consider in the osteopathy of the alcoholic is hormonal alterations. This is due in part to the cirrhosis, although the alcohol in itself, without the need for the coexistence of cirrhosis, provokes hypogonadism, altering the levels of vitamin D and the cortisol metabolism, even though the effects of these hormones are contained, in one way or another, in the FRAX® index.

FRAX® is, without a doubt, a useful tool. In fact, if we consider its value in the diagnosis of