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

Arantxa Cemborain¹, Inma Castilla-Cortázar¹, María García¹, Jorge Quiroga², Begoña Muguerza¹, Antonio Picardi¹,³, Santiago Santidrián¹ and Jesús Prieto²

Departments of ¹Human Physiology, ²Internal Medicine and Liver Unit, School of Medicine, University of Navarra, Pamplona, Spain, and ³Internal 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 CCl₄-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 CCl₄-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.
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 CCl₄-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 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 (CCl₄, 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, CCl₄-treated animals were randomly divided into two groups: group CI+IGF (n=12) which received rhIGF-I (2 µg/100 g bw×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 CCl₄ 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 N₂ 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 midpoint 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-HOL-OGIC QDR 1000TM, 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/cm² (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 (anteroposterior 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 cassette 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 molynbdate, respectively (29). In both determinations 200 mg of bone tissue were used. Femurs were calcinated by the colorimetric reaction of acidic ammonium molynbdate for phosphate and then dissolved in either 0.1% lanthane chloride for yttrate, 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 ×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

<table>
<thead>
<tr>
<th></th>
<th>Healthy control group (CO, n=12)</th>
<th>Cirrhotic group (CI, n=12)</th>
<th>Cirrhotic group before IGF-I treatment (CI+IGF, n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>501.4±10</td>
<td>403±10.8***</td>
<td>401.8±10.5***</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>42±2</td>
<td>360±52***</td>
<td>348±47***</td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>193±14</td>
<td>174±7</td>
<td>171±6</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.3±0.1</td>
<td>2.8±0.1**</td>
<td>2.8±0.1**</td>
</tr>
<tr>
<td>Total proteins (g/dl)</td>
<td>6.4±0.2</td>
<td>4.9±0.4**</td>
<td>4.9±0.3**</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>92±4.1</td>
<td>105±4.4*</td>
<td>106±4.2*</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td>167±14</td>
<td>242±24*</td>
<td>235±22*</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.39±0.02</td>
<td>0.67±0.03*</td>
<td>0.66±0.02*</td>
</tr>
</tbody>
</table>

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

### TABLE 2

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

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (CO, n=12)</th>
<th>Cirrhotic rats (CI, n=12)</th>
<th>IGF-I-treated cirrhotic rats (CI+IGF, n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>520.83±11.44</td>
<td>383.42±16.29***</td>
<td>373.73±13.01***</td>
</tr>
<tr>
<td>Femur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone weight (g)</td>
<td>1.25±0.03</td>
<td>1.07±0.03**</td>
<td>1.15±0.04</td>
</tr>
<tr>
<td>(/100 g bw)</td>
<td>0.24±0.51*</td>
<td>0.28±0.17**</td>
<td>0.31±0.76***</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>40.3±3.34</td>
<td>39.24±0.26*</td>
<td>39.2±0.38*</td>
</tr>
<tr>
<td>External-AP diameter (mm)</td>
<td>3.50±0.05</td>
<td>3.52±0.09</td>
<td>3.53±0.09</td>
</tr>
<tr>
<td>External-LM diameter (mm)</td>
<td>4.56±0.10</td>
<td>4.64±0.08</td>
<td>4.65±0.08</td>
</tr>
<tr>
<td>Densitometry (g/cm²):</td>
<td>0.34±3.4×10⁻³</td>
<td>0.31±3.1×10⁻³***</td>
<td>0.33±5.3×10⁻³##</td>
</tr>
<tr>
<td>Total densitometry</td>
<td>0.42±6.3×10⁻³</td>
<td>0.37±3.1×10⁻³***</td>
<td>0.38±6.3×10⁻³##</td>
</tr>
<tr>
<td>Distal epiphysis</td>
<td>0.35±3.6×10⁻³</td>
<td>0.30±3.5×10⁻³***</td>
<td>0.33±5.5×10⁻³##</td>
</tr>
<tr>
<td>Proximal epiphysis</td>
<td>0.31±2.2×10⁻³</td>
<td>0.28±3.0×10⁻³***</td>
<td>0.29±5.5×10⁻³##</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001 vs CO; #p<0.05 CI+IGF vs CI.
Osteopenia and IGF-I in experimental liver cirrhosis

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 (Boehringer-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 analysis 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.
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)

<table>
<thead>
<tr>
<th>Bone components</th>
<th>Healthy controls (CO, n=12)</th>
<th>Cirrhotic rats (CI, n=12)</th>
<th>IGF-I-treated cirrhotic rats (CI+IGF, n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyapatite</td>
<td>26.84±3.25</td>
<td>28.40±2.96</td>
<td>25.62±2.30</td>
</tr>
<tr>
<td>Calcium</td>
<td>26.52±1.02</td>
<td>30.50±2.55</td>
<td>26.56±2.46</td>
</tr>
<tr>
<td>Total proteins</td>
<td>47.14±4.51</td>
<td>42.24±0.64</td>
<td>44.99±2.42</td>
</tr>
<tr>
<td>Collagen</td>
<td>24.15±1.43</td>
<td>26.27±1.90</td>
<td>28.08±1.80</td>
</tr>
</tbody>
</table>
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 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
A. Cemborain et al.

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 CCL₄-induced tissue damage, MDA was determined in liver and bone homogenates from control and CCL₄-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 CCL₄-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 CCL₄ exposure was modified in two ways: 1) CCL₄ 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 CCL₄ 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 CCL₄ 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 CCL₄-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 CCL₄ 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 metabolism 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 CCl₄-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.

Acknowledgements

We thank Dr Anna Sköttner and Dr Jan Holmberg, Kabi Pharmacia, for providing rhIGF-I used in this study; Mr Javier Guillén for his efficient work in CIFA; and Dr Javier Pardo, Pathology Department of the University of Navarra, for his authorized help in histological classification of liver and bone samples. We thank the technical help of the staff of the Department of Nuclear Medicine, Clinica Universitaria, University of Navarra. We are indebted to Mrs M. Pilar Redín, Isabel Ordoqui and Cristina Chocarro for their secretarial and technical assistance. Finally, we thank Fundación Universitaria de Navarra and Fundación Echávarri for financial help.

Some of these results have been presented at the 31st Annual Meeting of the European Association for the Study of the Liver, Geneva, Switzerland, 26–29 August 1996.

References

7. Fiorelli G, Orlando C, Benvenuti S. Characterization, regula-

