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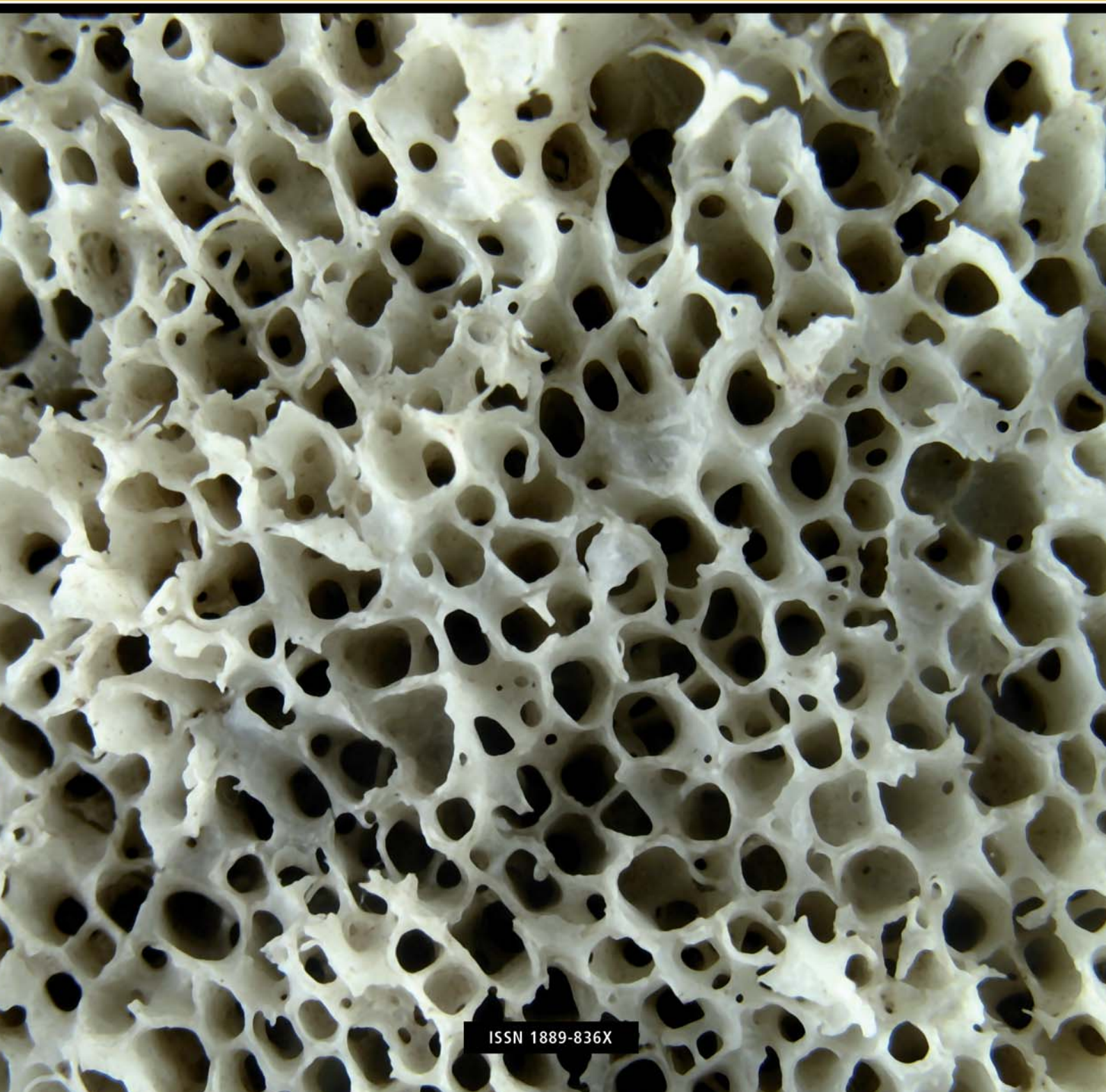

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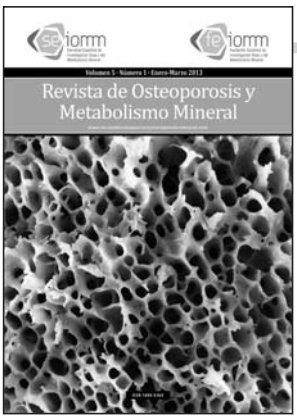

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SUMMARY

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Interdisciplinary prevention of hip fracture

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Spain has one of the highest hospital costs relating to hip fracture, at 9,936 euros for an admission related to this pathology[1]. To these economic-health costs we must add those arising in the patient's environment and, above all, the non-quantitative costs arising from the changes in lifestyle and the loss of productivity which fragility fractures produce, both for the patient, as well as for their families, and for society as a whole. These are difficult to quantify, pending the results of the ICUROS and PROA[2] studies, which have estimating these costs as their objectives.

If we take into account the high number of hip fractures treated annually, 720 cases annually for every 100,000 people over 60 years of age, it is not difficult to understand the serious public health problem this represents. However, the true problem is not the financial costs, but in the personal cost which results, and which is translated into raised levels of morbimortality.

Hip fracture, the outcome of loss of femoral resistance, and in many cases, of a fall, is the most serious example of the complications of osteoporosis. Its treatment should be based on resolving the functional problem, improving the nutritional and metabolic state of the injured person, on avoiding new falls and trying to recuperate and reinforce the bone structure.

If these actions are not carried out diligently the clinical and life prognosis will become more serious. Approximately a third of patients with hip fracture die as a consequence of it, the mortality index in autonomous patients without acute disease at admission, and having an intervention for hip fracture during the first or following day after hospital admission, being significantly lower than in those patients in whom the intervention is made later[3].

This mortality is increased in those patients with a deteriorated nutritional state[4,5], who have levels of albumen lower than <3.5g/dl and in patients with dementia, among other factors[6]. In spite of the fact that improved nutrition in patients is vital, and that it has been proved that the administration of protein supplements are effective in the recuperation of the patient and the prevention of complications[7], the prescription of recommended diets and of fall prevention is practically nonexistent in clinical histories[8]. But it is also necessary to curb bone loss and, as far as possible, recuperate the bone structure, since a second fracture may occur in up to 14% of cases in a period of less than 5 years, most of these being in the first 18 months. But it is not possible to treat a disease if it is not present. Firstly, there must be an awareness of underlying osteoporosis following a low energy hip fracture. However, the ABOPAP study indicated that 67.7% of doctors surveyed said that osteoporosis was not included in the preventative activities of their place of work[9].

Secondly, the establishment of drug treatment. The drugs most commonly used in recent years in treatment and secondary prevention have been the bisphosphonates. Their efficacy is such that they have been associated with a reduction in the frequency of hip fractures detected since the middle of the last decade[10], and in the appearance of secondary hip fractures[11]. However, the Record of Osteoporotic Fractures in Spain (Acta de Fracturas Osteoporóticas en España (AFOE)) study carried out by the Osteoporosis Study and Research Group (Grupo de Estudio e Investigación de la Osteoporosis (GEIOS)) of the Spanish society of Orthopaedic Surgery and Traumatology (Sociedad Española de Cirugía Ortopédica y Traumatología (SECOT)), proved that the diagnosis of osteoporosis prior to fracture was only token, and that only 18.4% of the patients had received any treatment for osteoporosis before the fracture. But, what was worse was that only 25.6% of patients received treatment on discharge. A subsequent initiative, the GIOS project (Gestión Integral de la Osteoporosis Integrated Management of

Osteoporosis), showed how it was possible to improve the prescription rate to 61.8% of cases[12], this successful outcome the result of the collaboration between traumatologists and primary care doctors, as the authors of this work recognise.

For all these reasons we must highlight the fact that nowadays, treatment of hip fracture cannot and should not be carried out only by the traumatologist. The combined action of professionals from different specialisms (traumatologists, geriatricians, rehabilitators, anaesthetists, nurses, social workers, etc)enables a resolution of the hip fracture which is more rapid, complete and satisfactory; collaboration based on scientific evidence, such as that proposed and developed by GEIOS along with other medico-surgical societies, and which nowadays are a reality in a great many centres in our country[13].

The work published in this number, and developed by Dr Herrera Pérez et al.[14], proves that the establishment of clinical pathways agreed by the different specialisms involved in the treatment of hip fractures, and the implementation of verification measures (checklists) to ensure secondary prevention of new fractures on discharge, contribute to adherence to treatment. This is interesting work which will need to be evaluated over the next few years to determine the impact it has had on the reduction in new fractures.

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Checklist for prevention of new hip fractures

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Summary
Introduction: Hip fracture is the worst complication of osteoporosis and especially affects postmenopausal women in the developed world. Previous studies have shown low rates of initiating osteoporosis treatment during hip fracture hospitalization.
Objective: To probe the effectiveness of the so-called hip fracture checklist in increasing the rate of the initiation of osteoporosis treatment compared with the previous two years.
Methods: Rates of initiating treatment among a population of one hundred postmenopausal women over 60 years of age surgically treated after suffering a hip fracture. Comparison of rates of prescription in our hospital before and after initiating the current study.
Results: In 2006, 1.66% of the patients were discharged from hospital with a new treatment for osteoporosis. In 2007, the rate was 6.9%. The age of our patients was 80.4 years. All of them were diagnosed during hospitalization with either osteoporosis (61.9%) or osteopenia (38.1%), but only 13% of them were previously diagnosed with osteoporosis, 10% of them were taking calcium and vitamin D, and 2% bisphosphonates. At the time of discharge, we prescribed calcium and vitamin D to all the patients (100%), and bisphosphonates (oral or parenteral) to the 94.6% of them.
Conclusions: The results show a significant increase in rates of antiosteoporotic drugs prescription compared with the previous two years. The implementation of the hip fracture checklist clearly increases the likelihood of starting osteoporosis treatment post hip fragility fracture.

Key words: *fragility hip fracture, osteoporosis, osteoporosis treatment.*

Introduction

Osteoporosis is characterised by the structural deterioration of bone tissue which leads to a loss in resistance which increases the susceptibility of suffering fractures. Both women and men are affected, although 80% are postmenopausal women[1]. The National Foundation for the study of Osteoporosis (NOF) estimates that 10 million people in the US suffer from osteoporosis and that approximately 34 million more have a greater risk of suffering from it due to low bone mass[2]. The data regarding osteoporotic fractures in Spain which started to circulate in the final years of the last century, referring to a period of one year, referenced approximately 30,000 to 40,000 fractures of the proximal end of the femur, 70,000 vertebral fractures and 25,000 fractures of the distal end of the radius. After the AFOE (Acta de Fracturas Osteoporóticas de España – Record of Osteoporotic Fractures in Spain) study endorsed by the GEIOS (Grupo de Estudio e Investigación en Osteoporosis de la Sociedad Española de Cirugía Ortopédica y Traumatología – Study and Research Group on Osteoporosis of the Spanish Society of Orthopaedic Surgery and Traumatology) and from the results of a survey carried out in 2003 in 77 hospitals in the different Autonomous Communities of Spain, it has been possible to confirm that these figures are already far from the reality, and have seen a notable increase, such that the number of fractures of the proximal third of the femur in those over 60 years increases to more than 60,000 a year[3].

Fractures of the hip are associated with a significant risk of mortality in the first year of between 15 and 33%, attributable to factors as variable as deep vein thrombosis, pulmonary embolism, pneumonia, a general state of deterioration and deficient rehabilitation. The ratio of mortality increases with the age of the patient (by 4% for each year of life), the period of delay before surgery (, 2 days: 4%; >4 days: 6.1%), and the comorbidity of admission (e.g., 40% of mortality in the first year in the case of congestive cardiac failure).

An osteoporotic hip fracture is very often the first sign of osteoporosis and always an alarm call. The follow up of patients with osteoporosis, above all if there is an earlier fracture, is very important in achieving a good development of the disease and, therefore, a lower level of incapacity in this type of patient.

Initial fragility fractures lead to an increased risk of suffering future fractures[1]. In a study carried out in more than 30,000 patients with home care in North Carolina (US), it was found that 23.9% of those patients with hip fracture and 15.1% of patients with other types of fracture experienced a second fragility fracture in the following two years[4]. In another follow up study of 22 years duration of 766 women with hip fractures, 45% suffered a second fracture[5].

The objective of this study is to assess the implementation of secondary prevention of osteoporotic fracture of the hip after raising the awareness of medical and nursing staff, and family and social workers through the introduction of a clinical pathway, discharge checklist or hip fracture checklist (Table 1).

Material and method

A unicentric prospective observational study, initiated as a consequence of the start of a doctoral thesis project by the first author of this article in a tertiary hospital. The study included 100 postmenopausal women over 60 years of age with osteoporotic fracture of the proximal third of the femur (after low intensity trauma), treated in our centre (University Hospital of the Canaries) during the period from January 2008 to June 2010. The inclusion and exclusion criteria were the following:

– *Inclusion criteria:*

1. Postmenopausal women over 60 years of age.
2. Admitted due to having presented with a low energy (fall from their own height or less) fracture of the proximal third of the femur.
3. Ability to walk before the fracture, and having both legs.
4. Signature of informed consent

– *Exclusion criteria:*

1. Hip fracture having been treated with surgical intervention with the presence of osteosynthetic material or prosthesis in the healthy hip.
2. Creatinine clearance <30 ml/min and corrected calcemia >11 mg/dl or <8 mg/dl.
3. Active neoplasm, active infection or bone metabolic pathology.
4. Life expectancy less than 6 months according to the researcher's criteria.

Activity protocol

Once the patient was stabilised from a medical point of view, the surgical intervention proceeded (osteosynthesis or partial or total substitution arthroplasty). A complete clinical history was taken, focusing on risk factors for osteoporosis, in addition to routine analytical tests and tests specific to bone metabolism, before and after surgery. Among the tests was one for blood vitamin D (25 dihydroxyvitamin D) using radioimmunoassay (BIOSOURCE®), a technique whose sensitivity was 0.6 ng/ml.

Bone densitometry was performed using dual energy X-ray absorptiometry (DXA), Hologic® QDR-2000 system (software version 5.54), when the patient was postoperatively mobile. The bone mineral content (BMC) and bone mineral density (BMD) were determined in the lumbar spine (L2, L3, L2-L4), and in the proximal femur of the non-fractured hip. Those patients who had a T-score lower than -2.5, were defined as osteoporotic according to the criteria of the WHO and incorporated in the principal guides[1]. The results are expressed in Table 1, taking as a reference the values described as normal for bone mass in our country[6].

The percentages of patients who had been diagnosed previously with osteoporosis, and whether they had taken any kind of treatment for it,

Table 1. Densitometric values of the patients studied

N=100	T-score neck	T-score trochanter	T-score total hip	T-score L2-L4	BMD neck	BMD total hip	BMD lumbar spine
Average	-2.17	-1.13	-1.89	-0.508	0.68147	0.72943	-0.90934
Median	-2.50	-1	-1.750	-0.450	0.68050	0.76700	0.89000
Deviation	1.1286	0.7208	1.0662	1.1432	0.126801	0.110397	0.166344
Minimum	-4.4	-2.8	-3.8	-2.8	0.420	0.497	0.552
Maximum	0.3	0.1	0.3	1.1	0.978	0.915	1.258

BMD: bone mineral density (g/cm^2).

were analysed. The sufficient level of intake of calcium was established at more than 2 rations a day, which the literature describes as ensuring an intake of at least 1,200 mg of calcium element a day[7].

Prior to the study, a retrospective review was carried out of patients with hip fracture discharged from our hospital in previous years, to see which were discharged with calcium/vitamin D and bisphosphonates. Specifically for the aims of this study, a hip fracture checklist was introduced (Table 2).

Statistical method

The data were analysed using the software programme SPSS 15.0. We proceeded first to determine if the variables had a normal distribution or not by means of the Kolmogorov – Smirnov test. Although most of the women had a Gaussian distribution, some hormones and markers for bone synthesis and resorption had a non-parametric distribution. Therefore, for the univariant inferential statistical analysis we used, with a normal distribution, Student's t test to compare one variable between two groups and the ANOVA test in the case of three or more groups, and subsequently the Student-Newman-Keuls test to discern between which groups differences were established, and Pearson's correlation to analyse the relationship between two quantitative parameters. Given the relationship between BMD and age, a covariance study was carried out with this parameter. In the case of non-parametric distributions, the Mann-Whitney U test was used to analyse the differences between two groups, and Kruskall-Wallis to analyse the differences between three or more groups, as well as Spearman's correlation.

Once the individual prognostic value for each parameter was established, a Cox regression study was then carried out to understand which of those showing a prognostic value in the univariant analysis also had it in the mutlivariant analysis.

There was also an analysis of whether the change in the values of the different variables determined at the start and at 6 months had any prognostic relationship through a general linear analysis model for repeated measurements. This analysis was also carried out to see the influence of alcoholic abstention on these variables. Subsequently, the patients were classified according to the gain or loss of bone mass at 6 months from the start of the study, analysing the prognostic value and the effect of abstinence using Kaplan and Meyer curves, the Log Rank test, and the Breslow test. Finally, we carried out a Cox multivariant test, including, among other parameters, abstinence, to compare the results obtained with the changes in the variables analysed and to confirm whether they had independent prognostic value or not.

Results

The patients in the study who had fractured their hip were elderly (average age 80.4 years), most living at home (where the fracture occurred), were sedentary, 40% of whom were receiving some kind of home help, and the same proportion had some degree of cognitive deterioration. 75% of them did not consume the minimum daily requirement of milk products, having an intake of two or less portions per day (Figure 1) and more than 50% had reduced values of vitamin D, meaning levels lower than 30 ng/ml (Figure 2).

After carrying out a DXA on admission, whose results are shown in Table 1, 61.9% had osteoporosis (Figure 3), but only 13% had previously been diagnosed with osteoporosis.

As regards the taking of treatment for osteoporosis previously, the results indicate that 10% received daily calcium and vitamin D and 2% oral bisphosphonates.

After the initiation of our study with the implementation of the checklist 94.6% of our patients were prescribed bisphosphonates on discharge:

Table 2. Checklist of the multidisciplinaryhip fracture team

ON ADMISSION:

1. Admission and preoperative phase: haemogram, coagulation study, ECG, thoracic X-ray, anteroposterior pelvic X-ray and axial X-ray of fractured hip.
2. Informed consent for surgery and blood transfusion.
3. Suspend antiaggregant/anticoagulant medication.
4. Provide the following appropriate medication:
   - Thromboembolic prophylaxis
   - Analgesia
   - Gastric protection
5. Carry out an interconsultation with Anaesthesiology.
6. Intervention within 48 hours improves medical conditions (interconsultation with Internal Medicine if associated comorbidity).

POSTSURGICAL:

7. Appropriate medication:
   - Endovenous analgesia
   - Thromboembolic prophylaxis
   - Gastric protection
   - Endovenous antibiotherapy (maximum of 48 hrs)
8. Endovenous vitamin D.
9. 24 hour serotherapy.
10. 24 hours seated.
11. Control X-ray and interconsultation with Rehabilitation.
12. Haemogram and basic biochemistry at 48 hours.
13. Remove drips, probes, first dressings and deambulation within 48 hours.

ON DISCHARGE:

14. Appropriate medicine:
   - Oral analgesic
   - Thromboembolic prophylaxis
   - Gastric protection
   - Home treatment
   - Calcium and vitamin D
   - Oral bisphosphonates (no earlier than two weeks from the fracture)
15. Appointment at the traumatology outpatients clinic.
16. Continuing rehabilitation treatment.
17. Appointments in other outpatient clinics requested.
18. Control X-ray.

19. ATTACHED RECOMMENDATIONS TO THE PATIENT OR FAMILY:
   - Lifestyle changes: regular exercise, stop smoking and minimise alcohol intake, stop taking sedatives and psychotropic drugs.
   - Assess the risk of falls in the home (slippery floors, poor lighting, domestic pets, etc.), eliminate sedative or psychotropic drugs, correct defective vision, use of crutches or walking frame.
   - List of questions for the patient to ask their FAMILY DOCTOR:

1. Have I had a recent fracture. Do I suffer from osteoporosis?
2. Do I need to have a test to discount it?
3. Do I need to take more calcium or vitamin D?
4. What can I do to prevent falls?
5. What will you prescribe me for osteoporosis?

80.7% taking a single weekly dose of 75 mg risedronate, and the remaining 19.3% i.v. zoledronic acid. All patients were prescribed calcium and vitamin D at discharge (a single daily dose of 2,500 mg of calcium carbonate and 880 UI of colecalciferol).

Although it did not form a part of the direct objective of this study, we recorded the percentage adherence to treatment at 6 months from discharge by means of a telephone survey of the patients or close family, obtaining the following results: 40% of patients had abandoned the calcium and vitamin D

and 10% the oral bisphosphonate. Of those who did not abandon the treatment, 56.10% and 59.74% of the patients who had taken calcium and vitamin D and bisphosphonates respectively took it correctly (optimum compliance considered to be when at least 75% of the recommended dose was taken). Logically, the compliance of the patients treated with endovenous bisphosphonates on admission was 100%.

Discussion

It is well known that after suffering a hip fracture there is a probability of between 14% and 20% of suffering another contralateral hip fracture[5,8], a history of fragility fractures being the most significant isolated clinical risk factor for the appearance of new fractures. It is also known that a patient who has suffered a fragility fracture of the femur has a fourfold greater risk of having a fracture in another location in the following three years. In addition, one of the few studies specifically designed to evaluate the risk of hip fracture among patients having suffered a previous hip fracture, showed that almost 15% of the 481 subjects suffered a second fracture[9]. While only 1% of the subjects would suffer a second hip fracture in the following 6 months, this figure increases to 8% in the first 5 years, and even to 12-15% in the first 10 years of follow up. In our sphere, the AFOE (Acta de Fracturas Osteoporóticas en España - Record of Osteoporotic Fractures in Spain, 2003)[3] study, carried out using data from 77 hospitals from across the whole country uses hip fractures recorded in each centre in the year 2002 and fractures of the hip, shoulder and wrist seen in the month of May 2003, contains very interesting data on this matter. It is not only that the number of fractures registered in the year 2002 was much higher than expected, but also that a third of those patients with hip fractures registered in May 2003 had suffered a previous fracture, 9.4% in the hip, 5.7% in the contralateral hip and 5.5% in the spine. 5.8% of the total number had suffered more than one fracture previously. However, only 23.7% of the patients with hip fracture received or had received any type of drug treatment for osteoporosis, and 60% of those, only calcium, with or without vitamin D.

Therefore, in spite of the strong scientific evidence relating osteoporosis with fracture and previous history of fragility fracture with a higher probability of new fractures, few patients with fragility fractures receive a complete evaluation of their osteoporosis. The directives of the National Foundation for the Study of Osteoporosis (NOF) were updated in 2008, and recommend that clinicians consider the initiation of treatment for osteoporosis in those patients who have suffered a hip or vertebral fracture[1]. However, various studies show a rate of prescription for treatment of osteoporosis in those patients which is uniformly low, between 11% and 53% (an average of 36%)[2,10]. In our series we observed a strong increase in the prescription of bisphosphonates on discharge: 94.6% of patients were prescribed bisphosphonate, in 80.7% of cases this was risedronate. In 100%

Figure 1. Intake of milk products in the patients studied

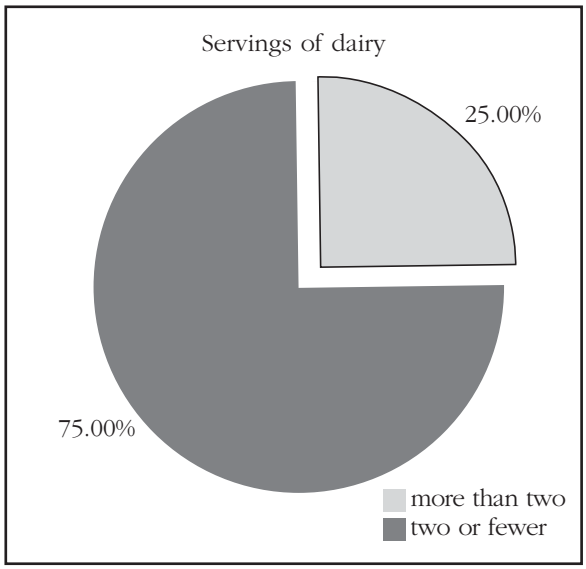

Figure 2. Levels of vitamin D (deficit if <30 ng/ml)

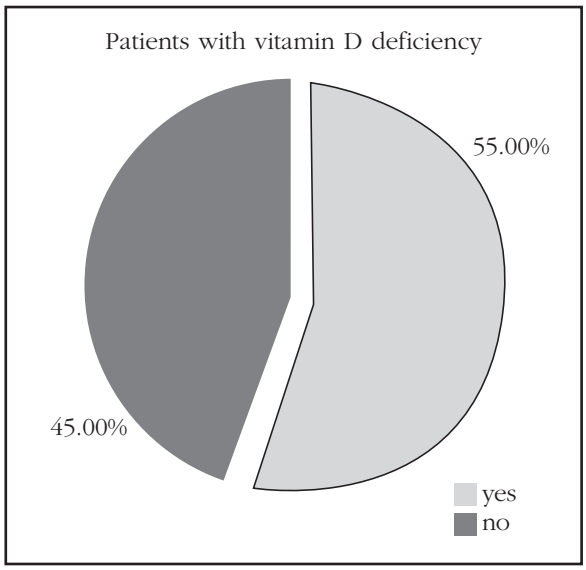

Figure 3. Bone mineral density (BMD) at admission

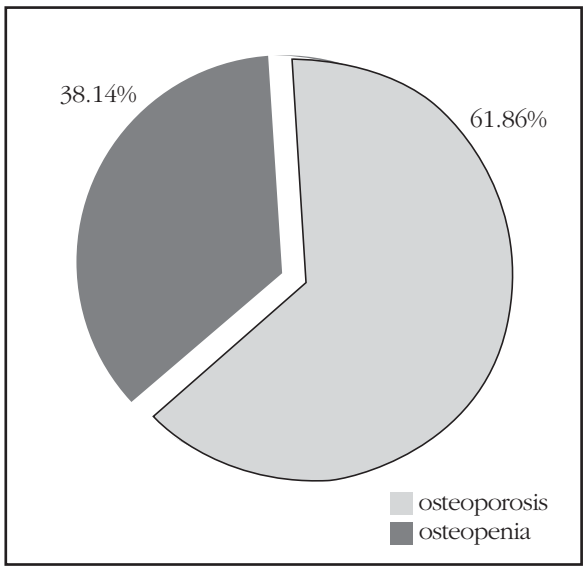

Table 3. Comparison of prescriptions on discharge from 2006 to 2010

Year	Hip fracture in women	Treatment at discharge bisphosphonates + Ca/vit D
2006	100	2
2007	110	8
2008	62	58
2009	69	66
2010	79	75
Our series	100	94

of our cases calcium and vitamin D were prescribed on discharge (a significant increase when compared with the 10% who were taking them prior to admission), for which we carried out a comparison of prescriptions in our centre in the years 2006 to 2010 (Table 3).

The prescription rate for treatments for osteoporosis for these patients on discharge varied according to the prescribing service, with the internal medicine service having the highest rate (58%), as against the lowest which was orthopaedic surgery (12%). A study showed that the probability of being prescribed this treatment in internal medicine is 8.33 times higher than in an orthopaedic surgery service[10]. And, specifically in the case of hip fractures, a shocking result that in only 1-9% of cases was treatment initiated for osteoporosis, with calcium with vitamin D the being the treatment most prescribed, and on rare occasions, bisphosphonates[11]. The role of internist includes not only the evaluation, assessment and stabilising of patients before surgery, but also the management and prevention of post-operative complications. This is reflected in their own MIR training plan, which literally states that *"the internist, as hospital generalist, should attend to the majority of medical problems that arise, either as consultant or as physician as part of multidisciplinary teams, thus contributing to the comprehensive care of patients admitted for surgery …".* The contribution of the internist within a multidisciplinary team has been studied in our field, notable being the article of Vidán et al.[12], which deals with the first randomised clinical trial carried out in our country on multidisciplinary geriatric intervention vs normal management in a group of patients over 65 years of age with hip fractures. The duration of stay, analysed as a primary objective, demonstrates a reduction of up to 2 days, and morbimortality and functional capability, analysed as a secondary objectives, showed a lower risk of death and complications, as well as a higher functional capacity after 3 months of follow up.

The benefits have been so notable that the role of a medical internist integrated into the traumatology service has been established in many tertiary level hospitals in European countries, resulting in a reduction in stay (total and postsurgical) of patients not receiving intervention due to medical complications, in mortality, in referrals and in calls on other medical services, and finally, achieving a saving of 60,000 euros a month for the reduction in stay alone[13].

In our centre, a tertiary hospital with 600 beds and a catchment area of 600,000 residents, thanks to the collaboration between the traumatology, orthopaedic and internal medicine services, since 2008 we have been able to count on an internist attached to our hospital departments dedicated to perioperative management of patients with osteoporotic hip fracture in the light of the accompanying high comorbidity in this group of trauma patients. This collaboration is already reflected in a significant reduction in perioperative complications in these patients, as well as in the rate of prescription of bisphosphonates and calcium with vitamin D on discharge.

In patients with osteoporotic hip fracture, the main role of the orthopaedic surgeon is to treat the fracture surgically, once they are in appropriate medical condition to withstand the aggression of surgical intervention. As the recommendations of GEIOS (Grupo de Especialistas en Osteoporosis – Osteoporosis Specialists Group) of SECOT (Sociedad Nacional de Cirugía Orthoédica y Traumatología - National Society of Orthopaedic Surgery and Traumatology) reflect[14], the fundamental objective of osteoporosis treatment is the avoidance of fragility fractures. Once these have occurred, we can then speak of the essential role of the orthopaedic surgeon in identifying this fracture as osteoporotic, and the prevention of the appearance of new fractures. For this, the following steps should be carried out:

1. Ensure the proper intake of calcium and vitamin D, as well as initiating treatment with antiresorptives, ideally bisphosphonates, for at least 2-3 weeks after the fracture being fixed.
2. Ensure early functional rehabilitation.
3. Introduce educative measures to the patient and their family, as well as enabling effective communication with primary care and social services, if required.
4. Insist on the implementation of all measures aimed at preventing new falls.

After the surgical intervention on the patient the second role the orthopaedic surgeon needs to take would be to implement a series of measures to reduce the morbidity and mortality of these patients. With this aim, in our centre we have designed the so-called discharge checklist, in the form of a clinical pathway from the admission of the patient until discharge, with special emphasis on medical treatment for osteoporosis if this had not yet been initiated, and on providing information for the patient and their family regarding the necessity of continuing this treatment in order to

avoid future fracture events. Following these steps will result not only in proper treatment but also continued care after discharge.

Participating in the implementation of this checklist are the traumatologist, the internist and the family doctor, who, after meeting with the family, is responsible for ensuring that good family support is in place for the patient with hip fracture prior to their discharge, and making contact with the social worker from our centre if this family support is not available.

With respect to pharmaceutical measures, the maintenance of optimum levels of calcium and vitamin D should be the basis of all antiosteoporotic treatment. However, the fact that the increase in bone turnover stimulated by the repair of the fracture and remodelling causes an increase in metabolism, and in the need for calcium and vitamin D, should be taken into account, as well as the fact that the elderly population which suffers hip fracture has a higher risk of having suboptimum levels of these elements, due essentially to lower exposure to sunlight, malabsorption, changes in the diet, etc. In addition, a meta-analysis carried out in recent years has shown the benefits of providing vitamin D as a secondary prevention strategy for fractures[15], by reducing the risk of falls and the risk of non-vertebral fractures.

On the other hand, the bisphosphonates constitute the treatment of choice at discharge, and over the long term, in patients who have suffered an osteoporotic hip fracture. Alendronate, risedronate, ibandronate and zoledronic acid have shown their efficacy in reducing the risk of osteoporotic fracture postmenopausal women in a number of well designed clinical trials[3]. Various studies with alendronate and zoledronate have also demonstrated a reduction in the rate of hip fractures. Morin et al. were the first to demonstrate how therapy with antiresorptive agents was capable of reducing the risk of new fractures occurring in patients who had suffered hip fractures[16]. This reduction in risk of new fractures may already be detected after the first 6 months of treatment[17]. Furthermore, treatment with oral bisphosphonates reduces global mortality by 8% per month of treatment, or approximately 60% for each year of treatment, as has been recently demonstrated[18]. Daily oral administration is effective; however, its compliance is low. Weekly or monthly administration appears to improve adherence to treatment, which, however, continues to be at suboptimum levels.

Once therapy is assessed and initiated, we consider that hospitalization due to hip fracture is a real clinical opportunity for the multidisciplinary hip fracture team, as studies which relate this early start to treatment on discharge with bisphosphonates with higher rates of compliance, demonstrate[8]. In addition, the orthopaedic surgeon also plays a crucial role, since it appears that compliance in treatment with bisphosphonates prescribed by the orthopaedist on discharge is higher than when it is prescribed by a primary care doctor: according to a randomised study of 162 patients with hip fracture followed up over 6 months, it was observed that 58% of patients treated by the orthopaedist maintained their treatment as against 29% of those whose treatment has been prescribed by a primary care doctor[19].

In conclusion, collaboration between services greatly improves the care given to the group of patients admitted with hip fracture to traumatology services. Thus we have developed a checklist on the clinical pathway model, with excellent initial results: thanks to the conscientiousness of medical staff, the percentage of patients treated with medication for osteoporosis increased significantly on discharge (Ca/vitamin D: from 10% to 100%; bisphosphonates: from 2% to 96%). We did not have more than 6 months of follow up data available, but it is our intention to study adherence to treatment and the incidence of new hip fractures after a year and two years from the start of this study.

Note: This study shows some of of the results developed in the doctoral thesis of the first signatory 20.

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Effects of calcium and vitamin D, with and without lactulose, in bone mineral density on postmenopausal women with osteopenia: Pilot randomized controlled trial

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Summary

We report the results of a randomized, double-blind, double-dummy, multicenter, parallel group pilot study, the objective of which was to assess whether the addition of lactulose to vitamin D and calcium supplementation for 12 months contributed to bone mineral density (BMD) maintenance in postmenopausal women with osteopenia (T-score –1 to –2.5 SD). Women in the lactulose group (n=19) received lactulose 15 mL/day (equivalent to 10.05 g), vitamin D3 400 IU/day and calcium carbonate 500 mg/day, and women (n=22) in the placebo group were administered lactulose placebo, vitamin D3 400 IU/day and calcium carbonate 1,000 mg/day. The baseline daily calcium intake was similar in both study groups. The primary endpoint was the BMD in the lumbar spine at the final visit. A generalized liner model was used to assess final versus baseline differences in BMD in both study groups. Differences in least-square means of BMD between lactulose and placebo were not statistically significant both in the per-protocol data set (–0.012, 95% CI –0.031 to 0.007, P=0.224) and in the intention-to-treat population (–0.005, 95% CI –0.025 to 0.016, P=651). As we have not found differences within the two study groups, the addition of lactulose to 500 mg of calcium carbonate associated with vitamin D supplementation could have similar effects on lumbar BMD as 1.000 mg of calcium carbonate. These findings may indicate that lactulose may improve calcium absorption in postmenopausal women. A long follow-up study with a greater number of subjects would be necessary to confirm these preliminary observations.

Key words: *lactulose, calcium, bone mineral density, osteopenia, postmenopausal women.*

Introduction

Osteoporosis is a very common disease in postmenopausal women and people of an advanced age, and is associated with an increased risk of fractures. Fractures related to osteoporosis are a very significant public health problem, having a consequence of high rates of morbidity and mortality, as well as a reduction in the quality of the lives of those who suffer from it. In addition, its ever increasing incidence (due, in part, to the gradual aging of the population) has renewed interest in the efficacy and safety of drugs available for the treatment of the reduction in bone mineral density (BMD) associated with osteoporosis. An adequate intake of calcium and vitamin D plays a critical and synergistic role in the maintenance of optimum musculoskeletal health, and is considered to be the first step in the treatment of osteoporosis[1]. Numerous studies support the importance of vitamin D deficit as a risk factor for osteoporotic fractures, and the beneficial effects of a treatment which combines vitamin D (of 700 to 800 UI/day) and calcium (of 1,000 to 1,200 mg/day) in avoiding non-vertebral and hip fractures[2-8].

It has been shown that a fractional decrease in the absorption of calcium in older women with a low intake of calcium increases the risk of hip fracture[9]. The proportion of calcium absorbed through the intestine varies enormously, from 10% to 70%[10-12]. However, the availability of calcium in the bone depends more on intestinal absorption than on the amount of calcium ingested. Other factors apart from vitamin D, such as the amount of fat[13] and non-absorbable sugars[14] in the diet, stimulate the intestinal absorption of calcium. Hence, the non-digestible oligosaccharides (such as raffinose, stachyose, the fructo-oligosaccharides, the polydextrins, the insulins and lactulose) and the prebiotics in general have received increasing attention due to their selective effects on the intestinal flora, which have beneficial effects on the wellbeing of the host and their health[15,16].

Lactulose is a synthetic disaccharide. It is composed of molecules of galactose bonded to molecules of fructose by means of a beta-1-4 link. The compound is synthesised by the isomerisation of lactose. Lactulose is not produced naturally; the human body does not have enzymes capable of hydrolysing lactulose from the monosaccharides galactose and fructose. Lactulose passes through the gastrointestinal tract and reaches the colon not having been modified, where it is broken down into short chain fatty acids (AGCC) (lactic, acetic, propionic and butyric acids) through bacterial degradation. The bacterial transformation of lactulose into AGCC acidifies the contents of the colon and induces various physiological changes in the colon, which are responsible for the preventative and therapeutic effects of the lactulose in constipation, portosystemic encephalopathy, enteritis due to salmonella and other potential indications. In experimental studies, the acidification of the colon which results from the hydrolysis of lactulose increases the concentration of soluble calcium and the absorption of calcium mediated by vitamin D[17-22]. However, data obtained from clinical studies are scarce. In 12 postmenopausal women who participated in a randomised study, with double-blind crossing, the consumption over 9 days of lactulose increased the absorption of calcium with a dose-responsive effect[23]. In a clinical trial of double-blind design, randomised, with crossing, in 24 healthy adult male volunteers, lactulose increased the absorption indices of calcium and magnesium[24]. To our knowledge, there are no studies which have examined whether the potential impact of lactulose on calcium absorption results in an increase in BMD.

One of the secondary effects of calcium at normal doses used in the treatment of postmenopausal osteoporosis is the digestive intolerance which in many cases necessitates the withdrawal of the drug, or is a reason for the abandonment of treatment.

Hypothesis

The combination of lactulose (10 g), vitamin D (400 UI/day) and calcium carbonate (0.5 g/day) is the equivalent of a regular dose of calcium carbonate (1 g/day) plus vitamin D (400 UI/day), and has the same effect on BMD after 12 months of treatment, which would hypothetically reduce the possible secondary effects of high doses of calcium and would improve adherence.

Objectives of the study

The primary objective was to evaluate BMD in postmenopausal women with osteopenia after 1 year of treatment with a combined regimen of lactulose (10 g), vitamin D (400 UI/day) and calcium carbonate (0.5 g/day) with a calcium placebo, against a second regimen of the same dose of vitamin D and a double dose of calcium carbonate (1 g/day) with a lactulose placebo, administered over 12 months.

The secondary objectives were the BMD in the femoral neck and total hip, as well as the effect of the treatment on the analytical parameters for bone remodelling, specifically, changes in the levels of blood calcium, phosphorus, parathyroid hormone, 25-hydroxyvitamin D and the urinary secretion of calcium, as well as changes in the values of bone alkaline phosphatase, blood CTx, and urinary NTx over the period of the study.

Subjects and methods

Design of the study

It consisted of a pilot prospective trial, phase IV, randomised, double blind, double simulation, of parallel groups. The study was carried out in the external clinics of the rheumatology and internal medicine services of two university hospitals with bone mineral metabolism units in Barcelona (Spain). The duration of the study was 12 months. Approval for the study was obtained from the national health authorities and from the committees for ethics and clinical trials of the participating hospitals. All the women gave their informed consent in writing.

Table 1. Baseline characteristics of the population of study (ITT population data)

	All women n=41	Study groups	
		Lactulose, n=19	Placebo, n=22
Age, years. Average (min-max)	58.5 (52-67)	57.6 (52-67)	59.4 (55-67)
Weight, kg. Average (min-max)	70.2 (52-110)	70.0 (56-110)	70.4 (52-90.5)
Height, cm. Average (min-max)	156.7 (144-169)	154.6 (144-163)	158.4 (146-169)
BMI, kg/m^2. Average (min-max)	28.6 (21.2-47.6)	29.4 (23.3-47.6)	28.0 (21.2-32.9)
Smoker			
Non-smoking	31 (75.6)	13 (68.4)	18 (81.8)
Former smoker	4 (9.8)	3 (15.8)	1 (4.5)
You smoke now	6 (14.6)	3 (15.8)	3 (13.6)
Physical exercise	36 (87.8)	18 (94.7)	18 (81.2)
Take a walk	32	17	15
Swim	4	3	1
Other	9	6	5
No exercise	5	1	4
Average food consumption, (SD)			
Dairy products, g/day	381.7 (206.9)	444.9 (251.1)	325.5 (142.7)
Total calcium, mg/day	698.7 (376.3)	825.8 (469.9)	585.8 (226.8)
Concomitant medication	36 (87.8)	17 (89.5)	19 (86.4)
Anti-inflammatory drugs	20	12	8
Pain relievers	17	6	11
Angiotensin renin inhibitors	11	8	3
Psychos-Analeptics	11	7	4
Psycholeptics	8	3	5
Lipid-lowering agents	6	3	3
Antacids	4	1	3
Antimicrobial	4	2	2
Beta-blockers	3	1	2
Calcium channel blockers	3	2	1
Mineral supplements	3	2	1
Other	17	8	9

Data such as numbers and percentages in parentheses unless stated otherwise.
ITT: intention-to-treat; SD: standard deviation.

Study population

Between June 2003 and March 2006 postmenopausal women between 50 and 70 years of age having had amenorrhea for a minimum of 5 years and osteopenia defined as BMD with a T-score of between -1 and -2.5 in the lumbar spine (L2-L4), and/or femoral neck or total hip were recruited[25]. The exclusion criteria were: suffering from any disease which would cause osteopenia or alterations in the metabolism of calcium or phosphorus, or any disease in which the taking of calcium and vitamin D or the use of laxatives were contraindicated; presence of galactosemia; treatment with corticosteroids, antacids which contain calcium, iron salts, or thiazides; continuing treatment with lactulose; treatment with vitamin D and/or calcium

supplements in 4 weeks prior to the study, or treatment with antiresorptives (bisphosphonates, hormone replacement therapy, raloxifene, etc.); known hypersensitivity to the drugs used in the study; serious illness, substance abuse, serious neurological disorders, psychiatric disease, or any disease which, in the opinion of the researcher could mean that the patient might not sufficiently comply with the protocol of the study.

Evaluation of bone mass and laboratory parameters

A pre-study visit (visit 1) was carried out in the month prior to the randomisation, which included: anamnesis and complete physical examination, evaluation of the intake of calcium, physical exercise and concomitant medicines, laboratory and bone densitometry tests. The evaluation of the calcium intake was carried out by means of a survey of the number of daily and weekly portions of different types of foods which were consumed (milk products, cereals, fruits, vegetables, fish and meat). Samples of blood and urine were obtained from all patients, at between 8 and 10 in the morning, after 12 hours of fasting. The laboratory tests included standard biochemical and haematological profiles, blood levels of calcium, phosphorus, parathyroid hormone, 25-hydroxyvitamin D, and urinary secretion of calcium (urine in 24 hours). In addition, the following markers for bone remodelling were measured: bone alkaline phosphatase, C-terminal telopeptide of type 1 collagen in blood (CTx) and N-terminal telopeptide of type 1 collagen in blood (NTx) (second sample of urine). To measure BMD in the femoral neck and total hip a Hologic® QDR-4500 (Hologic, Waltham, MA, US.) bone densitometer was used. The results were expressed in g/cm^2 (coefficient of variation of 1.3% in the lumbar spine and 1.65% in the femoral neck) and as T and Z score values.

In visit 2, after confirming the women's criteria of inclusion, no more than one month after visit 1, the two treatments of the study were assigned sequentially in a 1:1 proportion per treatment group using a centralised, computer-randomised list. The treatment administered in the lactulose group was: lactulose (Duphalac®, Solvay Pharma, Barcelona, Spain) (15 mL equivalent to 10.05 g), vitamin D_3 (colecalciferol) (400 IU/day), calcium carbonate (250 mg, twice a day) and placebo of calcium (250 mg, twice a day). The women assigned to the placebo group received a placebo of lactulose (15 mL), vitamin D_3 (400 UI/day) and calcium carbonate (500 mg, twice a day). It was recommended that the lactulose (or the lactulose placebo) be taken diluted in water or other appropriate liquid (orange juice, coffee, tea) and the placebo of calcium carbonate during dinner. The medications for the study were supplied to the subjects at the initial visit to cover the subsequent 3 months of the study.

The follow up visits were carried out at 30 days (visit 3), at six months (visit 4) and at 12 months (visit 5) after the initiation of the treatment. At the follow up reviews anamnesis, a complete physical examination, laboratory tests, and evaluation of concomitant medication and of adverse events were carried out. Compliance and adherence to the treatment were evaluated by means of a questionnaire and by counting the medicine used. A measurement of the women's BMD was made at 6 and 12 months (visits 4 and 5).

Parameters of efficacy and safety

The safety parameters were the incidence and gravity of adverse effects during the period of the study, measurement of vital signs, monitoring of complete blood count and blood biochemistry.

Statistical analysis

Due to the lack of previous studies which evaluated the efficacy of lactulose combined with vitamin D and calcium to conserve BMD in postmenopausal women, a sample size of 40 subjects was established for this pilot clinical trial, including abandonments and losses. The ITT population was defined as all the randomised women who had received at least one dose of medicine and who had BMD data available after the randomisation. The method was that the last observation registered was used to replace lost values. The PP population was defined as all those randomised women who complied with the inclusion/exclusion criteria, who had received the medicine being studied and who finished the trial as it was established in the protocol. The safety population included all those randomised subjects who received at least one dose of the drug in the study.

Different parametric and non-parametric statistical tests were used, such as Student's t-test, the Mann-Whitney U test, the Kruskal-Wallis test, the Wilcoxon test, the chi-squared test (χ^2), Fisher's exact test or Friedman's variance analysis (ANOVA) according to their correspondence. The analysis of the primary objective was carried out with the data of the PP population. The primary analysis was the difference between the values of BMD (L2-L4) between visit 1 (initial) and visit 5 (end of study) in both treatment groups. The differences in the measurement of BMD between the lactulose and placebo groups were analysed using a general linear regression model (ANCOVA), in which the value of BMD at visit 5 was the dependent variable, the value of BMD from initial measurements was the covariable (ANCOVA), and the treatment received, a fixed effect. The 95% confidence interval (CI) was calculated for the difference between the final and initial values of BMD. The primary endpoint was also analysed in the ITT population to confirm the results obtained in the PP population. The statistical significance was set at $p < 0.05$. For the analysis of data, the Statistical Analysis System (SAS Institute, Cary, NC, EE.UU. (version 9.1)) was used.

Results

Of the 68 potential participants, 21 did not comply with an inclusion criterion. Of the 47 remaining women included in the safety population, 6 were

Table 2. Results of bone densitometry measurements in PP and ITT populations

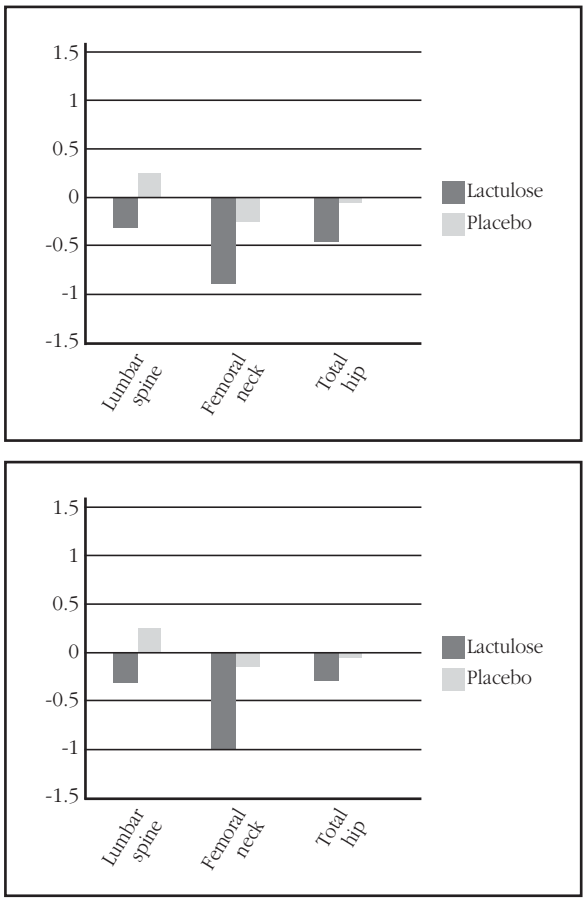
	Lactulose	Placebo
Population PP. Nº	**16**	**19**
BMD (L2-L4), g/cm². Average (SD)		
Visit 1 (commencement)	0.904 (0.058)	0.920 (0.082)
Visit 4 (6 months)	0.903 (0.057)	0.924 (0.088)
Visit 5 (at 12 months)	0.893 (0.064)	0.922 (0.092)
Change of the visit 1 to 5. %	-0.306	0.262
BMD FN, g/cm². Average (SD)		
Visit 1 (commencement)	0.753 (0.062)	0.732 (0.051)
Visit 4 (6 months)	0.744 (0.060)	0.725 (0.054)
Visit 5 (at 12 months)	0.746 (0.067)	0.730 (0.06)
Change of the visit 1 to 5. %	-0.884	-0.267
BMD CT, g/cm². Average (SD)		
Visit 1 (commencement)	0.897 (0.068)	0.869 (0.067)
Visit 4 (6 months)	0.887 (0.073)	0.874 (0.65)
Visit 5 (at 12 months)	0.893 (0.076)	0.869 (0.071)
Change of the visit 1 to 5. %	-0.447	-0.002
Population ITT. Nº	**19**	**22**
BMD (L2-L4), g/cm². Average (SD)		
Visit 1 (commencement)	0.934 (0.104)	0.922 (0.083)
Visit 4 (6 months)	0.938 (0.11)	0.928 (0.092)
Visit 5 (at 12 months)	0.912 (0.083)	0.929 (0.092)
Change of the visit 1 to 5. %	-0.306	0.262
BMD CF, g/cm². Average (SD)		
Visit 1 (commencement)	0.749 (0.062)	0.726 (0.049)
Visit 4 (6 months)	0.743 (0.064)	0.723 (0.05)
Visit 5 (at 12 months)	0.747 (0.065)	0.727 (0.057)
Change of the visit 1 to 5. %	-0.990	-0.157
BMD TC, g/cm². Average (SD)		
Visit 1 (commencement)	0.896 (0.074)	0.876 (0.065)
Visit 4 (6 months)	0.892 (0.079)	0.880 (0.063)
Visit 5 (at 12 months)	0.901 (0.079)	0.74 (0.069)
Change of visit initial at the end. %	-0.273	-0.084

Visit 1: pre-treatment; visit 4: six months of treatment; visit 5: 12 months of treatment (end of study).
PP: data by protocol; ITT: data by intention to treat; SD: standard deviation; FN: femoral neck; TH: total hip.

excluded from the analysis of efficacy since it was not possible to carry out the second measurement of BMD. In the ITT population were included 41 women, 19 allocated randomly to the lactulose group and 22 to the placebo group. Six women did not complete the study: two due to infractions of the inclusion criteria, three withdrew due to the appearance of adverse events and one due to there not being enough medication. Therefore, 35 women, 16 in the lactulose group and 19 in the placebo group, completed the study and were included in the PP data.

The average age of the women was 58.5 years (between 52 and 67 years of age) and the average body mass index (BMI) was 28.6 kg/m² (between 21.1 and 47.6 kg/m²). The total intake of calcium

Figure 1. Evolution of BMD in the lactulose and placebo groups between the initial visit and that at 12 months, in the PP (upper panel) and ITT (lower panel) populations. (Data expressed as percentage change)



was 698.7 (± 376.3 SD). The intake from milk and milk-derived products was 381.7 mg/day (± 206.9 SD), from cereals 50.4 mg/day (± 29.5 SD), from fruit 160.7 mg/day (± 72.1 SD), from fish 62.9 mg/day (± 82.9 SD) and meat 15.7 mg/day (± 9.4 SD). No patients declared having consumed more than 40 g/day of alcohol, and only 6 women were smokers during the study. Concomitant medication was recorded in 87.8% of the women, the most frequent being: non-steroid anti-inflammatories, analgesics, and hypotensives (Table 1). There were no statistically significant differences in these values between the two groups.

Efficacy

The results of the measurements of BMD in L2-L4, in the femoral neck and in the total area of the hip at the initial visit and after 6 and 12 months of treatment are shown in Table 2. The results were similar in both PP and ITT populations.

In terms of the measurement of the results of the main objective in the PP analysis, the minimum mean square (standard error – SE) for the difference in BMD in the lumbar spine (l2-L4) between that at 12 months and that at the initial stage was 0.902 (0.007) for the lactulose group, and 0.914 (0.006) for the placebo group; the difference between the two groups was -0.012 (95% CI, -0.031 to 0.007; p=0.224). The analysis of the ITT data gave similar results, with the minimum mean square for BMD of 0.917 (0.007) and 0.921 (0.007) in the lactulose and placebo groups respectively, p=0.652) (Figure 1).

With respect to the secondary objectives, no statistically significant differences were observed between the lactulose and placebo groups. In the PP data, the minimum mean square (SE) for the difference in BMD in the femoral neck between visits 5 and 1 was 0.734 (0.006) for the lactulose group and 0.740 (0.006) for the placebo group; the difference between the two groups was -0.006 (95% CI, -0.024 to 0.012; p=0.493). The analysis of the ITT data showed a minimum mean square (SE) of 0.731 (± 0.006) and 0.739 (± 0.005) in the lactulose and placebo groups respectively, and a difference between the two groups of the study of -0.008 (95% CI, -0.024 to 0.008; p=0.298). On the other hand, the measurement of BMD in the total hip showed a minimum mean square (SE) of 0.878 (0.005) and 0.882 (0.005) in the lactulose and placebo groups for the analysis of PP data (difference of –0.005, 95% CI, –0.019 to 0.009; p=0.485), and 0.885 (0.005) and 0.889 (0.004) in the lactulose and placebo groups for the analysis of ITT data (difference of –0.004, 95% CI, –0.016 to 0.009, p=0.565).

The changes in analytic parameters for the markers for bone remodelling are shown in Table 3. There were no statistically significant differences between the lactulose and placebo groups in the initial and final values of the study. All the parameters were within normal limits. The percentage change in blood calcium, in phosphorus, in bone alkaline phosphatase, in parathyroid hormone, in urinary calcium and in NTx after 12 months of treatment in the lactulose and placebo groups were not statistically significant. The percentage change in CTx in the lactulose group was not significant, but in the placebo group the average percentage change was – 13.3 ± 0.3 SD (p=0.046). The levels of 25-hydroxyvitamin D increased considerably in the lactulose group (percentage change of 41.4 ± 10.6 SD, p=0.006) and in the placebo groups (percentage change of 35.4 ± 10.7 SD, p=0.003).

No differences were observed in physical exercise or in consumption of milk products, nor in calcium derived from milk products, cereals, fruit, meat and fish in the data recorded for initial and final values for the study.

Safety

A total of 12 women (50%) from the lactulose group and 14 (60.9%) in the placebo group confirmed that they had suffered light adverse events. Only 7 women (3 in the lactulose, and 4 in the placebo group) reported having had more than two adverse events. The most common adverse events were: abdominal distension, urinary tract

Table 3. Changes in analytical parameters for bone metabolism in the two groups over the period of the study

	Initiation		At 6 months		At 12 months	
	Lactulose	Placebo	Lactulose	Placebo	Lactulose	Placebo
Calcium, mg/dL	9.1 (0.6)	9.3 (0.5)	9.2 (0.4)	9.2 (0.4)	9.3 (0.4)	9.1 (0.4)
Phosphorus, mEq/L	3.5 (0.3)	3.6 (0.7)	3.6 (0.4)	3.4 (0.4)	3.6 (0.4)	3.3 (0.4)
Bone alkaline phosphatase, ng/mL	12.3 (5.7)	11.7 (3.6)	11.9 (5.2)	10.9 (2.4)	12.9 (6.0)	11.4 (2.9)
CTx, ng/mL	0.4 (0.2)	0.5 (0.3)	0.3 (0.1)	0.3 (0.1)	0.3 (0.2)	0.3 (0.2)
Parathyroid hormone, pg/mL	48.9 (21.9)	46.6 (14.6)	51.8 (21.8)	48.1 (14.7)	47.6 (18.2)	41.2 (13.2)
25-hydroxyvitamin D, ng/mL	25.8 (7.6)	23.3 (8.0)	33.0 (8.5)	32.1 (7.6)	34.4 (9.8)	30.4 (9.6)
Urine calcium, mg/24 h	231.0 (159.8)	217.5 (130.5)	207.3 (127.1)	213.4 (89.8)	226.3 (117)	268.3 (94.9)
NTx, nM/mM	45.8 (13.8)	52.5 (26.1)	38.1 (10.3)	38.3 (14.5)	40.9 (13.4)	43.3 (17.5)

Data expressed as mean and (SD).

infection, back pain and arthralgia. The distribution of the adverse events by organ class and system were similar in both groups of the study. Three women discontinued the treatment at visit 4 due to these adverse events, which included a period of constipation which persisted after having stopped taking the treatment being studied in a patient assigned to the placebo group, and an episode of gastroenteritis and diarrhoea in two women assigned to the lactulose group. In the three cases the adverse events were of moderate intensity and possibly related to the drugs being studied. There were no serious adverse events or deaths during the study.

No significant changes in vital signs, or in the results of the laboratory tests, were observed. Adherence to the medication being studied was sufficient in 84.7% of the women in the lactulose group and 89.9% in the placebo group (p=0.685).

Discussion

Lactulose is a drug very commonly used in this population (postmenopausal women) as a laxative, with few secondary effects, and which may be of interest due to the effect of improving the intestinal absorption of calcium which it is known to produce[23,24]. This study is the first which has evaluated the effect of lactulose on BMD in osteopenic postmenopausal women. No differences were found between the two study groups, from which is may be concluded that the addition of lactulose to the 500 mg of calcium carbonate associated with vitamin D supplements could have a similar effect on lumbar BMD as 1,000 mg of calcium carbonate. Therefore, the results of this study can support the possible beneficial effects of this prebiotic non-digestible disaccharide on the maintenance of BMD, reducing the necessary dose of calcium. It is important to stress that this was a pilot study designed to detect possible changes, and that one of its limitations is the relatively small number of women and the short duration of the study. However, the results do show the maintenance of BMD. On the other hand, the combination of lactulose, vitamin D and calcium was well tolerated, and the safety profile in both groups was similar.

In terms of the possible mechanism through which the bone mass would be preserved in the women treated with lactulose, this may be related to an increase in the absorption of calcium. This is not possible to confirm conclusively, since the absorption of calcium was not really measured directly, for example, with the use of isotopic techniques. An indirect measurement is the urinary excretion of calcium, and in the study no significant changes were found in this parameter. In any case, it has been confirmed that the absorption of calcium induced by non-digestible oligosaccharides is not accompanied by a greater urinary excretion of calcium, which means that these compounds may also increase indirectly the reception of calcium by bone and/or inhibit bone resorption[14]. Consequently, not having found increases in the urinary excretion of calcium, does not weaken speculation that this is the mechanism involved in the maintenance of BMD found in this study.

An important point to note is that blood levels of 25-hydroxyvitamin D increased significantly in both groups in the study as a consequence of the vitamin D supplement, but with a higher tendency in the lactulose group. This observation may have clinical significance due to the low intake of calcium and vitamin D deficiency which a high percentage of postmenopausal women exhibit[25,26]. However, the effect lactulose or other prebiotics have on the absorption of vitamin D has not previously been investigated. In relation to the biochemical markers for bone turnover, no significant differences were found in the values of bone alkaline phosphatase in the blood, or in NTx in urine, in either of the two groups. However, the values of CTx were significantly lower at the end of the study in the placebo group, although the magnitude of the change was very modest (11%). Together, these results are similar to those observed in most of the studies with drugs for osteoporosis, when the placebo arm which included supplements of calcium and vitamin D are analysed. Thus, the changes in the placebo group of the sub-study of the Fracture Intervention Trial (FIT) and Fracture Reduction Evaluation of Denosumab in Osteoporosis (FREEDOM) studies showed modest reductions in values of bone alkaline phosphatase, which were 14% a year in the FIT study, or similarly non-significant reductions for bone alkaline phosphatase and CTx in the FREEDOM sub-study[27,28].

This pilot study suggests that, in postmenopausal women with osteopenia, the addition of 10 g/day of lactulose to 500 mg of calcium plus vitamin D, over 12 months, showed no differences in the conservation of bone mass from a supplement of 1,000 mg of calcium carbonate plus vitamin D. It would be necessary to conduct a study which was longer and had a greater number of subjects in order to be able to confirm these preliminary observations.

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PINP in patients with hepatic insufficiency: Comparison of two methods of measurement and association with different biochemical parameters

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Summary

Introduction: N-terminal propeptide of type 1 collagen (PINP) is a marker for bone formation. Blood PINP is found in trimeric and monomeric forms. There are two automated methods for its determination. R-PINP (Roche Diagnostics) determines both forms (Total PINP). IDS-PINP (IDS iSYS N-Mid® Vitro) determines the trimeric part (Intact PINP).
Objective: To compare the two methods.
Material and method: 81 patients (64 men and 17 women, average age of 53 ± 8 years) with terminal hepatic insufficiency were recruited. Creatinine, PTH, 25-OH-vitamin D, beta-crosslaps (β-CTX), desoxypyridinoline (Dpyr), hepatic function and PINP with both methods, were measured. Bone mineral density (BMD) was measured (Hologic®, QDR 4500) in the lumbar spine and femoral neck. The comparison between the two methods was carried out using a Bland-Altman and Passing'Bablok analysis.
Results: R-PINP showed higher values than IDS-PINP (85.03 ± 56.67 vs. 55.22 ± 32.81 ng/mL, p<0,001). The correlation between the two methods was r= 0.81 (p<0.01) and the Passing-Bablok regression analysis Y = 0.570 [0.475-0.669] X + 7.724 [2.130-12.542].
Conclusion: There is a good correlation between the two methods in patients with hepatic insufficiency, although not proportional or interchangeable.

Key words: *PINP, bone turnover markers, bone mineral density, liver insufficiency.*

Introduction

The markers for bone remodelling (MBRs) provide information around the risk of fracture and may be useful in monitoring the treatment of osteoporosis, both with anti-resorptive drugs and anabolics[1]. The first markers for bone formation used were osteocalcin and alkaline phosphatase (AP). Both have various limitations. Osteocalcin can be produced by other tissues, circulate in intact and fragmented forms, its expression regulated by $1,25(OH)_2$ D_3 and by the corticoids, is of limited value in patients with renal insufficiency and not very stable at room temperature[2]. With respect to AP, it may be affected by hepatic pathologies[3].

Type 1 collagen makes up 90% of bone protein and is synthesised as type 1 procollagen. In the extracellular processing of type 1 procollagen, the amino-terminal of type 1 collagen (PINP) and carboxy-terminal (PICP) fragments are released. These propeptides circulate in the blood and are used as markers for bone formation.

The pre-analytical advantages of PINP include low diurnal and inter-individual variability and stability at room temperature. It can be determined both in the blood and in the plasma, and, unlike other markers for bone resorption, its concentrations are not affected by the intake of food[4,5].

Currently, PINP is considered to be one of the markers for formation with the best clinical performance. Thus, it has been reported that in 14 patients, after the surgical menopause, PINP is the marker for bone formation which has the greatest diagnostic sensitivity, beating AP and osteocalcin[6]. The results reported in 51 patients with Paget's disease are similar, where a good correlation between the extension indices and the activity of the process was also observed[7].

There are two forms of PINP in the blood, intact or trimeric and monomeric[8]. The methods currently available measure the trimeric form (intact PINP) or the trimeric and monomeric forms (total PINP). There are few data published comparing the different methods of determining PINP, and even fewer in special populations, such as patients with chronic hepatopathy, candidates for liver transplant.

The aim of this study has been to compare two methods of automated determination of PINP: Cobas E 601, Roche Diagnostics (R-PINP) and IDS® Vitro (IDS- PINP) in patients with advanced chronic hepatopathy, candidates for liver transplant.

Material and method

The study included 81 patients with chronic advanced hepatopathy who were included on the waiting list for liver transplant of the 12th October University Hospital and studied in the clinic for bone metabolic diseases. The study was approved by the local ethics committee and carried out with the informed consent of all the patients. When the analyses were carried out the patients had received no treatment for their bone pathology. All the parameters analysed were determined using the same blood samples. The samples were taken in conditions of fasting and between 8.00 and 10.00 hours and stored at -70° C.

The analysis of total PINP (R-PINP) was carried out using an electro-chemoluminescence test using the ELECSYS 2010 (Roche diagnostics) equipment. This method detects the monomeric and trimeric forms (total). It has an analytical sensitivity <5,0 ng/mL. The intra- and inter-trial coefficients of variation (CV) vary between 2.3-3.7% and 1.8-2.9%, respectively. The normality range is 20 to 100 ng/mL. The analysis of the trimeric form of PINP (intact) (IDS-PINP) was carried out using automated chemoluminescence (IDS-iSYS). The intra- and inter-trial CVs vary between 2.6-3% and 4.2-5.3%, respectively. The normal values in adults are 27.7-127.6 ng/mL.

Also measured were: creatinine, glomerular filtrate, blood MBR, beta-crosslaps (β-CTX) and urinary MBR desoxypyridinoline (Dpyr), parathormone (PTH), 25 hydroxyvitamin D (25-OH D_3), and parameters for liver function (GOT, GPT, GGT, alkaline phosphatase, albumin and bilirubin). The calculation of glomerular filtrate was made using the formula CKD-EPI[9].

The marker β-CTX was determined by means of a sandwich type electro-chemiluminescence test using ELECSYS 1010 (Roche diagnostics) equipment. The intra- and intertrial CV is <4.1% and <5.7% respectively. The analytical sensitivity is 0.01 g/l. The normality range is from 0.20 to 0.70 ng/mL. The Dpyr analysis was carried out with a urine sample from the second micturation of the morning, performed using solid phase chemiluminescent immunoassay, using IMMULITE 2000 (SIEMENS) equipment. The results are expressed in nanomoles/litren(nM/l) of Dpyr and are normalised with reference to the excretion of urinary creatinine (mM/l). The analytical sensitivity is 6 nM/l of Dpyr. The method has a CV which varies between 2.5 and 11.8%. The normality range is from 2.3 to 7 nM-mM of creatinine.

The bone mineral density (BMD) was measured in the spinal column and femoral neck (Hologic®, QDR 4500), the T-score being calculated in accordance with a Spanish population of similar age and sex[10]. Osteoporosis and osteopenia are defined in accordance with the criteria of the WHO[11].

Statistical analysis

The marker is transformed logarithmically to reduce its asymmetry. The comparison study of the measurement processes was carried out using a linear regression analysis with the Passing-Bablok method[12]. If two methods are comparable and give similar results, the 95% confidence interval of the incline ("a") should include the value 1 and the 95% confidence interval of the ordinate at the origin ("b") should include the value 0. If "a" does not include the value 1 there are proportional systematic errors. If "b" does not include the value 0 there will be constant systematic errors. The degree of agreement between the two methods is calculated using the Bland-

Altman method[13]. The coefficients of correlation were calculated according to Pearson. The calculations were carried out using the statistical programme SPSS ((Statistical Package For Social Sciences, Waltham, USA) version 15.0 and CBstat (Statistical Analysis in Clinical Biochemistry) version 5.

Results

Table 1 summarises the characteristics of the population studied. 64 men and 17 women participated. The mean age was 53 ± 8 years. Of these patients, 44 (54%) had osteopenia and 37 (46%) densitometric osteoporosis. The values obtained with R-PINP were higher than the values of IDS-PINP (85 ± 56.7 vs 55.2 ± 32.8 ng/mL, p<0.001), according to the methodology (total vs intact PINP). The coefficient of correlation between the two methods was r= 0.81 (p<0.01). The Passing-Bablok regression analysis (Figure 1) showed that the 95% confidence interval of the incline did not include 1: a= 0.570 (0.475 ; 0.669) and that the 95% confidence interval of the ordinate at the origin does not include 0: b= 7.724 (2.130 ; 12.542); therefore there are proportional and constant systematic errors.

The difference of paired values (Bland Altman analysis) was -29.81 ± 4.34 (Figure 2). Correlations were found between R-PINP and creatinine (r= 0.36; p<0.01), R-PINP and β-CTX (r= 0.26 ; p<0.021) and between R-PINP and 25-OH D_3 (r= -0.27 ; p<0.017). The Dpyr showed a significant correlation with both R-PINP and IDS-PINP (r= 0.29 ; p<0.007). No correlation was found between PINP and the values of PTH, BMD (in any position) and parameters for liver function in either of the two methods.

A tendency to an increase in the difference between the two methods was observed, with high concentrations of PINP, as can be seen in Figure 1, as with the analysis of the ranges: R-PINP (9.4-259.2 g/mL). IDS-PINP (9.5-192.6 ng/mL). With values of PINP < 70 ng/mL, the comparison of the methods of R-PINP and IDS-PINP did not show any proportional systematic errors: a= 0.7973 (CI 95%: 0.6015 ; 1.1011), or constant systematic errors: b= 2.0609 (CI 95%: -7.4225 ; 8.4048).

In studying both methods classifying patients according to whether they had osteopenia (n= 44) or osteoporosis (n= 37), no significant differences were found in the values of intact or total PINP between them. However, it was observed that in patients with osteoporosis there is higher agreement between the two methods, there being neither proportional or constant systematic errors, as can be seen in the values of the incline and the ordinate at the origin obtained by Passing Bablok:

- Patients with osteopenia: a= 0.4583 (CI 95% : 0.3065 ; 0.6502), b= 14.679 (CI 95% : 3.4471 ; 21.2231).

- Patients with osteoporosis: a= 0.7062 (CI 95%: 0.4292 ; 1.2244), b= -5.9053 (CI 95% :-39.4974 ; 10.4624).

In classifying the patients according to renal function a better association between the two methods was observed when the renal function was normal. In patients with glomerular filtrate > 60 ml/min/1.73m^2 (n= 33)) no proportional systematic errors: a= 0.7007 (CI 95%: 0.4912 ; 1.0137), or constant systematic errors: b= 5.5611 (CI 95% : -7.2525 ; 14.3792) were found. However, in patients with glomerular filtrate <60 ml/m^2 (n= 48), in/1.73m proportional systematic errors were found: a= 0.5540 (CI 95% : 0.4391 ; 0.6563), but no constant systematic errors: b= 5.5698 (CI 95% : -4.4497 ; 13.1874).

Discussion

The use of MBRs in the management of bone metabolic pathology has increased notably in recent years. For example, recently the International Foundation for Osteoporosis and the International Federation of Clinical Chemistry and Medicine Laboratories have recommended the determination of blood PINP as the marker for bone formation of choice[14]. In this work two methods for the determination of PINP (total and intact forms) in patients with advanced chronic hepatopathy with osteopenia or osteoporosis were compared. Our data show that there is a good correlation between the two methods, although there is no direct transferability between the two results, since neither zero in the ordinate at the origin nor one in the incline of the regression line are included. There are few studies which compare different methods for determining PINP. It is not well established to what extent two forms of PINP circulating in the blood are recognized by the different methods. The clearing of intact PINP is characterised by a rapid capture by the hepatic endothelial cells, while the monomeric forms depend to a great degree on the renal function.

Koivula et al.[15] studied a manual method for intact PINP (RIA, Orion Diagnostics) as against the R-PINP method. The correlation between them in healthy subjects was 0.89, similar to that found in our study, and while the concentrations of PINP were similar between the two methods, this was in spite of the fact that one determined the intact form, and the other the total form.

The reason why we observed a greater difference between R-PINP and IDS-PINP as the value of PINP increases is unclear. Other authors have found similar results when comparing methods for the determination of PINP using RIA vs automated methods based on chemiluminescence. These authors make reference to a possible ceiling effect in using RIA at high concentrations of PINP, which could justify the difference between the two methods at these concentrations[15].

We found a weak significant correlation between the blood marker for resorption β-CTX and R-PINP, but not IDS-PINP, possibly because this only measures the trimeric form. However, the urinary marker for resorption, Dpyr, was correlated with both methods. Also found was a negative correlation between levels of 25-OH D_3 and the value of R-PINP, which may be due to the influence of the status of vitamin D in remodelled bone.

Table 1. Clinical and biochemical characteristics of the study population

	Median [P_{25};P_{75}]	Normal values
Sex M:76,5%/W:23,50%		
Age (years)	57 [49;62]	
Creatinine (mg/dL)	1.1 [0.8;1.4]	0.3-0.7
GOT (U/L)	26 [18;38]	5-37
GPT (U/L)	59 [39;101]	5-45
GGT (U/L)	129 [88;244]	8-61
PTH (pg/mL)	46 [33;84]]	15-51
25-OH Vitamin D (pg/mL)	15 [11;21]	15-54
β-CTX (ng/mL)	0.7 [0.5;1.1]	0.2-0.7
Bilirubin (mg/dL)	1.1 [0.8;1.8]	0.2-1.1
Albumin (g/dL)	3.4 [2.9;3.8]	3.5-5.0
Calciium (mg/dL)	8.8 [8.4;9.3]	8.4-11.0
BMD lumbar spine (mg/cm^2)	0.832 [0.761;0.885]	0.970-1.280
BMD femoral neck (mg/cm^2)	0.701 [0.618;0.770]	0.970-1.280
BMD total femur (mg/cm^2)	0.825 [0.716;0.892]	0.970-1.280
Osteopenia/Osteoporosis: 54% / 46%		

PTH: paratohormone; β-CTX: β-Crosslaps; BMD: bone mineral density.

The data found with respect to the positive correlation between creatinine and R-PINP are similar to those referred to by other authors. In healthy subjects, total and intact PINP show similar results, but in patients with chronic renal insufficiency the results differ. The increase is due to an increase in the monomeric forms, which accumulate in the blood of patients with renal insufficiency[16-18]. In patients in haemodialysis with terminal renal insufficiency, although the PINP showed an initial increase during the haemodialysis, at the end of the sessions no significant changes in intact or total PINP were observed[19-21].

Changes in MBR may be useful in monitoring treatment of osteoporosis, confirming therapeutic compliance and evaluating the efficacy of treatment[8]. In those patients with terminal hepatic insufficiency after transplant the use of bisphosphonates is indicated[22-24]. The values of PINP found do not appear to be affected by the hepatopathy, since they do not correlate with parameters for hepatic function in this group of patients with hepatic insufficiency who have not yet received post-transplant immunosuppressive, or anti-resorptive, treatment. We do not know if either method would be sensitive enough to detect in a comparable way the changes in bone remodelling which occur after hepatic transplant[25] and with anti-osteoporotic treatment[22-24]. Garnero et al. show that R-PINP, in comparison with manual RIA (intact PINP), detected an increase in bone remodelling in postmenopausal women and was sensitive enough to detect significant changes in bone remodelling induced by treatment with PTH 1-84 and with alendronate.

In summary, while we found a good correlation between the two methods of determining PINP (total and intact forms), there are differences which mean that their results are not consistent.

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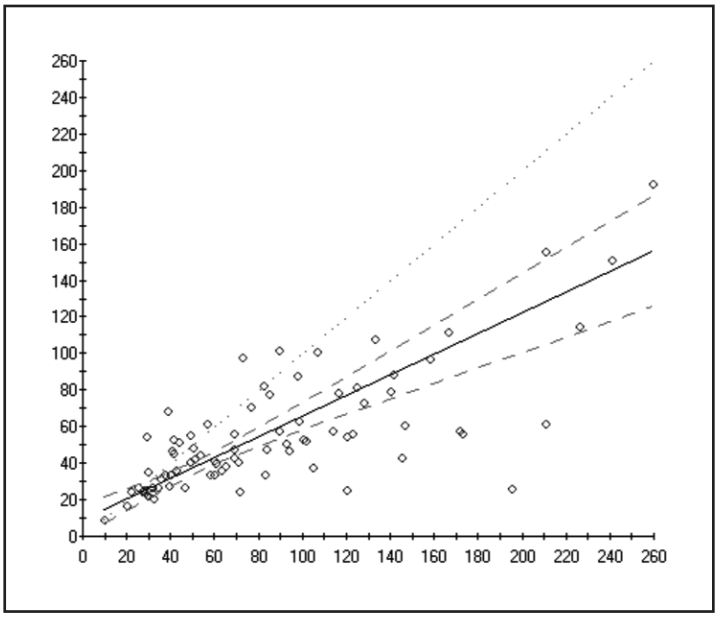
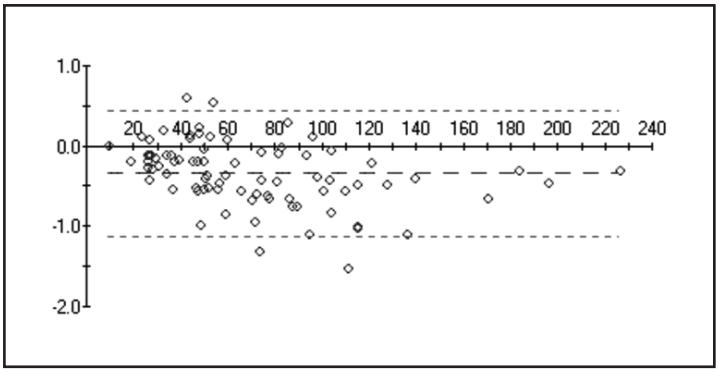
Figure 1. Passing-Bablok regression analysis



a= 0.570 [0.475-0.669]; b= 7.724 [2.130-12.542].
Linear regression: r= 0.81; p<0.01.

Figure 2. Bland-Altman analysis



R-PINP (on the x axis) µg/L).
Difference (R PINP – IDS PINP) (on the y axis) (µg/L).
Difference in paired values -29.81 ± 4.34.
Comparison of R PINP with IDS PINP in 81 patients with hepatopathy using Bland-Altman. The graph shows the differences between the values of PINP by both methods in each patient (y axis) in relation to the average values obtained by the two methods (x axis). The central line represents the average difference between the two methods and the upper and lower lines, the confidence interval at 95% of this difference.

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Epidemiology of hip fracture in Gran Canaria over the five year period of 2007-2011

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Summary

Background: Hip fracture is the most serious clinical complication of osteoporosis, due to its raised morbidity and mortality.

Method: We have studied the epidemiological and demographic characteristics of all the fragility fractures of the hip occurring in patients of ≥ 50 years of age recorded in Gran Canaria during the 5 year period of 2007-2011 from the admission, coding, emergency and traumatology services of all the hospitals in Gran Canaria, in both the public and private healthcare sectors.

Results: A total of 2,222 hip fractures were recorded, of which 1,593 (71.7%) occurred in women and 629 (28.3%) in men. The female/male ratio was 2.53. The average age at which the fractures occurred was 79 ± 9.7 years. Over the 5 years, the total number of fractures (men and women) varied between 402 (in 2007) and 504 (in 2010). The number of fractures increased with age up to the 90s. The annual global incidence was 150 cases/100,000 inhabitants ≥ 50 years: in women 205.4 cases with respect to the population of this sex and age, and in men, 89.1 with respect to the population of men ≥ 50 years. During the winter months 29.7% of the total fractures occurred, 7.5% more than those happening during the summer months (22.2%).

Conclusions: During the period 2007-2011 the incidence of hip fracture in Gran Canaria remained more or less stable, in every year being greater in women than in men, and increasing with age up until the 90s. The greatest number of hip fractures occurred during the winter months, with similar numbers in spring, summer or autumn.

Key words: *fracture, hip, osteoporosis, epidemiology, incidence, Gran Canaria.*

Introduction

The fracture of the proximal extremity of the femur, usually known as a hip fracture, is the most serious complication of osteoporosis[1,2]. This is due, on the one hand, to its mortality, both in the acute phase and in the following years, and on the other hand, to its morbidity, since a high proportion of patients after fracture require rehabilitation, continued assistance, or are institutionalised[3].

Fracture of the hip is an appropriate fracture with which to carry out epidemiological studies, given that all the patients who suffer from it are admitted to a hospital, and a huge majority usually require surgery. This factor is facilitated by the phenomenon of insularity of the island of Gran Canaria, which prevents the loss of cases due to movement or emergency treatment in neighbouring provinces.

We present in this study the epidemiological characteristics of all the cases of fracture occurring in the island of Gran Canaria during the period 2007-2011.

Patients and methods

In order to carry out this study we have consulted the archives and the discharge reports of the admissions, emergency, codification and traumatology services of all the hospitals of the island of Gran Canaria, both in the private and public sector.

Thus, all cases of hip fracture which were registered in the island of Gran Canaria from the 1st January 2007 to 31st December 2011 according to the criteria of the International Classification of Diseases (ICD 9), published by the Ministry of Health and Consumers Affairs[4], were recorded. All those cases in which: a) the patients lived in other autonomous communities or other countries, and who were only temporarily on the island; b) the fracture were the result of a high impact trauma: a traffic accident, a fall from a height greater than ones own feet, an attack, etc; and c) the fracture was pathological (neoplasms, Paget, bone cysts...).

Statistical analysis: the data were exported to an Excel® spreadsheet and later analysed using the statistical software package SPSS® version 18.0 (Statistical Package for the Social Sciences, Chicago, IL).

To study the incidence of cases, we obtained the data of the population aged ≥ 50 years, stratified by age and sex, from the public census data published by the Canarian Statistical Institute[5].

The distribution of frequencies was analysed in the case of discrete variables (sex, age groups), and mean ± standard deviation (SD) in the cases where they were continuous (age and hospital stay). The distribution of the variables was evaluated by means of the Kolmogorov-Smirnov test, and for the comparison of the means the Student's t-test was applied when the variable studies followed a normal distribution, and the Wilcoxon test when it did not. In all cases the significance level was established at 5% ($p<0.05$).

Results

A total of 2,222 fractures were recorded, of which 1,593 (71.7%) occurred in women and 629 (28.3%) in men. The female/male ratio was 2.53. The mean age of all the patients was 79 ± 10 years, 76 ± 11 years for the women, and 80 ± 9 years ($p<0.001$) for the men.

Analysing the data annually, the percentage of women who suffered from a hip fracture was always higher than 70%. The total number of fractures occurring annually varied between the 402, which occurred in 2007, and the 504, which happened in 2010 (Table 1).

In studying the number of hip fractures occurring each year as a function of age, it was observed that it increased for each decade of life in all the years recorded, until a maximum peak in the incidence when patients were in their 80s, and with a notable reduction in nonagenarians, also in all the years studied. Six cases of fractures of the hip were recorded in patients whose age was ≥ 100 years (Table 2).

Figure 1 shows the annual incidence of hip fracture during the years studied, expressed as the number of cases/100,000 inhabitants ≥ 50 years of age/year. The incidence was more than double in the women than in the men, both in each year studied and in the average of all the years. The average incidence of the 5 years studied was 89.1 cases/100,000 inhabitants in men and 205.4 cases/100,000 in women. Overall, including the whole population of both sexes, the annual incidence was 150 cases/100,000 inhabitants. The variation in the incidence of hip fracture over the 5 year period was small. The maximum overall incidence occurred in the year 2010 and the minimum in the year 2007, with a difference between them of 34.1 cases/100,000 inhabitants ≥ 50 years of age/year.

When the data were analysed taking into account the seasons of the year, it was observed that during the winter months the greatest number of cases occurred in each and every one of the years, while the number of hip fractures during the remaining seasons was variable depending on the year. Taking into account the figures for the 5 year period, summer was the season with fewer hip fractures (N=493) (Table 3).

The mean length of hospital stay was 8.8 ± 7.8 days, being significantly higher in men (9.4 ± 8.3 days) than in women (8.9 ± 7.3 days; $p<0.05$).

Discussion

Hip fracture is the most serious complication of osteoporosis, given that patients who suffer from it have a raise level of morbidity and mortality[1-3,6,7].

We have available many studies of the epidemiology of hip fracture in Spain, which were carried out above all in the decade of the 90s[8-11]. Also at this time, the results of the MEDOS study were published which in general terms described the incidence of cases of hip fracture in our country as being much lower than that which existed in other European countries[12,13], above all the Nordic countries, for which reason Spain was considered to be a zone of medium-low risk for hip fracture[12,13].

Table 1. Number and percentage of hip fractures occurring each year between 2007 and 2011 grouped by sex. The percentages refer to the total number of fractures each year

	Men (M)		Women (W)		Total (M + W)
Year	**Nº**	**%**	**Nº**	**%**	**Nº**
2007	119	29.6	283	70.4	402
2008	120	27.8	312	72.2	432
2009	133	29.2	322	70.8	455
2010	135	26.8	369	73.2	504
2011	122	28.4	307	71.6	429
Total	629	28.3	1,593	71.7	2,222

Table 2. Number of hip fractures in the population ≥ 50 years of age grouped by each year studied and by decades of age

	2007	2008	2009	2010	2011
	N	**N**	**N**	**N**	**N**
50-59	25	19	19	25	22
60-69	52	41	48	41	56
70-79	113	129	152	171	121
80-89	162	184	184	206	175
90-99	46	59	52	60	54
≥100	4	0	0	1	1

However, these incidence rates varied greatly from one place to another. Thus, in a review carried out in the year 2002[14] which referenced all the studies of incidence of hip fracture carried out in Spain, showed an overall incidence in older people of 517 cases per 100,000 inhabitants/year; 270 cases in men and 695 in women. In the Canary Islands the incidence adjusted for age and year was the lowest in the whole country, with 301 cases/inhabitants and year, while in Catalunia, for example, at the other extreme of Spain, the incidence was 897 cases/100,000 inhabitants/year in the population of both sexes. We should note that in this study the population of 65 years and over was selected, which means that the incidence figures given did not coincide with ours, since we analysed hip fractures in subjects over 49 years of age[15].

Islands are an ideal place to carry out epidemiological studies, both of hip fractures and any other serious pathology[16], because the insularity acts as a type of filter which prevents the loss of data, above all in a pathology such as hip fracture which necessitates urgent admission to hospital in all cases, and almost always a later surgical intervention[17].

Hip fractures continue to be a pathology pertaining to individuals of an advanced age. The average age of our patients was 79 years, being significantly higher in women than in men. This age is very similar to that reported in most of the series published in Spain[8-11,14]. The fact that the number of hip fractures increases with age up to 90 years of age is illustrative. The reason why the number of cases drops from this point lies in the fact that mortality at this age is high and, therefore, the population is less. Nevertheless, it should be noted that the high number of cases in nonagenarian patients, and even in those of 100 years of age or more, is as consequence of the progressive aging of our population and its better quality of life. In our series, all the patients aged more than 100 years underwent surgical intervention and were discharged from hospital, which perhaps means that these data suggest the necessity for a more interventionist and less conservative approach to the treatment of hip fracture in centenarians, although the discussion of this topic is beyond the objectives of our work.

Also, hip fracture continues to be a pathology which is more frequent in women, with the female/male ratio in our study being 2.53, very similar to that found both in most of the studies carried out in Spain, as well as by our group in an epidemiological study carried out 20 years ago in the population of Gran Canaria[15].

In a study carried out in another region of Spain (Cantabria) an increase in the incidence of hip fractures was observed after a period of 12 years[10]. The objective of this study was to describe the epidemiology of hip fracture in the period 2007-2011 in Gran Canaria, and while we don't yet have available the results which compare the current incidence with those of 20 years ago[15], this is a study we are working on.

On the other hand, the number of hip fractures was greater in the winter months. A factor which could have had an influenced on this is a lower production of vitamin D at this time of year. In spite of the fact that Gran Canaria enjoys adequate and consistent levels of sunshine throughout the year, in studies carried out by our working group in medical students in Gran Canaria, it was observed that 61.2% of them had values of 25(OH) vitamin D below 30 ng/ml[18], for reasons

Table 3. Number of hip fractures occurring during the different seasons of the year in the five years of the study

	Winter		Spring		Summer		Autumn	
	N	%	N	%	N	%	N	%
2007	131	32.6	110	27.4	84	20.9	77	19.2
2008	119	27.5	106	24.5	95	22	112	25.9
2009	140	30.8	106	23.3	97	21.3	112	24.6
2010	135	26.8	116	23	120	23.8	133	26.4
2011	136	31.7	124	28.9	97	22.6	72	16.8
Total	661	29.7	562	25.3	493	22.2	506	22.8

we are not able to determine[19]. Although to clarify this fact, it would be necessary to understand the circumstances in which hip fractures occurred. It is possible that sunshine has a significant role, given the lower number of hip fractures which occur on average in the summer, the period of greatest sunshine.

In recent years the average stay in hospital of patients with hip fracture in Gran Canaria has been below 9 days. These figures concur with current figures in the rest of Spain[20], and are much lower than those of 20 years ago in our population, when the average hospital stay was 15.9 ± 15 days[15]. This is indicative of better postoperative management of hip fracture and the awareness of the need for early rehabilitation outside the hospital environment to reduce the morbimortality of these patients. We have found no explanation for the shorter hospital stay in women, although their lower age at the time of the hip fracture may mean better postoperative recovery, and a greater facility for extra-hospital recuperation.

One of the limitations of our study is that, in the case of hip fractures registered in public hospitals, in being in line with the published codes for the International Disease Classification criteria[4], some cases could have been lost, since there is not a single code which codifies hip fractures, it being able to be assigned very different codes at the time of admission or discharge, a phenomenon which we have noticed recently[21]. If this were to happen, we think that it would only occur in a few cases and, above all, in public hospitals, given that the clinical characteristics of these patients in hospitals (high costs, complications, non-negligible levels of mortality and notably lower number of cases), makes us think that the number of cases of hip fracture lost in the private care system would be practically nonexistent.

As a conclusion to the results obtained in this epidemiological study of osteoporotic hip fracture in Gran Canaria over the five years 2007-2011, we may say that it continues to be more frequent in women than in men, that it occurs at a lower age in women, and that in both sexes it increases with age until 90 years of age, being more frequent in the winter months. Furthermore, there has been a notable reduction in average hospital stay, greater in women than in men.

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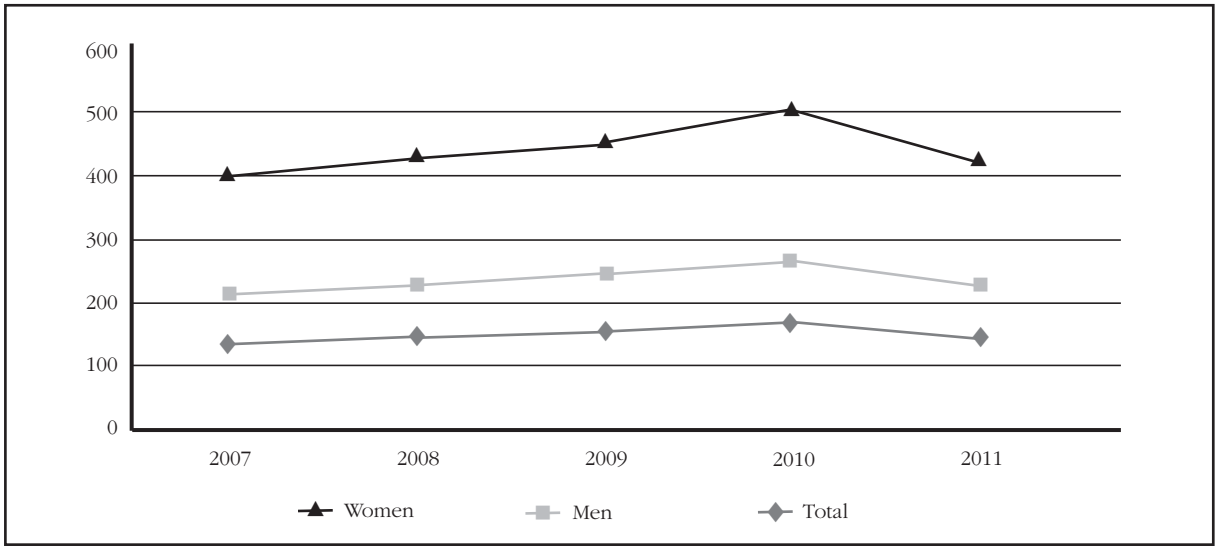
Figure 1. Annual and average incidence of hip fracture in Gran Canaria during the five years 2007-2011, adjusted to the population ≥ 50 years (cases/100,000 inhabitants ≥ 50 years/years) and by sex



	2007	2008	2009	2010	2011	Average
Women (W)	182.5	201.2	207.6	237.9	197.9	205.4
Men (M)	84.3	85	94.2	95.6	86.4	89.1
Total (M+W)	135.7	145.8	153.6	170.1	144.8	150

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Increased bone modelling as a presentation of Graves' disease

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Summary
The adverse effects of hyperthyroidism on bone have been described for years. Thyroid hormones are necessary for growth, maturation, metabolism and bone remodelling. However, untreated thyrotoxicosis causes increased remodelling, osteopenia or osteoporosis and increased fracture risk. Since the introduction of antithyroid drugs and radioiodine, hyperthyroid bone disease is less common. Here we present a rare case of an asymptomatic patient with thyrotoxicosis making its debut as increased bone remodelling.

Key words: *Graves' disease, bone remodelling, alkaline phosphatase, intact parathyroid hormone.*

Introduction

The biomechanical characteristics of bone depend on the bone mass density (BMD), the microarchitecture and the remodelled bone. An excess of thyroid hormones stimulates remodelling causing a loss of bone due to negative bone balance, with the expansion of resorption cavities and an increase in trabecular perforations. Therefore, untreated thyrotoxicosis can result in osteopenia, osteoporosis and high risk of fractures[1]. However, these may also be the consequence of a high risk of falls due to muscular weakness (myopathy) and of an agitated state in some patients. A significant increase in BMD after one year of anti-thyroid treatment has been reported[2]; however, years of euthyroidism are necessary in order to normalise levels of bone mass[3,4].

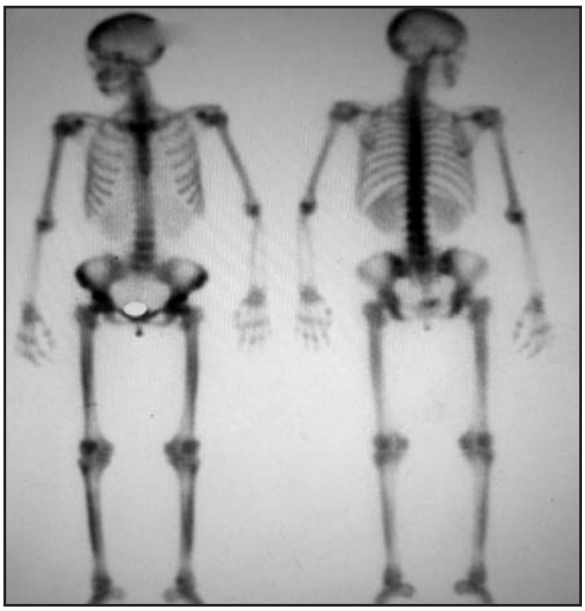
Clinical case

A woman of 33 years of age, native of China, without any medical history of interest, who was referred to the Bone Mineral Disease clinic due to her having been found in a routine analysis to have high levels of alkaline phosphatase. She reported pains in both legs of a month's duration, irregular menstruation and moderate bone loss in recent months. She reported not having had any changes in bowel habits. The physical examination showed no noteworthy abnormalities. Weight, 54 kg; height 1.56 m; body mass index (BMI), 21.39 kg/m^2. Neck without goitre or palpable adenopathies. In the analysis were observed: haemoglobin, 13.6 g/dl; alkaline phosphatase, 177 UI/l (normal values <120); calcium, 10.2 mg/dl (normal values between 8.2 and 10.6); phosphorus, 3.2 mg/dl (normal values between 2.5 and 5); hydroxycholecalciferol, 12.9 ng/ml (normal values between 20 and 50); intact parathormone (PTH), 36 pg/ml (normal values between 10 and 65), calciuria, 72 mg/24h; tubular reabsorption of phosphates, 89.41%; hydroxyproline/creatinine quotient, 0.278; carboxy-terminal telopeptide of collagen, 1.6. ng/ml (normal values between 0.064 and 0.548); amino-terminal propeptide of procollagen, 388.500 µg/l (normal values between 10.4 and 62); FSH, 9.90 mUI/ml; 17-Beta-stradiol, 25.90 pg/ml; thyroid stimulant hormone (TSH), <0.04 µUI/ml; free T4, 5.40 ng/dl; and free T3, >20 pg/ml. Antimicrosomal and anti-thryrotropin receptor (TSI) positive antibodies.

The thyroid gammagraphy was compatible with diffuse goitre, and the bone gammagraphy showed an increase in diffuse remodelled bone in the cranial shell and at the polyarticular level, without pathological elevations in metabolic activity which would suggest Paget-type disease (Figure 1). A study was competed with a gynaecological review, without evidence of alterations, and a Doppler sonography of the lower limbs, which revealed signs of chronic venous insufficiency.

The patient was diagnosed with primary hyperthyroidism due to Graves-Basedow disease. The patient received treatment with synthetic antithyroids (metimazol at decreasing doses) over a period of eighteen months, with which the thyroid function and levels of alkaline phosphatase were normalised. After a subsequent recurrence of the disease some months later, it was subject to definitive treatment with radioiodine.

Figure 1. Bone gammagraphy of the patient



The increase in bone remodelling was related to the thyroid disease. Currently the patient has clinical and analytic euthyroidism, with levels of alkaline phosphatase within normal limits.

Discussion

Thyrotoxicosis is the clinical syndrome resulting from the exposure of the tissues to an increase in thyroid hormones in circulation. The most common cause is Graves-Basedow disease (GBD) which constitutes 45-60% of all cases of thyrotoxicosis in Europe.

This pathology was described for the first time in 1825 by Parry. However, it took the name of the Irish doctor Robert James Graves, due to his descriptions in 1835, and of Karl Adolph von Basedow, because of his reports in 1840. It is an autoimmune disease defined by the production of antibodies to the thyropin receptor (TSH) in the thyroids. It is characterised by diffuse goitre and thyrotoxicosis, and may be associated with ophthalmopathy and, occasionally, infiltrative dermopathy.

As is the case with other causes of thyrotoxicosis, GBD is generally associated with an increase in the excretion of calcium and phosphorus in urine and faeces, demineralisation of bone, osteopenia or osteoporosis, an increase in bone remodelling and high risk of fractures[1]. In serious cases of thyrotoxicosis hypercalcemia maybe observed.On the other hand, blood concentrations of 25- hydroxycholecalciferol are usually diminished, which contributes to the reduction in the intestinal absorption of calcium, and in some cases, to osteomalacia[2].

The biochemical markers which reflect bone remodelling may be measured in urine or blood. There are markers for bone resorption, such as tartrate-resistant acid phosphatase, hydroproxiline, pyridinoline, and the N-terminal telopeptides of collagen type 1, and markers for bone formation (proteins synthesised by the osteoblasts), such as osteocalcin, alkaline phosphatase and carboxy-terminal propeptide of type 1 procollagen.

The markers for bone resorption may be increased by up to 7 or 8 times their normal value in patients with hyperparathyroidism[5]. Similarly, osteocalcin and bone alkaline phosphatase, may rise, although to a lesser degree, which suggests an imbalance between bone formation and resorption, with the consequent loss of bone in thyrotoxicosis[6].

In GBD total blood alkaline phosphatase (AP) is found to be raised in 67% of cases[7], essentially at the expense of its bone isoenzyme. AP is found in almost all tissues of the body, especially in the intestinal epithelium, renal tubules, bone, liver and placenta, In addition to thyrotoxicosis, there are other pathologies which may raise levels of AP: diseases of the kidney, bone (fractures in repair, bone metastases, sarcoma, myeloma, Paget's disease), liver (bilial obstruction, cholangitis, portal cirrhosis), septicaemia, ulcerous colitis, hyperparathyroidism and malabsorption (which causes a vitamin D deficit). Therefore, the clinical applications of this enzyme are, mainly, in obstructive hepatic disease and in bone metabolic disease, associated with an increase in osteoblast activity.

Increased bone resorption associated with GBD is corrected in 4-8 weeks once the thyroid hormones are normalised[8]. Bone formation, as reflected in the increased concentrations of bone isoenzyme of AP, remains elevated, despite the normalisation of the thyroid function, over a more prolonged period of time[8].

The singularity of this case resides in its atypical form of presentation of GBD, with few symptoms of thyroid hyperfunction and with biochemical data compatible with an increase in bone remodelling, which led to the initial discounting of bone metabolic pathology, such as Paget's disease or primary hyperparathyroidism. It is important, therefore, to consider the raised levels of AP in patients with GBD, before and after arriving at euthyroidism, to avoid unnecessary tests and misdiagnoses.

The authors declare that they have no conflict of interest.

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Zebra lines: Radiological repercussions of the action of bisphosphonates on the immature skeleton

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Summary
The bisphosphonates are used in the treatment of osteogenesis imperfecta, with a reduction in fractures in these patients observed with their use. However, the use of these drugs on the immature skeleton in these patients results in the formation of some radiologically visible hyperdense linear bands called zebra lines. We present the case of a patient with osteogenesis perfecta who started treatment with bisphosphonates at 10 years of age and after 2 years already showed these radiological images.

Key words: *zebra lines, bisphosphonates, osteogenesis imperfecta.*

Introduction

The use of bisphosphonates in the immature skeleton has been a great advance in the management of certain bone metabolic diseases, such as is the case with osteogenesis imperfecta, improving its clinical course and reducing the appearance of fractures. We describe the radiological repercussions which may be seen when these types of drugs are administered in growing bone, with the formation of a series of linear bands of increased density, known as zebra lines.

Clinical case

A male of 21 years of age diagnosed with osteogenesis imperfecta type IV-B. History of a tibial fracture and various femoral fractures between the ages of 6 months and 3 years (2 in the left femur and 2 in the right femur, treated conservatively. He received from the age of 10 to the age of 13 years a four-monthly infusion over three consecutive days of intravenous pamidronate (disodium pamidronate at a dose of 1 mg/kg/dose). From the age of 13 treatment with alendronate was initiated at a weekly dose of 40 mg, up to the age of 16, at which point the dose was reduced to 20 mg/week and maintained until he was 18 years of age. Analytical and densitometric controls were carried out. The last bone densitometry was carried out three years ago, with values within the normal range. The patient had not received any specific treatment in the last three years.

We show the radiological appearance of his left wrist at the age of 12 years, after approximately 2 years of treatment with pamidronate (Figure 1), and of his left femur at the current time (Figure 2). It is possible to see in both X-rays, in the metaphysary zone, some linear bands of increased density, called zebra lines, which correspond to the impact of the effect of the bisphosphonates on the growing skeleton. These lines shift towards the diaphysary zone, and may disappear or remain radiologically visible into adulthood, which may be seen in the X-ray of the same wrist carried out in the present, 9 years after the first (Figure 3).

Discussion

The use of bisphosphonates at a paediatric age has changed the clinical course of many bone metabolic diseases whose therapeutic management was limited to resolving its complications, as is the case with osteogenesis imperfecta[1]. These drugs increase bone mineral density, reduce the incidence of fractures and therefore secondary bone deformities, and improve functional parameters such as mobility and autonomy. They also diminish bone pain, above all dorso-lumbar pain, and definitely improve the quality of life of these patients[2]. In spite of there being questions to be answered, such as the age of initiation of treatment, its duration, the most appropriate type of bisphosphonates and their doses, the ideal form of administration and the long-term secondary effects, their use is already common in centres which treat this type of patient.

One of the consequences seen in patients with immature skeletons who receive treatment with bisphosphonates is the appearance of sclerotic lines, essentially metaphysary, parallel to the growth cartilage which have been called zebra lines[3]. In spite of their probable clinical insignificance, it is interesting to know of their presence at the time of radiologically evaluating these patients.

Their radiological pattern depends on the number of doses received: each cycle of pamidronate, for example, represents a dense band. If the doses are more frequent, the lines appear closer together. There are aligned circumferentially with the surface of the epiphysis, representing the progressive dose of the nuclei of secondary ossification, which means, on the other hand, that skeletal growth does not seem to be affected by the action of these drugs, which has also been corroborated clinically[3]. These finding are not found in bones of mature skeletons, being intimately related with metabolic activity: thus, in metaphysary regions with great growth potential, such as the distal section of the femur and the proximal section of the tibia, the lines are more prominent and marked. In zones of less activity, such as the pelvis or the calcaneum, the lines are finer and closer together, and less evident radiologically[4,5].

These bands have a great similarity with so-called Harris lines, described by Henry Albert Harris in 1931, who defined them as dense lines parallel to the physis, whose appearance is related to the temporary arresting of growth provoked by certain medical conditions, including diabetes mellitus, and which were called at the time growth arrest lines[6]. These lines also appear in the metaphysary-epiphysary zone of the immature skeleton, subsequently migrating towards the diaphysary zone, and which may disappear or remain visible into adulthood. They have been related with certain chronic diseases, above all, nutritional, and are considered to be the latent result of a situation of physiological stress. They also arouse interest in the archaeological analysis of bones of populations in which certain episodes of disease, epidemics or conflict situations have been historically documented which had a great impact on the health of the population of the time.

Although they are similar radiologically speaking, the differences between these two types of lines may be established first in their duration, the Harris lines being of greater duration and therefore more visible. Furthermore, the zebra lines are more extensive, appearing in all bones, while the others appear only in bone with high potential for growth, such as the distal section of the femur, the proximal and distal sections of the tibia and the proximal humerus. The Harris lines, may also appear in an isolated fashion in only one bone, for example in the reparative process of a fracture[3,7].

The fundamental etiopathogenic substrate is an interruption of osteoclast activity promoted by the bisphosphonates, there being a temporary disequilibrium in bone turnover in these zones of high

Figure 1. X-ray of the left wrist of the patient at the age of 12 years after approximately 2 years of treatment with pamidronate

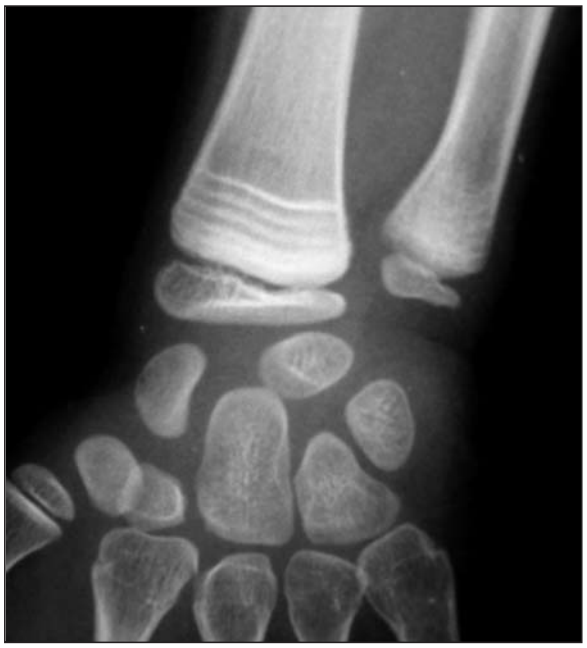


Figure 2. Radiography of the left femur of the patient at the moment

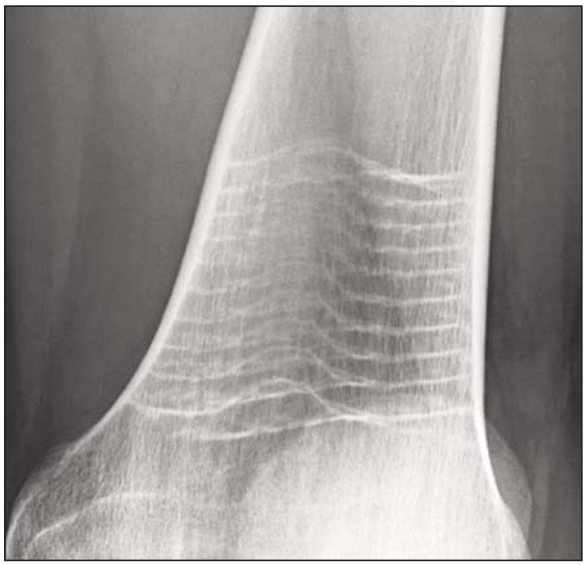
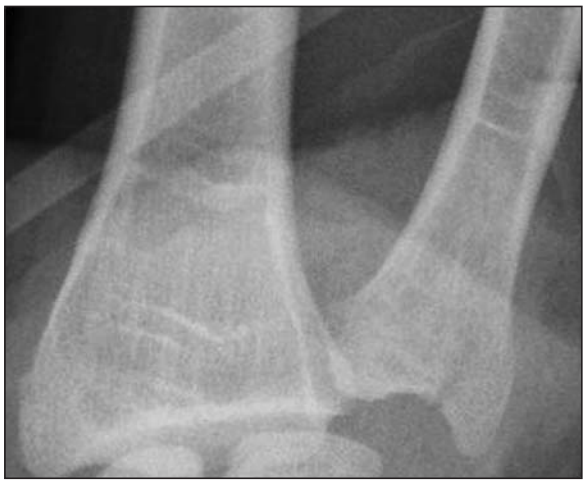


Figure 3. X-ray of the left wrist of the patient at this time



metabolic activity, which brings with it an increase in osteoblast function, and consequently in bone mineralisation, which becomes, radiologically, a radiolucid line parallel to the growth cartilage and which subsequently, with the growth of the bone, will shift to the diaphysary zone[8].

The diagnosis of the image is clear if there is a history of administration of bisphosphonates. If this is not the case, it would be necessary to suggest other options in the differential diagnosis, which may be, principally, nutritional deficiencies, and more rarely, lead or bismuth poisoning, congenital syphilis, scurvy, hypothyroidism or certain leukemias which may also result in the appearance of metaphysary radio-opaque bands.

Declaration of interest: The authors declare no conflict of interest.

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Biomechanics and bone (1): Basic concepts and classical mechanical trials

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Summary

The principals of classical mechanics are applied to the study of the resistance of materials to fracture when subject to a certain load. Bone has been, for a long time, the object of study in the field of mechanics in order to understand and resolve problems of fracture associated with deficient mechanical behaviour which may exist due to factors such as age or certain pathologies. The great quantity of specific vocabulary used in biomechanics, derived as it is from the terminology of mechanical engineering, makes it very difficult, on occasion, for researchers specialising in bone and mineral metabolism to interpret information available in the literature on the resistance of bone. The objective of this work is to describe as briefly and concisely as possible the main concepts and fundamental principles used in biomechanics, focused on their application to bone tissue. In addition, the main mechanical trials carried out on whole bones or on samples of trabecular or cortical bone are reviewed.

Key words: *biomechanics, bone tissue, bone strength, mechanical concepts.*

Introduction

Mechanics and materials science study the effects and the relationships between forces applied to a structure or rigid body and the strain produced. Bone, when it is studied, may be considered both as a tissue and as a structure, since it performs two basic functions: the control of the metabolism of Ca, P and Mg (physiological function) and the support of the organism and the protection of its organs (mechanical function). The mechanical complexity of bone tissue, composed of cortical and trabecular bone, each with distinct mechanical behaviours, exceeds that of most materials used in engineering.

The quantity of bone is defined as the mineral mass or bone mineral content (BMC in g), normally expressed per unit area as bone mineral density (BMD, in g/cm^2), the reference parameter which is used nowadays to determine bone strength. However, in recent years it has been confirmed that bone strength does not solely depend on its mass, but also on its geometric, structural and material properties (mineralisation and composition of the matrix), all this encapsulated in a concept known as bone quality. According to H.M. Frost, the geometric and material properties of bone are interrelated via feedback mechanism (Frost's bone mechanostate[1]). In turn, the structural properties are determined by the material and architectonic properties[2], which means whatever changes in the structural properties should be explained by changes in architectonic or material properties, or both[3]. Hence the densitometric variables (BMC, BMD, T-score and Z-score) are often insufficient to determine the mechanical properties of bone. Nowadays it is accepted that the strength of bone is determined by the integration of two variables: bone quantity and bone quality.

In order to Improve the treatments applied to osteodegenerative diseases such as osteoporosis, it is essential to optimise the diagnostic tools which are mainly based on establishing correlations between the biomechanical variables and the different variables which provide the analysis of the quantity and quality of bone[4-8]. With this work, we intend to present a review of the basic concepts of the mechanics of the materials which are key to understanding any determination or estimation of the biomechanical strength of bone.

Load and displacement

Force (F) or load (L) is a vector with a magnitude, direction and point of application, which when acting on a body changes its velocity or shape. In the SI system (System of International Units) it is measured in newtons (N). Depending on the angle and mode of application of the force, it may be classified as compression (when the change in shape of the object manifests itself as a shortening), traction or tension (if it manifests itself as a lengthening) and shear (if shearing of the object occurs). Although these are the three types of pure forces, in biomechanics there is often also the force of bending (which produces curvature of the object) (Figure 1). Bending stresses are really traction-compression forces in a normal direction to the force applied. Bending commonly takes place in the axial bones of the skeleton, causing forces of traction and lengthening in the convex side of the bone, and compression and shortening forces in the concave side[9].

The displacement (δ) experienced by the body or structure on which a force is exerted is proportional to its magnitude within the elastic limit, but this proportionality is not the same for all cases or for all directions. The mechanical characteristics of a material are measured in a test machine which subjects the object to a force of known magnitude and measures changes in its dimensions. When a mechanical test is carried out on an object a load-displacement curve is obtained which defines the total strain of the object in the direction of the application of the force. The load-displacement curve is used to measure the strength and rigidity of the structure, however, to compare different materials, standardisation by means of a stress-strain curve is necessary. The load and displacement may be normalised respectively as stress and strain using the dimensions of the object[10] (Figure 2).

Stress and strain

Stress (σ) is the internal resistance of an object to a force acting upon it, and is measured in pascals (Pa), 1 Pa being a force of 1 N distributed over an area of 1 m^2. In the case of bone, the physiological values of interest are found in the interval of millions of pascals (megapascals MPa)[11]. Strain (ε) is another concept necessary in describing the mechanical behaviour of materials and represents the changes in the dimensions of an object subject to the action of a force. Strain may be expressed in units of absolute length or in units of normalised length $\varepsilon = \Delta L/L$, where ΔL is the variation in length and L is the initial length, which means that in this case it is a non-dimensional magnitude (mm/mm). Strain is also usually expressed as a percentage.

On occasions, in subjecting a body to the action of a force, the body is capable of returning all the energy used in its deformation once this force ceases (elastic behaviour). However, in certain circumstances this is not possible, the strain suffered by the body being irreversible (plastic behaviour). If we subject a bone to the progressive action of a force, the two types of strain are produced successively (Figure 2), by which the bone is said to have an elastic-plastic behaviour. From the stress-strain curve we may obtain a great quantity of information on the properties of the material. It is possible to distinguish between a first area in which the stress and the strain are proportional (the linear area of the curve, which corresponds to the elastic area, in which Hooke's law of elasticity applies) and another area in which the original shape of the body does not recover even when the load ceases to be applied (the plastic zone or zone of irreversible strain).

The point of transition between the elastic area and the plastic area is called the yield point, which corresponds to the yield strain (ε_y) and to the yield stress or maximum elastic resistance (σ_y), which estimates the capacity of a material to become strained without suffering microfractures. In a certain area of the plastic region there is a point corresponding to the maximum stress (ultimate stress, σ_{ult}), from which microfractures occur which are responsible for the fact that even with a reduction in stress the deformation of the sample increases.

Mechanical properties of materials

The mechanical properties of a material are all those characteristics which allow it to be differentiated from others from the point of view of its mechanical behaviour.

Elasticity and plasticity

Elasticity is the property of a material to recover its initial form once a force has ceased to be a applied. Plasticity is the opposite property: plastic strain is maintained even when the force ceases. The proportions of total resistance borne in conditions of elastic and plastic behaviour may be expressed in the following way:

Elasticity = $\sigma_{ult} - \sigma_y$

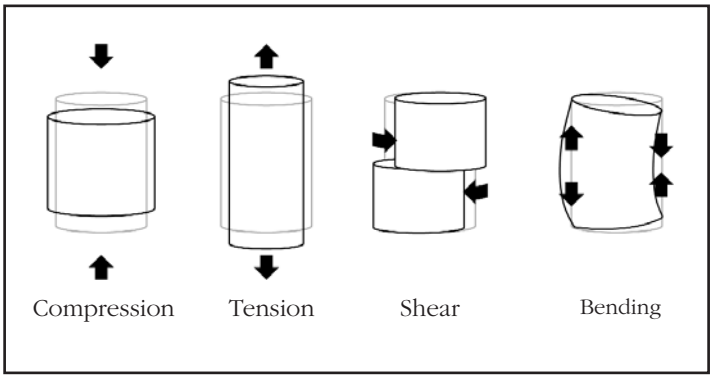
Plasticity = $(\sigma_{ult} - \sigma_y)/\sigma_{ult}$

An example of elastic material is rubber, while a plastic material would be, for example, plasticine.

Stiffness and flexibility

Stiffness is a characteristic of materials for which a major force is necessary to induce a small elastic strain in the material. It corresponds to the slope of the elastic area of the load-displacement curve (extrinsic stiffness, S), expressed as N/m; of the stress-strain curve (elasticity or Young's modulus, E), expressed as Pa. When speaking of stiffness it should be as a characteristic of the whole structure, while the stiffness of a material is indicated by Young's modulus. The concept of stiffness is commonly to be found in both contexts, which may give rise to confusion, which is why it is recommended that flexibility be used to describe the structural characteristic and modulus of elasticity for property of a material[11]. Flexibility is the property which is the opposite to stiffness. A flexible material is that which shows great strain in the elastic zone before getting to the plastic zone. A material with a low Young's modulus will undergo great strain with little stress, while a material with a high Young's modulus will suffer small strains with large stresses. Paper or cloth for example are flexible materials. On the other hand, ceramics or glass are stiff materials, since when they are bent they break.

Figure 1. Different types of forces to which bone may be subjected. Forces of compression, traction and shearing are pure forces, while that of bending is the result of a combination of various types of force acting simultaneously. The grey-coloured contour indicates the initial geometry of the sample, while the black-coloured contour shows the form after the force indicated is applied



Tenacity, work to break and resilience

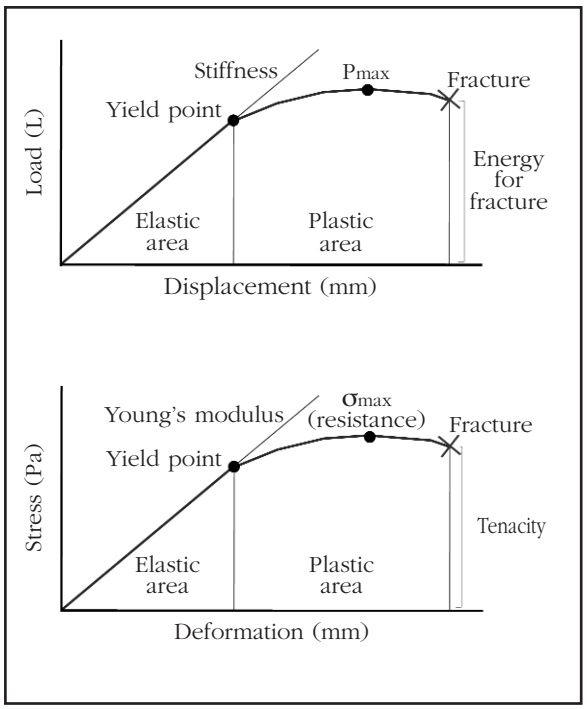
Toughness (or tenacity, u) is the capacity of a material to resist plastic strain. Toughness represents the quantity of energy absorbed up to the point of the appearance of a fracture[12]. It is obtained quantitatively by calculating the area under the curve which forms the elastic and plastic sections of a stress-strain graph. Those materials which, like iron, resist blows without breaking are called tough. If the information on toughness is found in the load displacement curve, which is to say that it refers to the structure and not the material, one speaks of the energy necessary for fracture, or work to break (U).

Toughness should not be confused with surface hardness, which refers to the resistance of a material to be scratched or dented. Toughness takes account of the energy absorbed up until the fracture happens, being calculated by means of the area below the curve from the initial point up to the breaking point, while resilience represents the energy which the material is able to absorb without experiencing permanent strain, which is to say it only takes into account the quantity of energy absorbed during the elastic strain (which would correspond to the area below the curve from the initial point to the yield point). Resilience is defined as the capacity of the material to resist elastic strain. A high degree of resilience is found, for example, in the cartilage of the joints.

Strength

Therefore the characteristics obtained from the load-displacement curve (maximum force, maximum displacement, extrinsic stiffness and work to break) will provide us with information relating to the extrinsic or structural properties, referring to the bone as structure. However, the information which we obtain from the stress-strain curve (maximum stress, maximum strain, Young's modulus and toughness) refer to the bone tissue as material, being known as intrinsic or material biomechanical

Figure 2. Biomechanical principles used in the determination of the mechanical properties of bone. The load-displacement curve (above) and stress-strain curve (below) after the normalisation of the former using the dimensions of the object under test



properties. But what then is bone strength? Strength estimates the effective opposition of a material to losing its integrity, which is to say, of being fractured, being defined as the force necessary to trigger the mechanical break of the material under specific load conditions. From the yield point in the stress-strain graph starts what is called the plastic zone, in which small increments in stress provoke relatively large increases in strain/deformation, which indicate that a part of the structure has begun to break. Maximum strength, or simply, strength ,is no more than the maximum stress necessary to fracture the material. The maximum force is used also on occasions as an indicators of the strength of an object, but it is necessary to take into account that this should only be used to compare samples of the same composition and size.

Concepts referring to the dimensions of the material

Poisson coefficient

If an object is subjected to a uniaxial compression force its dimensions reduce in the direction of the force (longitudinal direction) and increase in the transverse direction. If, on the other hand, we apply to the object a force of traction or tension, the dimensions of the object reduce in the transverse, and increase in the longitudinal, direction (Figure 1). The relationship between the strains in the two directions is given by the Poisson ratio, ν, such that:

$$\nu = \varepsilon_{transv}/\varepsilon_{long}$$

Moment of inertia

Inertia is the property of an object to resist a change in its movement and is described in Newton's first law of motion ("Any body will continue in a state of rest or uniform and rectilinear motion unless obliged to change this state due to forces acting on it). Any object which spins on an axis develops rotation inertia, which is to say, a resistance to change in velocity or in its axis of spin. The inertia of a rotating object is determined by its moment of inertia, (I), which is the resistance with which a rotating body opposes a change to its spin velocity. Considering a large bone to be a cylinder of bone, the moment of inertia of the elliptical transverse section may be calculated as[10,13-15].

$$I = \frac{\pi}{64}[(x_1 y_1^3) - (x_2 y_2^3)]$$

where x_1 is the greatest external diameter of the transverse section at the point of application of the force, and y_1 is the smallest external diameter, x_2 the greatest internal diameter and y_2 the smallest internal diameter (Figure 3).

Inertia may be interpreted as an analogue of mass in uniform rectilinear movement. The moment of inertia would reflect, therefore, the distribution of the mass of a body with respect to its axis of spin. The moment of inertia depends exclusively on the geometry of the body and the position of the axis of spin (and not on the forces which intervene in the movement), in such a way that the greater the distance between the mass and the centre of spin, the greater its value.

Viscoelasticity

While the mechanical behaviour of many solids approximates Hooke's law (elastic behaviour) and that of many liquids to Newton's law (viscous behaviour), both laws are idealisations. In applying a load on an elastic solid this is strained until the force ceases and the strain returns to its initial value. If the load is applied to a viscous fluid it is also strained, but does not recover once the load ceases. In the case of a viscoelastic material the object to which the force is applied recovers part of the strain. Viscoelasticity is a phenomenon which describes the mechanical characteristics of materials as a function of time. Bone, as with most biological materials, is a viscoelastic material. To quantify the mechanical properties of a viscoelastic material we need to take into account stress relaxation and creep. Stress relaxation is the reduction in tension in a material subject to a constant strain, while creep is the gradual increase in the strain of a material subject to a constant load[16].

Viscoelastic behaviour is described using three variables: storage modulus, E', loss modulus, E" and loss tangent, tan δ). In viscoelastic materials the complex modulus, E* is calculated, which is a measure of the resistance with which the material opposes the strain and combines the elastic response, through the storage modulus (related to the storage of energy), and viscosity, through the loss modulus (related to the dissipation of energy).

Fracture and fatigue

As has already been seen, when a force is exerted on bone a deformation will occur in elastic conditions first and in plastic conditions later,
until the point in time at which a fracture or mechanical break of the bone occurs. However, fractures commonly appear in bone without it having reached the maximum stress which it can endure. Fatigue is the damage which occurs in a material due to repeated stress below the maximum level. The cycles of load on a material may provoke a failure even though these loads are below the value for rupture. For example, in a human bone a stress may provoke a microfracture without the bone breaking completely. If this stress is repeated over a number of consecutive cycles, the microfracture will propagate, causing total rupture of the structure.

Mechanical trials of compression and traction

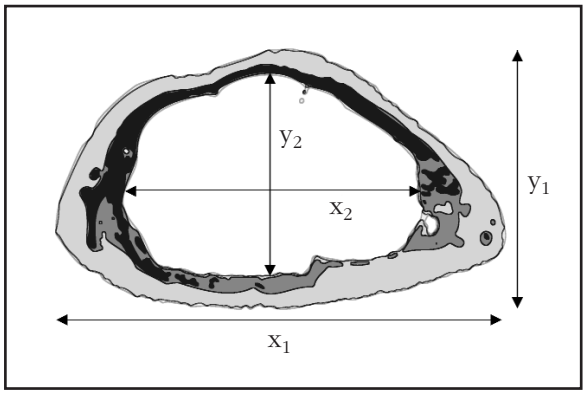
Mechanical trials of compression and traction are standardised tests in which a sample is subjected to a uniaxial force in a universal machine for controlled force or displacement tests (Figure 5).

The specimens for traction or tension trials need to be cylindrical or prismatic in shape, with widened ends, both to facilitate their subjection to the test machine, and to ensure their rotation in within the area of smallest section (Figure 4). Although the traction trial is one of the most precise methods to determine the mechanical properties of bone, the obtaining of bone samples for these trials is highly complex. In the case of trabecular bone samples, which may fracture easily when subjected to the instruments of the test machine, the ends of the samples are usually coated in plastic resin. The proportions of the different measures of the specimen are derived from the ASTM American Society for Testing and Materials) standards .

In the case of compression trails, the samples commonly consist of 8 mm cubes or cylinders of 8 mm in diameter. In compression trials of trabecular bone it has been shown that the Young modulus is found to be low due to the effects of friction between the plates and the surfaces of the sample, and the damage done to those surfaces while the sample is being obtained[17]. To minimise these effects cylindrical samples are recommended with a length-diameter ratio of 2:1[18]. It is very important to ensure that the end surfaces are parallel to each other to avoid errors during the test. There are now even autoadjustable compression plates available to compensate for a lack of alignment of the surfaces.

Both in the compression trials and those of traction, the use of en extensometer is normal, which can be set to the measurements of the object to be tested. This fact, in addition to allowing the determination of the deformation occurring in the specimen, reduces possible errors in the measurement by excluding deformation caused by the grips, the plates of the machine, etc. The sample is subjected to compression or traction at a constant force (N/s) or displacement (m/s) and the data relating to the force and shortening or lengthening of the sample are gathered by means of a force transductor and an extensometer.

Figure 3. The transverse section of a long bone considered as a hollow ellipse for the calculation of the moment of inertia. The external (x_1 and y_1) and internal (x_2 and y_2) dimensions used for the quantitative calculation



The stress may be calculated as:

$$\sigma = \frac{P}{A}$$

where P is the load applied, and A the area of transverse section of the sample. The strain is calculated as:

$$\varepsilon = \frac{\delta}{L_0}$$

where δ is the displacement of the sample and L_0 its initial length. Hence we can obtain a stress-strain curve. From this curve we can calculate Young's module as the incline of the linear region of the curve (elastic zone).

$$E = \frac{\Delta\sigma}{\Delta\varepsilon}$$

The area under the stress-strain curve gives us the value of toughness of the material (u). The value of maximum stress (σ_{ult}) will give us the strength of the bone under force of traction or compression.

Mechanical trials of torsion

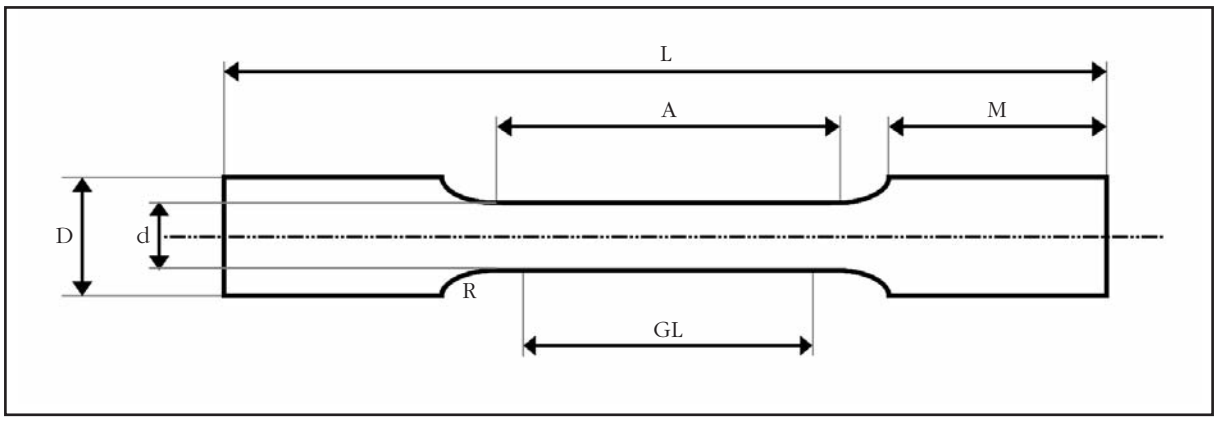
Trials of torsion are carried out to determine the mechanical properties of an object when a shear stress is applied. The samples for torsion trials (normally with a circular transverse section) are fixed at the ends to the test machine supports and twisted in opposite directions from their ends, producing shear stress until the sample snaps[19]. The torque, T, is measured by means of a transductor and the twist angle, ϕ, using a sensor, both incorporated into the test machine. With these two variables and the dimensions of the sample being tested, we can calculate the shear stress, τ:

$$\tau = \frac{Tr}{I_p}$$

where T is the torque, r the radius of the sample, I_p the polar moment of inertia of the transverse section. The shear strain, γ, will be:

$$\gamma = \frac{\phi r}{L}$$

Figure 4. Geometry of a sample for a traction test. L: total length; A: parallel length; GL: calibrated length; M: end length; D: diameter of the sample at the ends; d diameter of the sample in the test area; R: radius of curvature



where ϕ is the twist angle, r the radius of the sample and L its length.

The shear modulus, G, is obtained from the incline of elastic region of the curve:

$$G = \frac{\Delta\tau}{\Delta\gamma}$$

As with the compression or traction tests, the maximum stress (τ_{ult}) indicates to us the resistance of bone to torsion.

Mechanical tests of bending

There are two normal types of bending tests: bending at three points and bending at four points (Figure 5). In both cases the sample is fixed on two supports, but for the bending at three points the force is applied on the upper part in the centre of the specimen (being applied at the centre, the maximum moment of bending), while for bending at four points, two equal forces are applied symmetrically on the upper side, in such a way that the moment of bending is spread uniformly in the area between the two points of application[19]. These tests are often used to determine the strength of long bones. Due to the fact that it is relatively simple to obtain samples, it is used extensively. When a bone is loaded while bending it is subject to a combination of compression (which act on one side of the bone) and traction forces (which act on the opposite side). As bone is less resistant to traction, the fracture is initiated on the surface which undergoes traction forces, propagating towards the compression surface and provoking the appearance of shearing forces, until a "butterfly wing" fracture occurs (with two oblique lines of fracture which form an angle between them and delimit a triangular fragment), characteristic of the two bending tests.

Using the theory of beam bending and assuming that bone has an elastic linear behaviour, we calculate the stress and strain in a flexion test at three points in the following manner[10,16,20]:

$$\sigma = \frac{PLc}{4I}$$

where P is the load applied, L is the distance between the supports, *c* is half the lowest external diameter of the transverse section of the bone at the point of application of the force (mid section of the bone diaphysis), and I the moment of inertia of the elliptic transverse section. The moment of inertia for a hollow ellipse may be calculated in the way we have seen in the section "Concepts referring to the dimensions of the material". The strain will be obtained thus:

$$\varepsilon = \delta\left(\frac{12c}{L^2}\right)$$

An estimate of the elasticity modulus may be calculated from the load-displacement curve obtained[21], calculating the moment of inertia (I) and with the value of the distance between supports L, as:

$$E = \frac{PL^3}{48I\delta}$$

Similarly, for the bending tests at four points, we calculate the stress as:

$$\sigma = \frac{(P/2a)c}{I}$$

where a is the distance between one support and the nearest point of application of the force. The elasticity modulus is estimated with the following formula[16]:

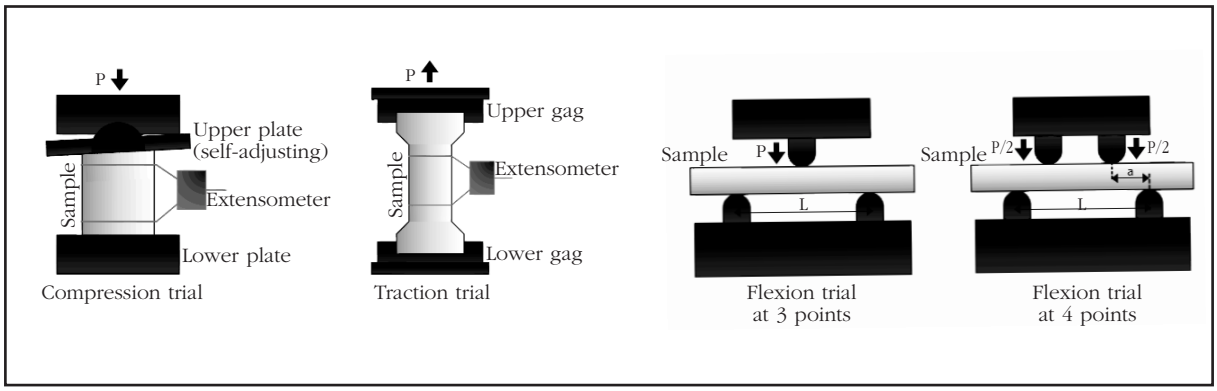
$$E = \frac{P/2a^2(3L-4a)}{6I\delta}$$

Conclusions

The biomechanical behaviour of bone is extremely complex due to its heterogeneous, anisotropic and viscoelastic character. In this work the basic concepts of material mechanics, as well as certain properties characteristic of bone, are presented. These are all necessary in characterising this behaviour, whose understanding is important in interpreting the great quantity of information which we may find in the literature referring to the mechanical properties of bone.

In a mechanical trial the relationship between the force which we apply to a body and the dis-

Figure 5. Schematic representation of the principal classic mechanical trials



placement it undergoes is studied. The relationship between force and displacement is known as stiffness, while the maximum work carried out by the force in deforming the body is known as work to break. All these concepts are used frequently in mechanical tests with samples of bone. However, it should be taken in to account that these parameters describe an effect on which the structure of bone has a great influence. Thus, force, displacement, stiffness and work to break are known as extrinsic or structural properties. Let us imagine a cylinder of titanium and a cylinder of wood of the same diameter. The cylinder of titanium will be capable of resisting much greater forces than the cylinder of wood, since it is a stronger material. However, if we carry out a mechanical test on a piece of titanium wire and a very thick log of wood, the latter will withstand greater forces, which is not to say that from a material perspective wood is stronger than titanium. For this reason it is necessary to eliminate the contribution of the geometry of the sample to the biomechanical measures, calculating the intrinsic or material biomechanical properties of the body under test. This is done by normalising the force applied by dividing it between the area to which it is applied, obtaining the stress, and dividing the displacement by the initial length of the body, obtaining the strain. The relationship between them will give use the elasticity modulus and the area under the curve will indicate the toughness of the material. Due to the anisotropic character of bone, its biomechanical properties vary as a function of the direction in which the force is applied. Thus, the bone will show different strength depending on whether forces of compression, traction or shearing are applied. Compression tests are often used for trabecular or cortical bone samples, or for vertebral bodies. The long bones such as the femur or tibia are usually subjected to traction, torsion or bending tests. In these, there is a combination of compression forces on the side to which the force is applied and of traction forces on the opposite side.

The relationship between structural properties, material properties and the mechanical behaviour of bone is complicated and this is a challenge. An understanding of this relationship is of great importance, since it helps us to understand of the behaviour of bone subjected to constant physiological loads, identifies the areas most susceptible to fracture and allows the prediction of different pathologies in relation to bone strength, and their treatment. In a second part to this work we analyse the hierarchical structure of bone and the biomechanical tests which are carried out nowadays in different areas, as well as alternative techniques to the classic biomechanical trials for the determination of bone strength.

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Cellular and molecular mechanobiology of bone tissue

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Summary
Several data support the concept that skeletal homeostasis, repair and adaptation to daily life depend on mechanically-induced signals that promote appropriate responses of bone cells. This review considers the cells that are responsive to mechanical signals within the bone environment, and the molecular mechanisms involved in mechanotransduction, the process by which cells convert mechanical stimuli in biochemical signals and subsequently modify biological activity. Understanding the cellular and molecular mechanisms underlying bone responses to mechanical loads will positively impact current knowledge on basic bone biology and pathophysiology and will likely contribute to the development of new interventions to improve bone strength.

Key words: *osteocytes, mechanical adaptation, mechanotransduction, mechanosensors.*

Introduction

The skeleton is a strong, hard and tough organ which is formed by specialised connective tissue which is characterised by having an extracellular calcified bone matrix in which are embedded different types of cells which give functionality to the tissue. In general, it is possible to attribute four basic functions to bones. On the one hand they have a structural function, providing internal support to the body and protecting vital organs. They also have a role in locomotive function as a result of the interaction between the bones, the muscles and the joints. On the other hand, they are responsible for the production of certain essential components for the differentiation and survival of the haematopoietic mother cells. Finally, bones are an important store of calcium and phosphates, both their deposit and their mobilisation contributing to the maintenance of mineral homeostasis. For some time now, advances in the understanding of bone biology have suggested that bone may also be considered as a key endocrine organ, capable of participating in the regulation of different physiological processes such as energy metabolism or reproduction[1].

Taking into account the important role which this tissue plays in the physiology of the organism, it is of vital importance that both its composition and its mechanical resistance are maintained throughout life. Hence, bone is constantly renewed by a process known as bone remodelling[2], which replaces old bone with new. This renovation takes place through the balanced action, coordinated in time and space, of the osteoblasts and the osteoclasts[3,4]. It is possible that part of this process occurs at the specific points of the bone which require renovation[5,6], although it is thought that most of it happens randomly, resulting in a complete renovation of the skeleton approximately every 10 years.

Bone is a tremendously dynamic tissue, its structure and size changing from birth until consolidation in adulthood. Furthermore, bone has the capacity to change to adapt to new functional demands which may arise in an individual on a day to day basis. Hence, besides remodelling, there is another process, called bone modelling, which allows bones to acquire their normal shape and structure, and which modify them at certain points through the action, independent and not linked, of the osteoblasts and osteoclasts[7]. Bone modelling can take place during the growth phase, or even in adulthood, to change the shape of bone in response to mechanical load, a process known as mechanical adaptation[8]. It is known that daily physical stimulus of the skeleton induces an anabolic effect in bone tissue, facilitating the maintenance bone mass and reinforcing resistance in those areas which receive a greater mechanical load[9]. A clear example of this effect is seen in the forearm of tennis players, which show an increase in bone mass up to 10% in the arm which wields the racquet[10]. And contrarily, a reduction in physical demands, such as prolonged periods in bed, space voyages or situations of paralysis or relative immobility provoke losses in the quantity and quality of bone, and as a consequence, an increase in the risk of fracture[11]. The main objective of this work is to offer a general vision of the types of cells and the molecular mechanisms responsible for regulating the adaptive response of bone to its physical environment.

Bone cells sensitive to mechanical stimulus

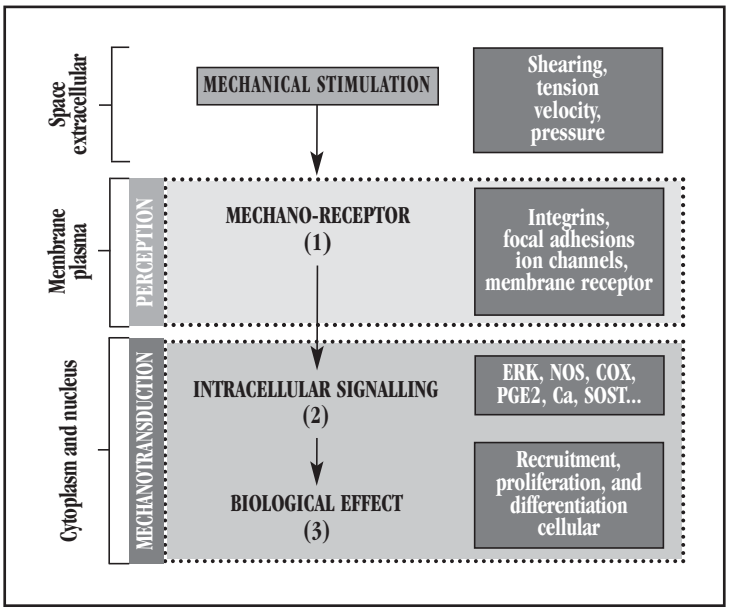
The process of mechanical adaptation requires that the cells are capable of detecting the mechanical signals and transforming them into biological signals, a phenomenon known as mechanotransduction. Ultimately it will be these signals which will direct the necessary changes in the bone architecture. The mechanisms responsible for the response to the physical stimuli in bone are still little understood, but everything points to the fact that there are various types of bone cells involved. It is possible that both the osteoclasts, and the mesenchymal progenitors, osteoblasts and osteocytes are capable of perceiving or being affected by mechanical stimuli coming from the environment. To what extent the responses which occur in each of these types of cells is the result of direct or indirect mechanisms is something which is not totally clear at present. But in any case, it seems evident that the interactions between all these cells are key to the regulation of the recruitment, proliferation and differentiation of the osteoblasts and osteoclasts, events which will ultimately determine the changes in the bone tissue.

Due both to their disposition and abundance in bone (90% of all osteoblast cells), as well as the network of canaliculi which connect them themselves and with other bone cells, the osteocytes are considered to be the main cells charged with mechanotransduction[12]. Hence, Tatsumi et al. observed that the specific elimination of the osteocytes and their dendritic processes in rats blocked bone loss induced by the absence of mechanical stimulation, supporting the essential role of these cells in mechanotransduction[13].

It is worth mentioning that osteocytes are found deeply embedded within the bone matrix. This suggests that these cells may be exposed to a wide range of stimuli which may include tension, shearing, changes in pressure or flow of fluids[14]. Furthermore, certain characteristics of these stimuli, such as their magnitude or frequency, may also fundamentally influence the cell response. Given the intrinsic characteristics of bone tissue, high magnitude mechanical stimuli deriving from daily activity generate relatively small deformations (0.1% deformation from the original state). On the other hand, the skeleton is continuously subject to stimuli of very low magnitude (deformations <0.0005% from the baseline position) and high frequency (10-50Hz), a product of the constant muscular contractions required to maintain posture[15]. In most of these cases, the stimuli are incapable of acting directly on the cells embedded in the matrix. It is thought rather that these stimuli indu-

ce changes in the interstitial fluid which flows in the extensive network of canaliculi which connect the osteocytes. The movement of fluid within this system may be influenced by mechanical stimuli in the environment and generate shearing forces, changes in the velocity or pressure exerted on the bone cells, which would be capable of activating a whole battery of membrane receptors which will be those responsible for initiating the cascade of intracellular signalling which direct the biological responses need to respond to a certain mechanical stimulus. In addition to being the receptive medium, this system of canaliculi contributes to the amplification and distribution of the signal to adjacent cells. There is a range of experimental evidence which supports this idea. On the one hand, a flow of fluid has been observed around the osteocytes in the tibias of rats which have been mechanically stimulated[16]. Similarly, Price et al. have shown how there is a movement of fluid in the canaliculi in response to certain mechanical stimuli[17]. That said, it is important to mention that the possibility cannot be completely excluded that the osteocytes are responding directly to the pressure coming from the mineralised matrix after the physical stimulus[18,19]. It is even possible that the same stimulus may provoke the simultaneous appearance of a number of these forces. Although the composition of the mineralised matrix and the functional interactions between the cells which are embedded in them are becoming better understood, the mechanisms which underlie the perception and the subsequent transduction of these physical signals are for the moment the object of intense scientific debate.

Figure 1. Transduction of the mechanical signal in bone tissue. The process of mechanotransduction converts the mechanical stimuli into a sequence of cellular events which are finally translated into a biological effect (e.g. an increase in cell proliferation, initiation of programmes of cell differentiation...). The transduction of the signal starts in the membrane, by means of different structures sensitive to changes in the mechanical characteristics which surround the bone cells (1). These receptors activate various intracellular pathways (e.g. ERK, ion flow, G proteins...) which ultimately provoke changes in the expression in certain key genes in the cell biology of bone (2). Variations in the levels of these genes ultimately modify the proliferation, differentiation and recruitment of bone precursors (3).

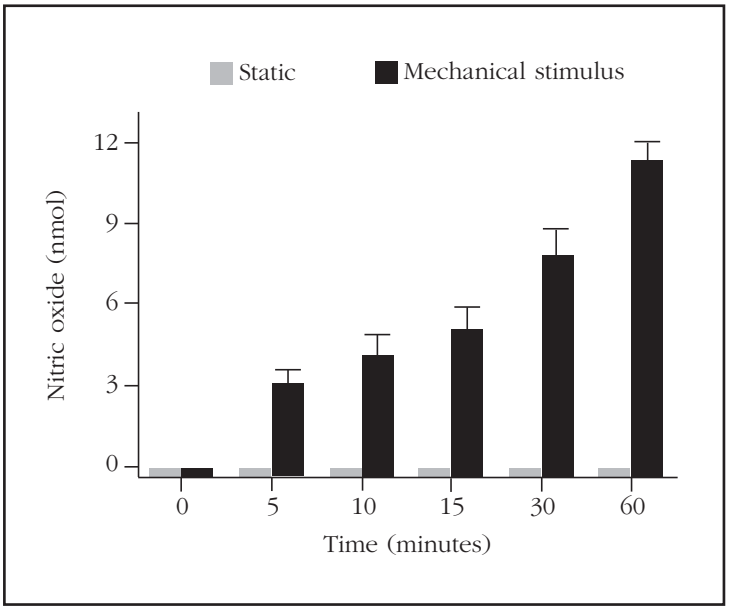


Transduction of the mechanical signal

The ability of the bone cells to perceive mechanical signals in their mineralised environment requires the presence of mechanoreceptors, in other words, molecules, protein complexes or biological structures capable of detecting changes in the different forces associated with mechanical load (e.g. pressure, fluid flow...). In theory, these structures should 1) connect the cells with the extracellular space, allowing it to "sense" the pressure provoked in the extracellular mineralised matrix, or 2) be situated in the plasmatic membrane to detect changes in the pressure or flow of fluid which surrounds these cells. Among the elements which have been postulated as being responsible are different integrins, focal adhesions, ciliary structures and different membrane proteins. In fact it has been shown experimentally how the structures capable of anchoring the cells in the extracellular space which surrounds them, such as the aforementioned integrins or focal adhesions, are necessary for the perception of mechanical stimuli[20,21]. Similarly, the canals sensitive to physical stimuli, such as the calcium canals or the connexins, also play an important role in the reception and subsequent transduction of the signal, generally allowing the entry or exit of different factors charged with mediating the cellular response to physical stress[22]. Finally, the mechanosensory organs, such as cilia are increasingly being seen to be important in this area. So much so that it has been suggested that the release of prostaglandin E2 (PGE2) after the perception of the mechanical signals may be, at least in part, regulated by these types of structures[23,24]. It has also recently been suggested that the cytoskeleton, which connects the cell interior with the extracellular surroundings may be a critical element in determining how the osteocytes "sense" these forces[25].

Once the stimuli have been perceived, they should be transformed into biological signals which promote changes in cell activity, such as phosphorylation, translocation of transcription factors or changes in gene expression. Among the mediators which join the perception of the signals through the aforementioned structures and these

Figure 2. Production of nitric oxide in response to mechanical stimulus. The application of a pulsing flow directly on the cell membrane of osteoblast cells (HOS-TE85) induces a marked secretion of nitric oxide into the medium (black bands), these increasing with the duration of the stimulus. On the other hand nitric oxide was not detected in those cells which were not mechanically stimulated (white bands).
nmol=nanomoles



effectors are found different kinases, receptors associated with the G protein and second messengers such as calcium or cyclical AMP[8,14] (Figure 1).

In spite of the fact that not all the molecular mechanisms which mediate the transduction of the signal are completely understood, we now have a better idea of which factors are ultimately in charge of modulating the activity of the different types of cells. Thus it is known that mechanical stimuli provoke changes in the expression of certain target genes such as sclerostin, Wnt ligands, nitric oxide synthases or prostaglandins, among others[26-29]. Although there may be various molecules involved, the effect of the mechanical load on the bone is characterised principally by a reduction in the expression of sclerostin on the part of the osteocytes[26,30,31]. Sclerostin is a powerful inhibitor of bone formation, which inhibits the signalling of the Wnt ligands by bonding with LRP-type co-receptors[32]. In support of the significant role being given to sclerostin in this process, it has been observed that rats deficient in this gene are resistant to loss of bone mass in the hind limbs induced by the absence of mechanical stimulation[31]. However, in spite of the important role of this molecule in the adaptation of bone, hardly anything is known about what are the mechanisms which provoke the reduction in its expression in response to mechanical stimulus. Hence, our group has shown in *in vitro* experiments that the lowering of transcriptional levels of SOST may be, at least partly, mediated by the production of nitric oxide[33] (Figure 2). It has recently been suggested that the estrogens may also be involved in the modulation of the transcriptional levels of this gene in response to mechanical stimuli[34]. Although, as has already been mentioned, the response is mainly led by the levels of sclerostin, it seems that it is not possible to discount the idea that there are various molecules and signalling pathways involved in mechanical adaptation. In fact, the production and subsequent signalling mediated by PGE2, as well as the role of nitric oxide and the synthesis of Wnt ligands, also appear to play a significant role in the formation of bone promoted by mechanical forces[27,28,35].

Conclusion

The combination of mechanisms which underlie mechanical adaptation are even today little understood. The wide range of physical stimuli to which cells may be subjected, as well as the diversity of biological responses and the possible interactions between the different types of cells involved in the process increase exponentially the complexity of studying the mechanisms involved. The use of animal models has helped advance understanding of the mechanobiology, although on occasion the results are difficult to interpret, mainly due to the impossibility of isolating other biophysical components of the load applied, or because of the difficulty in the choice of an appropriate mechanical stimulus. There have been various advances in this field achieved through the use of *in vitro* techniques, since these provide greater control of the different factors which may have an influence on the response. However, these experiments eliminate the natural environment of the bone in which the mechanosensory cells are found. It seems, therefore, that it will be necessary to approach its study from various experimental angles, combining the investigation of individual molecules in certain types of cells, with functional studies in animals. Although not mentioned earlier, since it was not the objective of this review, it is nevertheless important to take into account role that muscle, and in particular the factors produced by this tissue, may have in mechanical adaptation[36].

There is no doubt that the study and understanding of the molecular mechanisms which regulate the capacity of bone to respond to functional demand may lead to the development of new and more effective therapeutic strategies for musculoskeletal disorders , covering a wide range from the establishment of optimum physical exercise regimes to medicines which take advantage of the main signalling pathways involved in mechanical adaptation.

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