Osteopenia in rats with liver cirrhosis: beneficial effects of IGF-I treatment

Arantxa Cemborain[1], Inma Castilla-Cortázar[1], María García[1], Jorge Quiroga[2], Begoña Muguerza[1],
Antonio Picardi[1,3], Santiago Santidrián[1] and Jesús Prieto[2]

*Departments of [1]Human Physiology, [2]Internal Medicine and Liver Unit, School of Medicine, University of Navarra, Pamplona, Spain, and
[3]Internal Medicine, Libero Istituto Universitario Campus Bio-Medico, Rome, Italy*

Background/Aims: **Liver cirrhosis is associated with osteopenia and also with low levels of IGF-I. This hormone has been reported to stimulate bone formation in states of undernutrition and low bone turnover. Our aims were to evaluate whether osteopenia develops in male Wistar rats with CCl$_4$-induced cirrhosis and whether IGF-I is effective in the restoration of bone mass in these animals.**

Methods: **Cirrhotic rats were distributed into two groups: group CI ($n=12$) which received placebo and group CI+IGF ($n=12$) which was treated with human recombinant IGF-I (2 μg/100 g bw/day, sc, 21 days). Twelve normal animals which received placebo constituted the control group. On the 22nd day, the animals were sacrificed, and bone parameters were analyzed in femur and/or tibia.**

Results: **Posterior-anterior and latero-medial diameters were similar in all groups. Also, no significant differences were observed in bone contents of calcium, total proteins, collagen and hydroxyapatite in CI rats as compared with controls. However, CI rats showed significant reductions in bone weight (-13.5%, $p<0.001$), total bone density (-9.28%, $p<0.001$), and increased perimedullar bone resorption and urinary levels of deoxypyridinoline (a marker of bone resorption). In CI+IGF rats these parameters improved significantly as compared with CI animals.**

Conclusions: **Osteopenia characterized by loss of bone mass and preserved bone composition is found in rats with CCl$_4$-induced cirrhosis. This bone disorder is partially corrected by treatment with low doses of IGF-I. Since osteoporosis seems to be the predominant form of osteopenia in patients with cirrhosis, IGF-I should be considered as a possible therapy for this disorder.**

Key words: **Insulin-like growth factor-I (IGF-I); Liver cirrhosis; Osteoporosis; Undernutrition.**

INSULIN-LIKE growth factor I (IGF-I) is an anabolic hormone produced in the liver upon growth hormone stimulation (1). Several organs other than the liver, including bone, also synthesize IGF-I which acts locally in an autocrine and/or paracrine manner (2–4). Bone is, in turn, one of the major target organs for IGF-I, not only during growing but also in adult life. In fact, IGF-I stimulates osteoblastic proliferation and differentation (2–5), acting through specific receptors which are present in bone cells of the osteoblastic lineage (4–6) and in bone-derived endothelial cells (7). It is thought that IGF-I plays a key role in the process of bone remodeling and therefore in maintaining bone mass (2,4). Furthermore, clinical studies have shown beneficial effects of recombinant human IGF-I (rhIGF-I) administration on bone in a variety of clinical conditions (4,8–10).

In patients with liver cirrhosis the prevalence of osteopenia is significantly higher than in an age-matched normal population (11–14). As a consequence, these patients are exposed to an increased risk of bone fractures, which are a source of morbidity in advanced stages of the disease and after liver transplantation (12–15). The pathogenesis of osteopenia in liver cirrhosis is not clearly understood, although malabsorption, undernutrition, vitamin D deficiency, reduced levels of sexual hormones and alcohol toxicity appear to be some of the factors involved in disturbed bone metabolism (16–21). In addition to these defects, in advanced liver cirrhosis IGF-I levels decrease (22,23) as a result of diminished hepatocellular biosynthetic function and progressive loss of growth hormone receptors on the

Correspondence: Jesús Prieto, Department of Internal Medicine and Liver Unit, Clínica Universitaria, Av. Pio XII, s/n, 31080-Pamplona, Spain. Tel: 48-255900. Fax: 48-296785.

liver cell membrane (22,23). Although low IGF-I levels characterize advanced liver cirrhosis, the role of IGF-I deficiency in the genesis of osteopenia and the possible therapeutic effect of IGF-I in this condition have not yet been established. Thus, in this study we aimed to evaluate whether rats with CCl_4-induced liver cirrhosis develop osteopenia and whether low doses of IGF-I could increase bone mass in these animals.

Materials and Methods

Animals and experimental design

Thirty-six 4-week-old male Wistar rats, weighing about 110–120 g, were included in this protocol. Animals were housed in cages placed in a room provided with a 12-h light-darkness cycle and at constant humidity and temperature (20°C). Both food (standard semipurified diet for rodents, purchased from B.K. Universal, Sant Vicenc dels Horts, Spain) and water were given *ad libitum* until rats were sacrificed at the end of the study. All the experimental procedures were performed in conformity with *The Guiding Principles for Research Involving Animals* (24).

Induction of liver cirrhosis

Liver cirrhosis was induced in 24 rats by intraperitoneal injection of carbon tetrachloride (CCl_4, Merck, Darmstadt, Germany). The organic solvent was mixed with mineral oil (1:1) and administered twice a week at doses of 0.4 ml/100 g bw, for 8 weeks (25,26).

Experimental period

Ten days after the last dose of the toxic agent had been administered, CCl_4-treated animals were randomly divided into two groups: group CI+IGF ($n=12$) which received rhIGF-I (2 μg/100 g bw$\times$day, subcutaneously, in two divided doses) for 21 days; and group CI ($n=12$) which received vehicle (saline) following the same schedule. Twelve age-matched healthy rats maintained under the same conditions during the period of the study, excluding CCl_4 administration, were used as the control group (CO). These rats were given saline, as in group CI. The day before starting the administration of IGF-I or placebo, body weight was recorded and blood samples were obtained from the retro-ocular venous plexus with capillary tubes (70 mm, Marienfeld, Germany). Serum was stored at -20°C for analytical determinations. Animals were housed in individual wire-bottomed metabolic cages during the last week of the experimental period and 24-h urine (day 21) was collected and stored at -20° C for further analysis.

On the 22nd day, animals were sacrificed by decapitation. Immediately thereafter, liver, femurs and tibias from all animals were carefully dissected out and weighed. Samples of both the left major liver lobe and the tibia were then excised and processed (fixed in Bouin solution) for histological examination. Tissue specimens were immediately frozen by immersion in liquid N_2 and stored at -80°C until analysis. Plasma and sera were stored at -20°C.

Histological liver sections (4 μm), stained with Masson's trichrome, were used for histopathological confirmation of liver cirrhosis. The only requirement for the retrospective inclusion of rats from groups CI and CI+IGF for data analysis was the existence of fibrous septa delimiting full-shaped regenerative nodules with either micronodular or mixed micro-macronodular pattern (established liver cirrhosis).

Methods

Bone morphometry, densitometry and X-ray studies, as well as analysis of the biochemical composition of bone were performed in the femur, while histopathological-morphometric studies were done in the tibia.

Morphological parameters and densitometry of bone

Femur length was measured from the major trochanter to the end of the distal epiphysis. Anteroposterior (AP) and lateromedial (LM) diameters were assessed at the mid-point of the medial diaphysis. All measurements were performed with a precision calliper, Mituyoto® (±0.05 mm).

Bone density was determined by dual-photon absorptiometry using a DP-3 instrument (DXA-HOLOGIC QDR 1000™, Hologic, INC, Waltham, MA, USA). Measurement stability was controlled by scanning a phantom every time. Femur densitometries were performed at environment temperature. Bones were placed on a polyester resin cell, nonopaque at X-ray. An *Ultra High Reach Program* (NEC APC IV, Power Mate 2 Boxboro, MA, USA) was used for processing the data. Bone density results were expressed as g/cm^2 (27).

Histopathological studies in bone

Histopathological analysis was carried out in tibias. After demineralization, tibias were cut longitudinally and placed in paraffin. Longitudinal sections (4 μm) were stained with Masson's trichrome and H&E stains. Morphometrical measurements were made at three points of diaphysis from each bone, using a light projection microscope (Micro Promar Leitz GMBH, Wetzlar, Germany).

X-ray studies

Radiographs were performed on dissected bones, which were carefully aligned (anteposterior and lateral

positions successively) on Min-R™, E, MRE-1 chassis (10×24 cm) (Kodak Diagnostic Film, Windsor, Colorado, USA), using a tube of X-rays (Siemens, München, Germany), coupled to a Polumat-PC computer, 100/60 with Farb-Monitor MCM 1404 (Siemens, Nixdorf, China). Bones on the cassete were located 1.15 cm from the X-ray tube, and to obtain a higher magnification they were elevated to 90 cm.

Analytical methods in bone tissue
Bone hydroxyproline was determined in whole bone tissue according to the method of Woessner (28) and it was expressed in mg/100 mg dry bone tissue. Total bone calcium content was assessed by atomic absorption spectrophotometry (29) and total bone phosphate by the colorimetric reaction of acidic ammonium molybdate, respectively (29). In both determinations 200 mg of bone tissue were used. Femurs were calcinated and then dissolved in either 0.1% lanthane chloride for calcium assessment or bidistilled water for phosphate determination. Results were expressed in mg/100 mg of dry bone. All determinations were made in triplicate.

Total collagen and hydroxyapatite can be indirectly assessed from hydroxyproline and phosphate values, respectively (30). Total nitrogen content was assessed by Kjeldalh's method in triplicate, and $<5\%$ intraassay variation was considered to be acceptable. Protein content was calculated from these data, using $\times 6.25$ as constant (31).

Lipid peroxidation in liver and bone
Malondialdehyde (MDA), measured after heating samples at 45° C for 60 min in acid medium, was used as an index of membrane lipid peroxidation (32). This product was quantitated by colorimetric assay in liver and bone homogenates, using a novel chromogenic reagent (LPO-586, Bioxytech®, OXIS International, France) which, after reacting with MDA, generates a stable chromophore that was measured spectrophotometrically at 586 nm.

Analytical methods in sera and urine
Basic biochemical parameters were determined in sera obtained on the day before beginning treatment (day

TABLE 1

Baseline biochemical data and body weight the day before the treatment period in the three experimental groups

	Healthy control group (CO, $n=12$)	Cirrhotic group (CI, $n=12$)	Cirrhotic group before IGF-I treatment (CI+IGF, $n=12$)
Body weight (g)	501.4±10	403±10.8***	401.8±10.5***
AST (IU/l)	42±2	443±74***	440±63***
ALT (IU/l)	29±2	360±52***	348±47***
Glycemia (mg/dl)	193±14	174±7	171±6
Albumin (g/dl)	3.3±0.1	2.8±0.1**	2.8±0.1**
Total proteins (g/dl)	6.4±0.2	4.9±0.4**	4.9±0.3**
Cholesterol (mg/dl)	92±4.1	105±4.4*	106±4.2*
Alkaline phosphatase (IU/l)	167±14	242±24*	235±22*
Bilirubin (mg/dl)	0.59±0.02	0.67±0.03*	0.66±0.02*

Values are mean±SEM. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs Control (CO); AST: aspartate aminotransferase; ALT: alanine aminotransferase.

TABLE 2

Bone morphometric and densitometric data in the three experimental groups at the end of the treatment period (21 days)

		Healthy controls (CO, $n=12$)	Cirrhotic rats (CI, $n=12$)	IGF-I-treated cirrhotic rats (CI+IGF, $n=12$)
Body weight (g)		520.83±11.44	383.42±16.29***	373.73±13.01***
Femur	Bone weight (g)	1.25±0.03	1.07±0.03**	1.15±0.04
	(/100 g bw)	0.24±5.41^{-3}	0.28±0.17**	0.31±7.76^{-3}***
	Length (mm)	40.3±0.34	39.24±0.26*	39.2±0.38*
	External-AP diameter (mm)	3.50±0.05	3.52±0.09	3.53±0.09
	External-LM diameter (mm)	4.56±0.10	4.64±0.08	4.65±0.08
	Densitometry (g/cm²):			
	•Total densitometry	0.34±3.4×10^{-3}	0.31±3.1×10^{-3}**	0.33±5.3×10^{-3}*#
	•Distal epiphysis	0.42±6.3×10^{-3}	0.37±3.1×10^{-3}***	0.38±6.3×10^{-3}**
	•Proximal epiphysis	0.35±3.6×10^{-3}	0.30±3.5×10^{-3}***	0.33±3.5×10^{-3}#
	•Diaphysis	0.31±2.2×10^{-3}	0.28±3.0×10^{-3}***	0.29±5.5×10^{-3}*

*$p<0.05$, **$p<0.01$, ***$p<0.001$ vs CO; #$p<0.05$ CI+IGF vs CI.

Fig. 1. Decrement in femur weight in cirrhotic rats receiving either IGF-I (CI+IGF) or placebo (CI) for 3 weeks. Values are expressed as mean±SEM and represent percent decrement with respect to mean values of the control group (CO) taken as 100%. n=12 in each group.
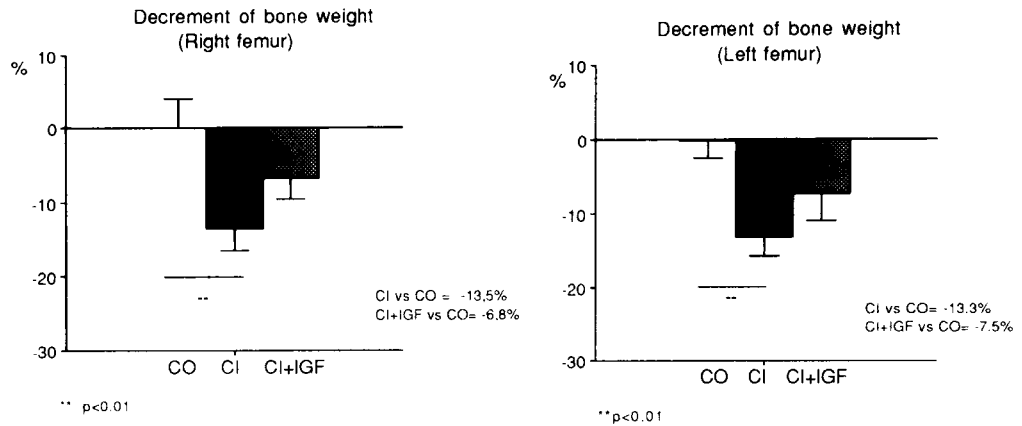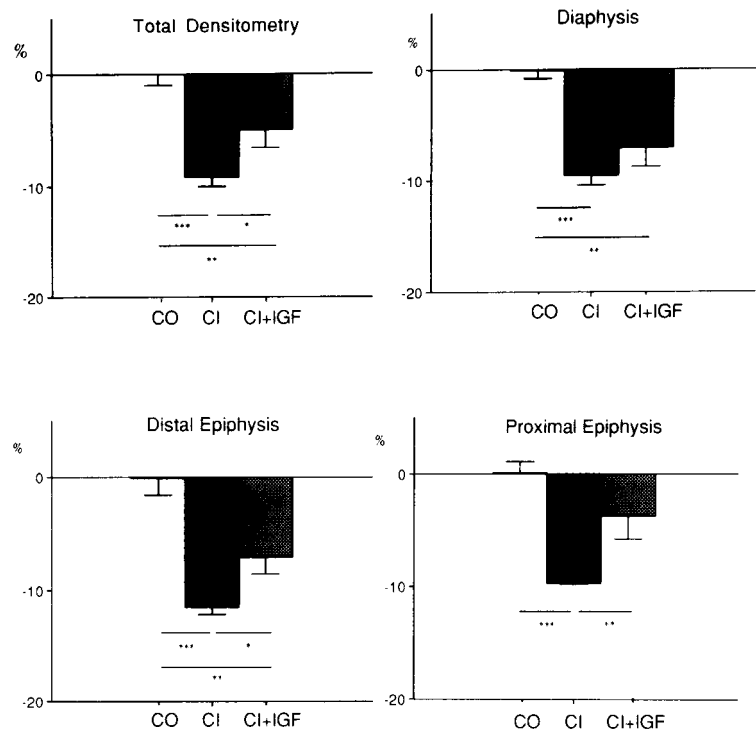

Fig. 2. Decrement in femur densitometry in cirrhotic rats receiving either IGF-I (CI+IGF-I) or placebo (CI) for 3 weeks. Values are expressed as mean±SEM and represent percent decrement with respect to mean values of the control group (CO) taken as 100%. n=12 in each group.

0) from all animals. Serum levels of total proteins, albumin, glucose, cholesterol, bilirubin, alkaline phosphatase and aminotransferases (AST and ALT) were determined by routine laboratory methods using a Hitachi 747 autoanalyzer (Boerhringer-Mannheim, Germany). Urinary deoxypyridinoline cross-links were assessed using a commercial kit from Metra Biosystems, Inc (Mountain View, CA, USA). Values were expressed as nM/mM of creatinine.

Statistical analysis
Data are expressed as mean±SEM. Non-parametric tests were used for sample size: Kruskall-Wallis analy-

sis was used for simultaneous comparisons of the three groups, and the group-to group significance was evaluated by an *a posteriori* Mann-Whitney test. Statistical significance was accepted at $p<0.05$. Calculations were performed with SPSSWin v.6.0. Program PC.

Results
Diagnosis of liver cirrhosis and baseline biochemical data
The existence of liver cirrhosis was verified histopathologically in all rats from the CI and CI+IGF groups at the end of the study. Therefore, no animal was excluded from evaluation of the results.

A

AP radiograph of femur

Control   Cirrhotic   IGF-Treated
rat   cirrhotic rat

(x 1.28 magnification)

B

Lateral radiograph of femur

Control   Cirrhotic   IGF-Treated
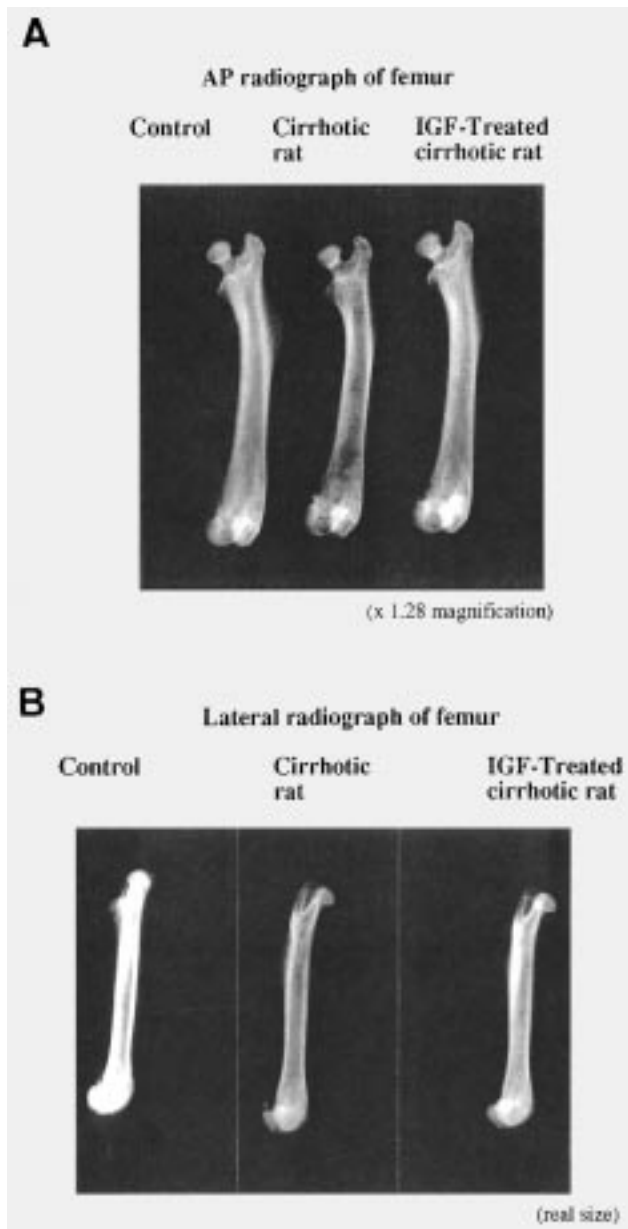rat   cirrhotic rat

(real size)

Fig. 3. Demonstrative anterior-posterior (3A) and lateral (3B) radiographs of the femur in a healthy control rat, untreated cirrhotic rat and cirrhotic rat treated with IGF-1. The thickness of cortical and bone density are diminished in CI as compared with CO. Intermediate bone density is observed in CI+IGF.

The day before starting the experimental period, the two groups of cirrhotic rats showed, as compared with control animals, significantly lower body weight and significantly abnormal serum levels of aminotransferases, albumin, total proteins, cholesterol, alkaline phosphatase and bilirubin (Table 1). The extent of these alterations was the same in the two cirrhotic groups, thus indicating the absence of differences in the severity of liver disease and malnutrition at baseline.

Bone morphometric, densitometric and radiological studies

Table 2 summarizes morphometric and densitometric data in the three groups of animals. At the end of the study, femur weight was significantly reduced, as compared with controls, in CI rats but not in CI+IGF animals. However, if bone weight is expressed as mg/100 g of rat body weight, control rats exhibited significantly lower values than both groups of cirrhotic rats, due to the marked deficiency in body weight observed in cirrhotic animals (Table 2).

Femur length was minimally but significantly reduced in both cirrhotic groups as compared with controls, while no significant differences were observed in antero-posterior and latero-medial diameters between groups (Table 2).

Densitometry studies (Table 2) demonstrated a significant reduction in bone mass of the whole femur and of its distal epiphysis in both CI and CI+IGF groups as compared with control animals. However, in these locations, bone mass was significantly lower in nontreated than in IGF-I treated cirrhotic rats. At the proximal epiphysis of the femur, only CI rats showed significantly decreased densitometry, whereas rats treated with IGF-I exhibited values similar to those of controls and significantly higher than those found in the CI group. Mean densitometric values of the entire femur were significantly lower in the two groups of cirrhotic animals than in controls; however, IGF-I treated animals showed significantly higher densitometry values than untreated cirrhotic rats (Table 2 and Fig. 2).

Figures 1 and 2 show the comparison between

TABLE 3

Bone composition in the three experimental groups at the end of the treatment period (21 days)

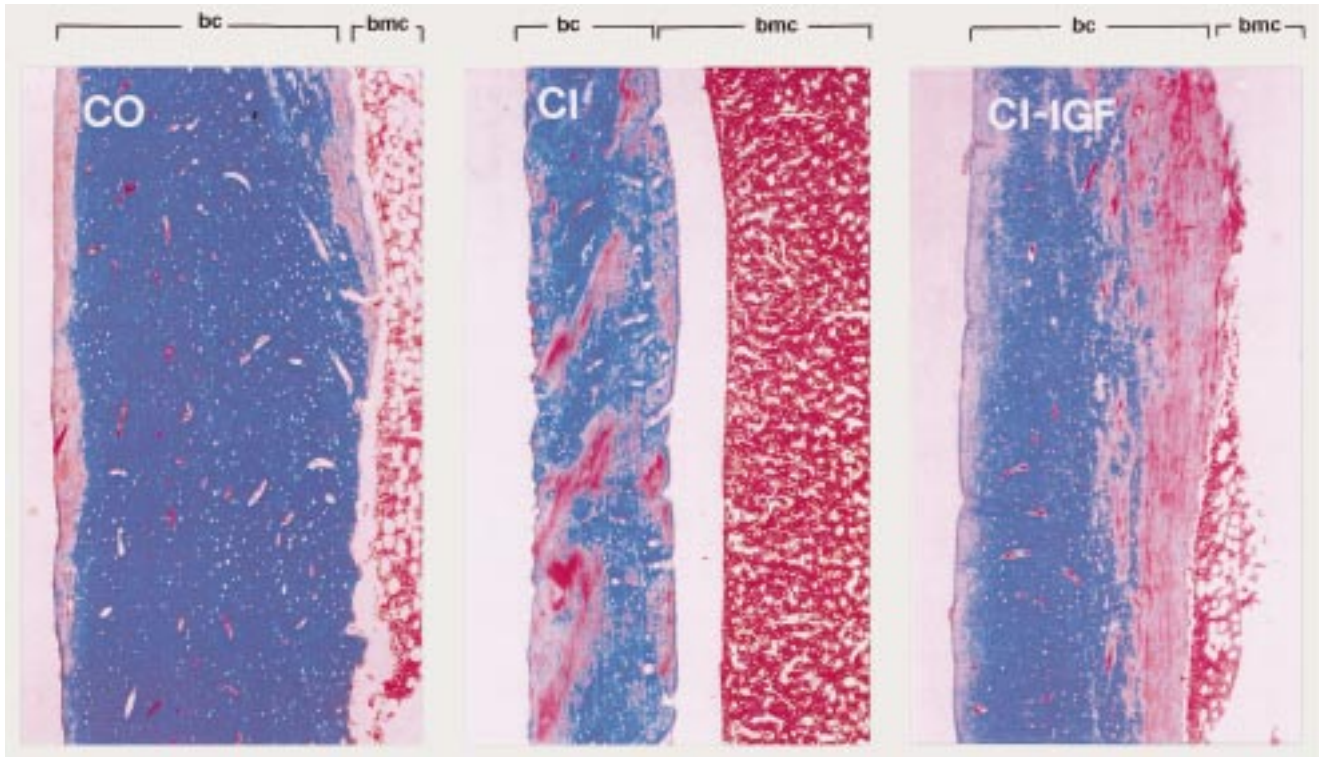|	Healthy controls (CO, *n*=12)	Cirrhotic rats (CI, *n*=12)	IGF-I-treated cirrhotic rats (CI+IGF, *n*=12)
Bone components	(% of dry bone tissue)	(% of dry bone tissue)	(% of dry bone tissue)
Hydroxyapatite	26.84±3.25	28.40±2.96	25.62±2.30
Calcium	26.52±1.02	30.50±2.55	26.56±2.46
Total proteins	47.14±4.51	42.24±0.64	44.99±2.42
Collagen	24.15±1.43	26.27±1.90	28.08±1.80

Fig. 4. In each panel longitudinal sections of the diaphysis of the tibia show bone cortical (bc) and bone marrow cavity (bmc) (×150 magnification) stained with Masson's trichrome in control rat (CO), untreated cirrhotic rat (CI) and cirrhotic rat treated with IGF-I (CI+IGF). Decreased cortical thickness can be seen in CI animals, while increased newly formed osteoid tissue (pink colour) in the inner surface of the bone cortical can be observed in CI+IGF.

groups CI and CI+IGF regarding femoral weight (Fig. 1) and densitometry (Fig. 2) as percent decrement with respect to mean values of the control group (considering control values as 100%). As shown in Fig. 1, a significant decrement of bone weight was observed in CI rats while CI+IGF animals exhibited non-significant changes. With respect to densitometric values (Fig. 2), IGF-I-treated cirrhotic rats showed significantly less decrement than nontreated cirrhotic rats, with the exception of bone diaphysis where differences did not reach statistical significance.

Demonstrative X-ray films in Fig. 3 show that cortical femoral density was lower in CI animals than in control rats, whereas in the CI+IGF group bone density was intermediate between the other two groups. Although X-ray images cannot replace quantitative measurements, they are useful to illustrate bone architecture in the three experimental groups (see below).

Bone composition
There were no significant differences among groups regarding bone content of hydroxyapatite, calcium, total proteins and collagen, expressed as percent of dry bone weight. As a result, the protein to mineral ratio of bone was similar in the three groups (Table 3).

Bone histopathology
Bone histopathology (Fig. 4) was evaluated in longitudinal sections of the diaphysis of the tibia. Cortical bone thickness was significantly diminished in untreated cirrhotic rats (0.707 ± 0.034 mm; $n=12$) as compared with both controls (1.070 ± 0.045 mm, $n=12$) and cirrhotic rats treated with IGF-I (0.950 ± 0.095 mm, $n=12$) ($p < 0.001$ for both comparisons). Although cortical thickness was greater in this last group of animals than in CI rats, it remained below normal values (CI+IGF vs CO $p < 0.05$).

Urinary excretion of deoxypyridinoline cross-links
The urinary excretion of deoxypyridinoline cross-links, the most reliable marker of bone resorption, was significantly increased in nontreated cirrhotic rats as compared with both CO animals and IGF-I-treated cirrhotic rats (Fig. 5). Values in these last two groups were
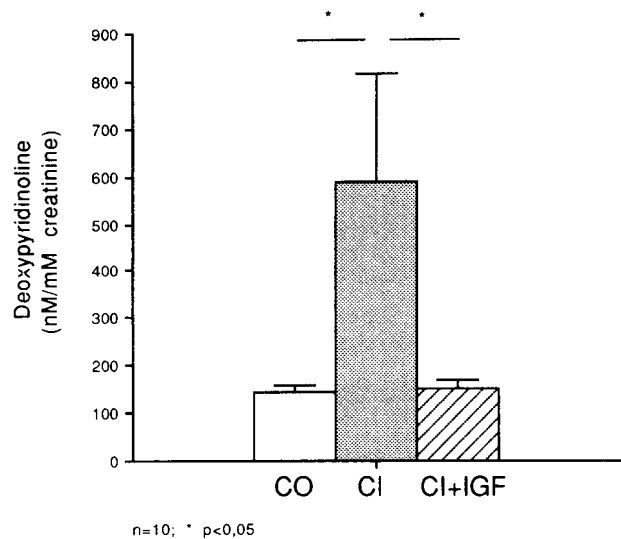
Fig. 5. Urinary excretion of deoxypyridinoline, in control rats (CO), untreated cirrhotic rats (CI) and IGF-I treated cirrhotic rats (CI+IGF). Values are mean±SEM, n=12 in each group.

similar, suggesting that the accelerated bone resorption occurring in cirrhotic rats had been halted in the IGF-I-treated group.

Malondialdehyde (MDA) in liver and bone homogenates

Since lipid peroxidation is a major mechanism of CCl_4-induced tissue damage, MDA was determined in liver and bone homogenates from control and CCl_4-treated rats to assess whether lipid peroxidation could be involved in bone changes observed in cirrhotic animals. Tissue MDA was significantly increased in liver homogenates from CCl_4-treated rats (70.85±6.53 nmol/g tissue, $p<0.01$) as compared with controls (42.60±3.37), but products of lipid peroxidation were undetectable in bone homogenates from control and cirrhotic animals (<0.25 nmol/g tissue, which is the limit of detection of the assay).

Discussion

The present study has demonstrated that osteopenia occurs in experimental liver cirrhosis and that this bone disturbance can be partially corrected by a short-term course of IGF-I at low doses.

In our study osteopenia in cirrhotic rats was characterized by reduced bone weight and total bone mass with preserved bone composition. Changes in both cortical and cancellous bone account for these deficiencies, as shown by histopathological and densitometric data. This scenario closely resembles the type of osteopenia

which occurs in patients with chronic parenchymal liver disease (11–13,16–18) in whom osteoporosis constitutes the commonest associated bone disorder. Thus our animal model might be useful to study the osteopenia which develops in patients with cirrhosis.

Although bone weight was significantly reduced in CI rats as compared with controls, when values were normalized by body weight the ratio was higher in CI rats than in controls. This finding indicates that the malnutrition which occurs in cirrhotic rats has a greater impact on striated muscle and fat than on bone. In fact, reductions of fat stores and muscle mass have been reported to be the main contributors to weight loss in clinical malnutrition (33–36), and we have recently shown that significant muscle wasting occurs early in the course of experimental liver cirrhosis (37).

In the present study the usual technique of cirrhosis induction by CCl_4 exposure was modified in two ways: 1) CCl_4 was given intraperitoneally, instead of by inhalation, in an attempt to minimize extrahepatic damage (25,26); and 2) phenobarbital, usually added to the drinking water to potentiate CCl_4 toxicity (38), was not used in order to avoid changes in bone metabolism and osteopenia induced by barbiturates (38–41). However, a question arises as to whether direct toxicity of CCl_4 to bone could contribute to osteopenia. This possibility has been reasonably ruled out by showing that MDA, a marker of lipid peroxidation, was undetectable in bone homogenates from both controls and CCl_4-treated rats, suggesting that lipid peroxidation does not occur in bone under the experimental conditions used in this study. These findings are in keeping with the absence in bone of cytochrome P450 (42), an enzymatic activity which is crucial for initiating CCl_4 toxicity (39).

The mechanisms responsible for osteopenia in our experimental model of liver cirrhosis cannot be fully ascertained from present data. It seems possible that osteopenia might be related, at least in part, to malnutrition (19,35,36). On the other hand, the marked increase in deoxypyridinoline cross-links in untreated cirrhotic rats indicates enhanced bone resorption and osteoclastic activity. Under these conditions the maintenance of bone mass would require a compensatory increase in osteoblastic function. However, histopathological data suggest that new bone formation is not increased in the cirrhotic group of animals. Since IGF-I is an important anabolic hormone for bone formation, decreased bioavailability of this hormone may play a role in the osteopenia accompanying liver cirrhosis.

There are two sources of IGF-I acting on bone: the growth hormone-dependent IGF-I, produced by the

liver, which circulates bound to IGF-I binding proteins (IGFBP′s), and the locally produced IGF-I, arising from osteoblasts in response to growth hormone and other signals (2,3,6,43). This locally produced IGF-I is also bound to IGFBP′s and acts as autocrine or paracrine messenger (43). No information is available concerning this form of IGF-I in liver cirrhosis. Regarding hepatic IGF-I, its levels are preserved or moderately low in early stages of cirrhosis and markedly reduced in advanced disease (22,23,37).

In the present study rhIGF-I was able to attenuate the decrease in bone weight and densitometry observed in CI rats, restoring densitometric values to near those found in normal animals. The changes in cortical density of the femur induced by IGF-I could be perceived even visually on the X-rays films of bones after denudation of surrounding soft tissues. Serum markers of osteoblastic activity were not measured in the present work; however, our histopathological findings suggest an increased bone-forming activity in the tibia in CI+IGF as compared with CI rats. This is in agreement with the known effects of IGF-I on bone (2–6,8–10). *In vitro,* IGF-I enhances bone collagen and matrix synthesis and stimulates the replication of cells of the osteoblast lineage (2,3,5). In addition, IGF-I lowers the transcription of the collagen-degrading protease, MMP-13, thus inhibiting bone collagen degradation (44). Although the effect of IGF-I on bone resorption is controversial, our observation that deoxypyridinoline cross-links returned to normal in IGF-I-treated animals suggests that the hormone may reduce bone resorption in cirrhotic animals. This is in agreement with recent *in vitro* studies using bone organ cultures, which indicate that IGF-I has an inhibitory effect on stimulated bone resorption by inhibiting osteoclasts or by interfering with ostoblast-derived factors which stimulate osteoclast activity (45).

Different studies have shown an improvement in osteopenia or bone turnover in response to a wide range of IGF-I doses (2,3,4,8–10,46,47). Interestingly, a recent study in elderly women demonstrated that both GH and high doses of IGF-I enhanced both bone formation and resorption, while a low dose of IGF-I, similar to that used in our work, increased bone formation but caused no changes in parameters of bone resorption (46). It should be considered that side effects of IGF-I may occur when high doses of IGF-I are used. In the present work the dose was chosen after preliminary studies demonstrating that it did not induce hypoglycemia in cirrhotic rats. Hypoglycemia was avoided in order to fully preserve the anabolic effects of IGF-I since the systemic anabolic activity of this hormone could be important in restoring bone metab-

olism in individuals with cirrhosis. It seems possible that the positive effects of low doses of rhIGF-I on nutritional state, intestinal absorption and liver function, which have been previously demonstrated in rats with liver cirrhosis (37,48,49) might have contributed to improvement of bone metabolism.

In summary, rats with CCl_4-induced liver cirrhosis exhibit osteopenia of osteoporosis-type. A short treatment course with low doses of IGF-I increases bone weight and density, probably by inhibiting bone protein degradation and inducing bone formation. Since in patients with liver cirrhosis osteopenia constitutes a source of added morbidity, which, at present, cannot be treated effectively, IGF-I should be considered as a possible therapeutic approach for this complication.

References

1. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16: 3–34.
2. Mohan S, Baylink DJ. Autocrine-paracrine aspects of bone metabolism. Growth Genet Horm 1990; 6: 1–9.
3. Eriksen EF, Kassem M, Langdahl B. Growth hormone, insulin-like growth factors and bone remodelling. Eur J Clin Invest 1996; 26: 525–34.
4. Rosen CJ, Donahue LR, Hunter SJ. Insulin-like growth factors and bone: the osteoporosis connection. PSEBN 1994; 206: 83–102.
5. Slootweg MC, Hoogerbrugge CM, de Poorter TL, Duursma SA, Van Buul Offers SC. The presence of classical insulin-like growth factor (IGF) type-I and -II receptors on mouse osteoblasts: autocrine/paracrine growth effect of IGFs? J Endocrinol 1990; 125: 271–7.
6. Bautista CM, Mohan S, Baylink DJ. Insulin-like growth factors I and II are present in the skeletal tissues of ten vertebrates. Metabolism 1990; 39: 96–100.
7. Fiorelli G, Orlando C, Benvenuti S. Characterization, regula-

tion, and function of specific cell membrane receptors for insulin-like growth factor I on bone endothelial cells. J Bone Miner Res 1994; 9: 329–37.

8. Grinspoon AK, Baum HBA, Peterson A, Klibanski A. Effects of rhIGF-I administration on bone turnover during short-term fasting. J Clin Invest 1995; 96: 900–6.

9. Ebeling PR, Jones JD, O'Fallon WM, Janes CH, Riggs BL. Short-term effects of recombinant human insulin-like growth factor-I on bone turnover in normal women. J Clin Endocrinol Metab 1993; 77: 1384–7.

10. Johansson AG, Lindh E, Ljunghall S. IGF-I stimulates bone turnover in osteoporosis. Lancet 1992; 339: 1619.

11. Diamont T, Stiel D, Lunzer M, Wilkinson M, Roche J, Posen S. Osteoporosis and skeletal fractures in chronic liver disease. Gut 1990; 31: 82–7.

12. Quiroga J, Beloqui O, Castilla A. Liver cirrhosis. In: Prieto J, Rodés J, Shafritz, editors. Hepatobiliary Disease. Berlin, Heidelberg: Springer-Verlag; 1992. p. 323–415.

13. Bonkovsky HL, Hawkins M, Steinberg K, Herrsh T, Galambos JT, Henderson JM, et al. Prevalence and prediction of osteopenia in chronic liver disease. Hepatology 1990; 12: 273–80.

14. Hodgson SF, Dickson ER, Wahner HW, Johnson KA, Mann KG, Riggs BL. Bone loss and reduced osteoblast function in primary biliary cirrhosis. Ann Intern Med 1985; 103: 855–60.

15. Pimstone NR, Goldstein LI, Ward R, Ruebner BH. Liver transplantation. In: Zakim, Boyer, editors. Hepatology: a Textbook of Liver Disease, Vol. 2, 2nd ed. Philadelphia: W.B.Saunders Company; 1990. p. 791–821.

16. Stellon AJ, Davies A, Compston J, Williams R. Osteoporosis in chronic cholestatic liver disease. Q J Med 1985; 57: 783–90.

17. Bikle DD, Genant HK, Cann C, Recker RR, Halloran BP, Strewler GJ. Bone disease in alcohol abuse. Ann Intern Med 1985; 103: 42.

18. Hay JE. Bone disease in cholestatic liver disease. Gastroenterology 1995; 108: 276–83.

19. Heaney RP. Nutrition and osteoporosis. In: Favus JF editor. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 3rd ed. Philadelphia; Lippincott-Raven; 1996. p. 262.

20. Compston JE. Hepatic osteodystrophy: vitamin D metabolism in patients with liver disease. Gut 1986; 27: 1073.

21. Stellon AJ, Webb A, Compston J, Williams R. Low bone turnover state in primary biliary cirrhosis. Hepatology 1987; 7: 137.

22. Schimpf RM, Lebrec D, Donadieu M. Somatomedin production in normal adults and cirrhotic patients. Acta Endocrinol 1977; 86: 355–62.

23. Hattori N, Kurahachi H, Ikekubo K, Ishihara T, Moridera K, Hino M, et al. Serum growth hormone-binding protein, insulin-like growth factor-I, and growth hormone in patients with liver cirrhosis. Metab Clin Exp 1992; 41: 377–81.

24. The Guiding Principles for Research Involving Animals. National Academy of Sciences and published by the National Institute of Health (NIH), revised in 1991.

25. Pérez Tamayo R. Is cirrhosis of liver experimentally produced by CCl$_4$ an adequate model of human cirrhosis? Hepatology 1983; 3: 112–20.

26. Ariosto F, Riggio O, Cantafora A, Colucci S, Gaudio E, Machelli C, et al. Carbon tetrachloride-induced experimental cirrhosis in the rat. A reappraisal of the model. Eur Surg Res 1989; 21: 280–6.

27. Ostlere SJ, Gold RH. Osteoporosis and bone density measurements methods. Clin Orth 1991; 271: 149–63.

28. Woessner JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this aminoacid. Arch Biochem Biophys 1961; 93: 440–47.

29. Willis JB. The determination of calcium in blood serum by atomic absorption spectrophotometry. Spectrochim Acta 1960; 16: 259–64.

30. Robyt JF, White BJ. Biochemical techniques, theory and practice. Monterey, California: Brooks and Cole Publishing Company; 1987.

31. Wuzke A. A century of Kjeldahl's nitrogen determination. Z Med Lab Diagn 1985; 26: 383–7.

32. Esterbauer H, Schaur RJ, Zollner J. Chemistry and biochemistry of 4-hydroxynonemal malondialdehyde and related aldehydes. Free Radic Biol Med 1991; 11: 82–128.

33. McCullough AJ, Tavill AS. Disordered energy and protein metabolism in liver disease. Semin Liver Dis 1991; 11: 265–77.

34. Romijn JA, Endert E, Sauerwein HP. Glucose and fat metabolism during short-term starvation in cirrhosis. Gastroenterology 1991; 100: 731–7.

35. Müller MJ, Lautz HU, Plogmann B, Bürger M, Körber J, Schmidt FW. Energy expenditure and substrate oxidation in patients with cirrhosis: the impact of causes, clinical staging and nutritional state. Hepatology 1992; 15: 782–94.

36. Mezey E. Liver disease and nutrition. Gastroenterology 1978; 74: 770–83.

37. Picardi A, Costa de Oliveira A, Muguerza B, Tosar A, Quiroga J, Castilla-Cortázar I, et al. Low doses of insulin-like growth factor-I improve nitrogen retention and food efficiency in rats with early cirrhosis. J Hepatol 1996; 24: 267–79.

38. Muguerza B, Lecaroz C, Picardi A, Castilla-Cortázar I, Quiroga J, Cemborain A, et al. A potential experimental model for the study of osteopenia in liver cirrhotic rats. J Physiol Biochem 1996; 52: 113–29.

39. Gitlin N. Clinical aspects of liver diseases caused by industrial and environmental toxins. In: Zakim, Boyer, editors. Hepatology a Textbook of Liver Disease, Vol. 2, 2nd ed. Philadelphia: WB Saunders Company, 1990: 791–821.

40. Guichot-García MR, Lluch-Fernández MD, Ramos-Sánchez I. Evaluation of bone mass by dual-photon absorptiometry in children under proled anticonvulsant therapy. An Esp Pediatr 1992; 37: 109–13.

41. Teodore JH. Steroid and drug-induced osteopenia. In: Murray JF, editor. Primer on the Metabolic Bone Diseases and Disorders on Mineral Metabolism, 2nd ed. New York: Raven Press; 1993. p. 250–4.

42. Lewis DFV. Cytochromes P-450. Structure, Function and Mechanism. Bristol: Tailor & Francis Ltd; 1996.

43. Canalis E. Regulation of Bone Remodeling. In: Favus JF, editor. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 3rd ed. Philadelphia: Lippincott-Raven, 1996. p. 29–34.

44. Canalis E, Rydziel S, Delany A, Varghese S, Jeffrey J. Insulin-like growth factors inhibit interstitial collagenase synthesis in bone cell cultures. Endocrinology 1955; 136: 1348–54.

45. Jonsson KB, Wiberg K, Ljunghall S, Ljunggren O. Insulin-like growth factor I does not stimulate bone resorption in cultured neonatal mouse calvarial bones. Calcif Tissue Int 1996; 59: 366–70.

46. Ghiron LJ, Thompson JL, Holloway L, Hintz RL, But-

terfield GE, Hoffman AR, et al. Effects of recombinant insulin-like growth factor I and growth hormone on bone turnover in elderly women. J Bone Miner Res 1995; 10: 1844–52.

47. Bagi CM, Brommage R, Deleon L, Adams S, Rosen D, Sommer A. Benefit of systemically administered rhIGF-I and rhIGF-I/IGFBP-3 on cancellous bone in ovarectomized rats. J Bone Miner Res 1994; 9: 1301–12.

48. Castilla-Cortázar I, García M, Muguerza B, Perez R, Quiro-ga J, Santidrián S, et al. Hepatoprotective effects of insulin-like growth factor-I in rats with carbon tetrachloride-induced cirrhosis. Gastroenterology 1997 (in press).

49. Castilla-Cortázar I, Prieto J, Urdaneta E, Pascual M, Nuñez M, Zudaire E, et al. Impaired intestinal sugar transport in cirrhotic rats: correction by low doses of insulin-like growth factor I. Gastroenterology 1997 (in press).