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# Low doses of insulin-like growth factor-I improve nitrogen retention and food efficiency in rats with early cirrhosis

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***Background/Aims:*** **In order to ascertain whether malnutrition is an early-onset feature of liver cirrhosis and whether the anabolic hormone insulin-like growth factor I (IGF-I) could be useful in the treatment of this complication, we analyzed the nutritional alterations present in rats with early-stage liver cirrhosis and the effects of IGF-I on nutritional parameters in these animals.**

***Methods:*** **After a 24 h fast, a $^{15}N$-enriched diet was administered for 5 days to normal control rats and to cirrhotic rats receiving subcutaneous injections of vehicle (Group 1) or IGF-I, 2 μg·100 g bw$^{-1}$·day$^{-1}$, (Group 2) during the 5 experimental days. $^{15}N$, a stable N isotope, was measured in biological samples by mass spectrometry.**

***Results:*** **Compared with control rats, Group 1 animals showed significant reductions in N intake and food efficiency ($p<0.05$, both). In addition, the weight of the gastrocnemius muscle, its total N content and the dietary N content of this muscle were significantly lower in Group 1 than in control animals ($p<0.05$, all). In rats from Group 2, mean values of N intake, food efficiency, gastrocnemius N content and the amount of dietary N incorporated into this muscle were similar to those in control rats, and (with the exception of gastrocnemius N total content) significantly higher than those in non-treated cirrhotic rats ($p<0.05$, all).**

***Conclusions:*** **A variety of nutritional disturbances were detected in rats from the early stages of liver cirrhosis. Low doses of IGF-I were found to reverse most of these changes. These results stimulate further studies to determine whether IGF-I might be useful in the correction of the malnutrition present in patients with liver cirrhosis.**

***Key words:*** **Food efficiency; IGFBP; Insulin-like growth factor-I (IGF-I); Liver cirrhosis; Malnutrition; Nitrogen balance; N intake; Stable isotope $^{15}N$.**

In patients with liver cirrhosis, derangements in intermediary metabolism involving a variety of biochemical pathways (1–5) lead to a progressive deterioration of the nutritional state resembling protein-calorie malnutrition or starvation (4,6–9). This has been consistently observed in both human and experimental cirrhosis, especially in advanced disease, when ascites develops. A host of factors contribute to malnutrition in advanced cirrhosis, including the complications which occur in the course of the disease such as infections or hemorrhages. In fact, the disturbances linking liver damage and impairment of the nutritional status are ill understood. Reduced food intake, impaired digestion and/or absorption of nutrients, overall increased energy expenditure and impaired protein disposal are thought to be some of the factors involved (2).

In contrast with patients with advanced cirrhosis, patients with well compensated cirrhosis rarely exhibit clinical signs of malnutrition (2,5). However, some alterations in intermediary metabolism such as insulin resistance are more prevalent in patients with compensated cirrhosis than in the general population (5,10). It seems possible that the basic mechanisms ultimately leading to protein-calorie malnutrition in highly evolved cirrhosis are already present in the early stages of the disease

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At present, the only effective procedure to correct the protein-calorie malnutrition occurring in patients with cirrhosis is liver transplantation. There are few reports related to the treatment of malnutrition status associated with liver cirrhosis (11). Although no hormonal treatment has been proposed to improve the nutritional status in liver cirrhosis, insulin-like growth factor-I (IGF-I) might be a candidate, since its plasma levels are low in advanced cirrhosis (12) and it has been shown to possess a significant anabolic potential and to be effective in patients with malnutrition due to a variety of conditions (13–17). The effect of IGF-I on the nutritional disturbances of cirrhosis has not yet been explored.

The aim of this study was, first, to investigate whether nutritional alterations are present in the early stages of liver cirrhosis and, second, to test the effect of low doses of IGF-I on the nutritional abnormalities, if any, observed in rats with non-advanced liver cirrhosis.

## Materials and Methods

*Reagents*

Lactalbumin, corn oil, choline bitartrate, sucrose, carbon tetrachloride, and all other chemical substances for analytical purposes were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Luminal® was purchased from Bayer (Leverkusen, Germany), urethane from Fluka Chemie AG (Buchs, Switzerland), salt and vitamin mixtures AIN-76 from Dyets (Bethlehem, PA, USA), and standard non-purified diet for rodents from B.K. Universal (S. Vicenc dels Horts, Spain). Metabolic cages were purchased from Tecniplast Gazzada (Buguggiate, Italy), analytical precision scales Mettler AE 200-S from Mettler Instruments (Zurich, Switzerland), and HPLC equipment Pico-Tag® from Waters Milford (MA, USA). Atomic absorption spectrophotometer 305B was from Perkin-Elmer (Norwalk, CT, USA). $^{15}N$-labeled bean seeds (cultivar Aroana) were supplied by the Centro de Energia Nuclear na Agricultura (C.E.N.A., Piracicaba, SP, Brazil), and $^{125}I$-recombinant human IGF-I by Du Pont NEN® Research Product (Bad Homburg, Germany). Autoradiographic films were purchased from Fuji Medical X-Ray Film, (Tokyo, Japan). Bio-Rad Protein Assay Dye Reagent Concentrate and nitrocellulose filters were purchased from Bio-Rad (Madrid, Spain). Free fatty acids determination kit NEFA-C from Wako Chemicals GmbH (Neuss, Germany). Recombinant human IGF-I (rhIGF-I) was a kind gift from Kabi Pharmacia (Stockholm, Sweden).

*Animal preparation*

*Induction of liver cirrhosis*: Liver cirrhosis was induced in male Wistar rats, aged 5 weeks with a body weight of 100–120 g, by carbon tetrachloride ($CCl_4$) inhalation twice a week (18). The time of exposure to $CCl_4$ was progressively increased (from 30 s to 5 min), during a 10-week period. Phenobarbitone was added to the drinking water (400 mg/l) from 1 week before starting $CCl_4$ exposure until the end of the experimental period. Before and during the experimental period, rats were provided a 12 h light-dark cycle and a constant room temperature (20°C). The study protocol begun 7 days after the last dose of $CCl_4$. At this time, rats were 16 weeks old and weighed about 350 g; the animals may therefore be considered adults.

All the experimental procedures were performed in conformity with the Guiding Principles for Research Involving Animals.

*Animal diet*

Before starting the study, all rats were allowed free access to a standard nonpurified diet. For N balance assessment, a semipurified diet was prepared and supplied following the recommendations of the American Institute of Nutrition for Nutritional Studies (19,20). Briefly, the experimental diet contained 11% protein, 5% lipids (corn oil), 5% fiber, 3.5% salt mixture, 1% vitamin mixture, 0.2% choline bi-tartrate, and up to 100% carbohydrates as a 1/1 (w/w) sucrose and corn starch mixture. The percentage (%) of diet ingredients is in reference to diet weight. The protein source was a 50% mixture of lactalbumin and vegetable $^{15}N$-enriched proteins from a specific kind of bean (*Phaseolus vulgaris*) (21).

$^{15}N$-enriched bean seeds were obtained from plants cultured on soil containing ammonium sulfate enriched with $^{15}N$ 10 atoms% excess.

Bean seeds, from the same harvest, were washed, immersed for 24 h in tap water, and cooked under pressure for 30 min. Finally, the cooked beans were freeze-dried, and ground to 40-mesh to prepare the semipurified diet.

*Study design*

Twelve nonascitic cirrhotic rats and six normal matched control rats were used. Cirrhotic rats were randomly assigned to two different groups to receive the vehicle (Group 1, $n=6$) or rhIGF-I (Group 2, $n=6$).

All rats were housed in individual wire-bottomed metabolic cages and fasted for 24 h prior to the administration of the diet containing $^{15}N$ to reduce isotope dilution by intestinal content. Thereafter, rats

were fed *ad libitum* with the $^{15}N$-enriched food during the 5-day experimental period.

rhIGF-I was administered s.c. to rats from Group 2 during the 5-day experimental period (2 µg/100 g bw per day, administered in two divided doses). Total volume per injection did not exceed 250 µl. Control rats and Group 1 rats received the same volume of vehicle (saline) with the same administration scheme. rhIGF-I doses were given at 0900 hours and 1800 hours. Free access to drinking water and food was allowed before and throughout the study.

Basal body weight was recorded at zero time of the first experimental day. At the end of the 2nd and the 5th experimental days, rats were weighed, water and food consumption recorded, and feces and urine collected, weighed and stored for further analysis.

The last dose of rhIGF-I was administered right at the end of the 5-day experimental period. After 24 h fasting, rats were killed by decapitation, blood was collected from the neck in ice-cooled tubes containing EDTA and centrifuged at 4 °C. Aliquots of plasma samples were stored at –20 °C until tested.

Immediately after decapitation and blood collection, liver and gastrocnemius muscles were carefully dissected and weighed up to the fourth decimal digit. A sample of the left major liver lobe was then excised and processed for histological examination. Tissue specimens were immediately frozen by immersion in liquid N and stored at –80 °C until analysis.

*Analytical methods*

Percent N content of all samples was determined by Kjeldahl's method (22) in triplicate, and <5% intra-assay variation was considered acceptable. The percent of $^{15}N$ content in every sample was determined in the ammonium sulphate from the titrated and acidified solution derived from Kjeldahl's procedure (23) by atom mass spectrometry (C.I.E.M.A.T., Madrid, Spain) using a mass spectrometer with a 90° magnetic sector VG, model G-30 (Isogas, Manchester, England). All specimens were analyzed in duplicate, and determinations were performed three times in each sample. Sample delivery and data processing were accomplished automatically under computerized control.

Total plasma levels of IGF-I (rat IGF-I in control group and Group 1, and rat IGF-I+rhIGF-I in Group 2) were determined by RIA at the Kabi Peptide Hormone Laboratory, Stockholm, Sweden, as described elsewhere (24,25).

IGF binding proteins (IGFBPs) were studied using Western blot analysis which was performed as described by Hossenlopp et al. (26) and Hardouin et al. (27). Briefly, rat plasma (3 µl) was diluted up to 50 µl in 0.06 M Tris-HCl and 0.15 M NaCl, pH 6.8, with 5% SDS, 20% glycerol and 0.02% bromophenol blue. The solution was submitted to a 5–15% gradient SDS-PAGE. Proteins were blotted to a 0.2 µm nitrocellulose filter (Bio-Rad). Nitrocellulose filter was dried for 5 min at 37 °C, followed by quenching firstly in Tris buffer alone, and then in Tris buffer with 3% NP-40 (30 min at 4 °C). Finally, the filter was soaked in Tris buffer with 1% BSA and 0.1% Tween-20 for 60 min, and was then incubated overnight with 200000 cpm $^{125}I$-IGF-I. After washing several times with buffer, the filter was dried and, lastly, exposed to an autoradiographic film for 4 days.

Liver hydroxyproline was determined in whole tissue in triplicate (28). Briefly, 50 mg aliquots of liver tissue were hydrolyzed for 22 h at 110 °C in 1 ml 6 N HCl, and hydroxyproline content was quantified by HPLC using the Pico-Tag® method for amino acid analysis. Hydrolysate, 25 µl, was derivatized with phenyl isothiocyanate at pH 9–10 to produce phenyl thiocarbamyl–amino acid derivatives. After derivatization, samples were dried under vacuum and redissolved, immediately before chromatographing with 0.01 M disodium hydrogen phosphate in 5% acetonitrile, buffered at pH 7.40. Finally, samples were introduced into a 300×3.9 mm HPLC chromatographic column (Waters), with a 10 µm particle stationary phase, at 46 °C. An automatic injection system was used to introduce samples in the column. As a mobile phase, 70 mM sodium acetate in acetonitrile:methyl alcohol:water (45:45:10%), buffered at pH 6.46, was used. Commercially available standard amino acid solutions were processed similarly, and used as external standard to calculate hydroxyproline concentrations in the experimental samples.

Hydroxyproline content was expressed as µmol/mg liver protein (instead of as µmol/g liver weight) to avoid the influence of fatty degeneration in cirrhotic livers. Liver protein concentration was determined in liver tissue homogenates (20 mg of tissue in 1 ml of water) by Bradford's colorimetric method (29).

*Calculations:* Absolute values of all parameters measured represent the cumulative value of the 5-day experimental period. Parameters obtained by calculation and their respective units and formulas were as follows:

Retained N = N intake–[fecal N+ urinary N] (expressed as $g \cdot 5\ days^{-1}$).

Absorbed N = N intake–fecal N (expressed as $g \cdot 5\ days^{-1}$).

Percent endogenous N content of samples, as previously defined (20), was calculated according to the following formula:

$$\%\text{Endogenous N} = [(F^{15}N - S^{15}N)/(F^{15}N - {}^{15}NNA)] \times 100$$

where $F^{15}N$ was $^{15}N\%$ content of food, $S^{15}N$ was $^{15}N\%$ content of the sample studied and $^{15}NNA$ was $^{15}N\%$ natural abundance (0.370 $^{15}N$ atoms%).

The corresponding percent dietary N content of each sample was obtained by subtracting percent endogenous N from 100%. The absolute amount of endogenous or dietary N (in grams) contained in samples was obtained from percent endogenous or exogenous N content and total N content of each sample.

Body weight gain (in grams) was calculated by subtracting basal body weight from body weight at the end of the 5th experimental day.

Food conversion efficiency (FE) was calculated as follows:

FE=[body weight gain/food intake]×100, and expressed as percent.

Sodium balance was obtained by subtracting total urine sodium output from total sodium intake during the last 3 days of the experimental period. Sodium content in the prepared food (0.079 mEq/g) was measured by atom absorptiometry.

*Liver histopathology*

Livers from carbon tetrachloride-treated rats were scored from 0 to 4, according to histopathological findings: 0 – normal liver, 1 – pericentral venous fibrosis, 2 – fibrous septa not forming full-shaped regenerative nodules, 3 – established cirrhosis with fibrous septa delimiting full-shaped regenerative nodules of variable size (mixed micro/macronodular pattern), 4 – established cirrhosis with fibrous septa delimiting full-shaped regenerative nodules with a diffuse micronodular pattern (30).

The pre-established criteria for retrospective inclusion of animals were the presence of histologically proven liver cirrhosis (score 3 or 4 of the classification) and absence of ascites confirmed by direct examination of the abdominal cavity. In addition, portocaval anastomosis could be excluded since no sign of diminished testicular size was observed. It is known that testicular atrophy may develop in cases of portocaval shunt (31).

*Statistics*

Data are expressed as mean±SEM. Regarding the sample size ($n<10$), nonparametric tests have been applied: Kruskal-Wallis analysis has been used for simultaneous comparisons of the three groups, and the significance within each group was determined by *a posteriori* Mann-Whitney test. Regression analysis was performed by the least squares method. Statistical significance was accepted at $p<0.05$.

## Results

*Liver histology and liver hydroxyproline content*

Liver tissue samples from all rats were evaluated histologically, and lesions in livers were scored from 0 to 4 as described in the Methods section. Only rats exhibiting established liver cirrhosis (score 3 or 4) were considered for data analysis. As a result, one rat from Group 2, which showed incomplete cirrhosis, was excluded from analysis.

The histopathological score was the same in Groups 1 and 2 (3.2±0.2 vs. 3.2±0.2, $p$=NS). Control rats did not show abnormal histological findings in the liver. Liver hydroxyproline was not significantly different between Group 1 (5.2±0.5 μmol/mg liver protein) and Group 2 (4.1±0.5 μmol/mg liver protein), both values being significantly higher than that in control rats (1.1±0.2 μmol/mg liver protein).

*$^{15}N$ content in food, urine, feces and tissues*

The naturally occurring amount of $^{15}N$ accounts for 0.370% of total N atoms. The $^{15}N$-enriched food specifically prepared for this study contained 0.819 ±0.010 atoms%.

At the end of the study period, after administering an $^{15}N$-enriched diet to the animals for 5 days, in feces this stable N isotope represented 0.706±0.006 atoms% in the control group of normal rats, 0.680±0.009 atoms% in Group 1 of cirrhotic rats and 0.721±0.012 atoms% in Group 2 of IGF-I treated cirrhotic rats ($p<0.05$ vs. Group 1). No significant differences were observed in the urinary content of $^{15}N$ among groups, being 0.578±0.008, 0.557±0.011 and 0.584±0.010 atoms% in the control group, Group 1 and Group 2, respectively. Regarding the tissue content of $^{15}N$, no significant differences were found in gastrocnemius muscle (control group: 0.394±0.001; Group 1: 0.389±0.002; Group 2: 0.394 ±0.002 atoms%) but the liver $^{15}N$ content was slightly higher in Group 2 (0.499±0.006 atoms%) than in Group 1 (0.480±0.005 atoms%, $p<0.05$), with no significant differences being observed with respect to control rats (0.487±0.005 atoms%). Thus, in the different specimens tested, all groups achieved a $^{15}N$ concentration above the naturally occurring value. The slight, but statistically significant, differences observed in $^{15}N$ content in feces and liver between

TABLE 1

Biochemical data in the three experimental groups of rats

| | Control ($n$=6) | Group 1 ($n$=6) | Group 2 ($n$=5) |
|---|---|---|---|
| GPT (IU/dl) | 38±1 | 161±71* | 96±15* |
| Glycemia (mmol/dl) | 6.1±0.2 | 5.8±0.3 | 5.5±0.2 |
| Total proteins (g/dl) | 6.5±0.2 | 6.3±0.2 | 6.5±0.3 |
| Albumin (g/dl) | 2.8±0.1 | 2.5±0.2 | 2.8±0.3 |
| Insulin (U/ml) | 16.2±2.1 | 17.3±2.7 | 18.7±1.3 |
| FFA (mEq/l) | 1.36±0.16 | 1.03±0.04* | 1.03±0.05* |
| Lactate (mmol/dl) | 5.8±1.0 | 6.5±1.1 | 5.7±1.9 |
| Creatinine (mg/dl) | 0.6±0.1 | 0.6±0.1 | 0.5±0.1 |
| Urea (g/l) | 0.21±0.03 | 0.20±0.03 | 0.22±0.02 |
| $Na^+$ (mEq/l) | 125±2 | 128±1 | 124±42 |
| UCr (mg·3 $d^{-1}$) | 55.3±3 | 45.7±1* | 52.7±4 |
| Creatinine Clearance(ml/min) | 2.287±0.194 | 1.964±0.154 | 2.333±0.307 |
| UUrea (g·3d $^{-1}$) | 1.058±0.11 | 1.036±0.096 | 1.227±0.179 |
| $UNa^+$ (mEq·3 $d^{-1}$) | 4.244±0.629 | 3.60±30.198 | 5.099±0.609 |
| $Na^+$ Balance (mEq·3 $d^{-1}$) | 2.005±0.266 | 2.289±0.657 | 1.630±0.703 |

Values are means±SEM; *$p$<0.05 vs. Control. UCr, UUrea, $UNa^+$=urinary output of creatinine, urea or sodium. The remaining values correspond to plasma

Groups 1 and 2 probably reflect differences in food intake between these two groups (see below).

*Biochemical characteristics*

Compared with control rats, cirrhotic rats from Groups 1 and 2 showed significantly higher values of serum GPT, and significantly lower levels of serum free fatty acids. In addition, urinary creatinine excretion was significantly lower in Group 1 than in control rats. There were no significant differences among groups in the remaining parameters measured except for free fatty acids, which were significantly reduced in Groups 1 and 2 ($p$<0.05) as compared with control rats (Table 1).

*Food intake, weight variation and food efficiency*

No significant differences in basal body weight were observed among groups. During the 5-day experimental period, water intake (–26%, $p$<0.05) and dry feces weight (–30%, $p$<0.05) were significantly reduced in nontreated cirrhotic rats (Group 1) compared with the control group; food intake and urine volume were also lower in Group 1 as compared to controls (–20% and –29%, respectively) but differences were just below the limit of statistical significance (Table 2). In contrast, in rhIGF-I-treated cirrhotic rats (Group 2) the mean values of all these parameters were similar to those observed in controls and food intake and dry feces weight were significantly higher than in Group 1 ($p$<0.05 in both cases; Table 2).

At the end of the experimental period, body weight gain was significantly lower in Group 1 than in the other two groups, which showed similar values (Fig. 1). The difference in the increase in body weight between Group 1 and the other two groups of animals was due not only to differences in food intake but also to a significantly lower FE in nontreated cirrhotic rats. Fig. 1 shows that both FE and body weight gain are significantly higher in Group 2 than in Group 1 and that these parameters are similar in Group 2 and in control animals.

*Nitrogen intake, excretion and balance*

Nitrogen intake, fecal and urinary N excretion and N absorption during the 5-day experimental period are summarized in Table 3.

Nitrogen intake was significantly lower in Group 1 than in Group 2 ($p$<0.05) while fecal N was significantly lower in Group 1 than in both the control group and Group 2 ($p$<0.05, both). No significant differences were found in urinary N excretion among groups.

The amount of absorbed N was significantly

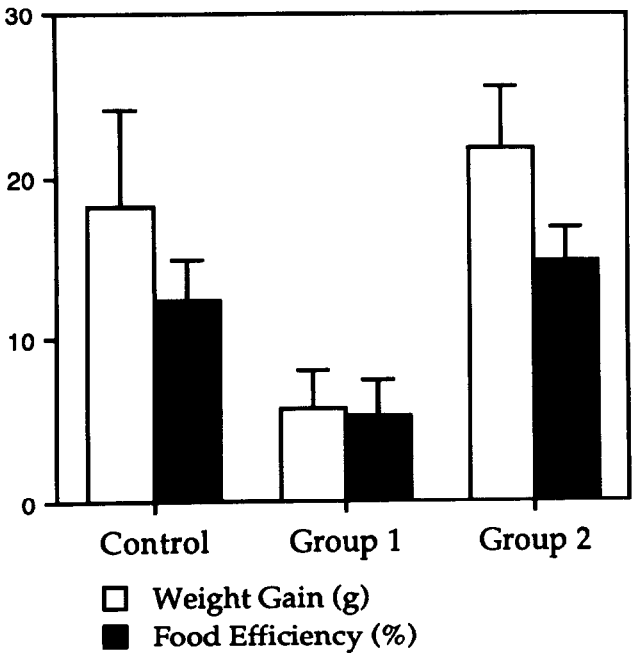


*Fig. 1. Body weight gain and food efficiency. Three groups of rats (Control rats, n=6; Group 1, n=6; Group 2, n=5) were fasted for 24 h and then refed for 5 days. Cirrhotic rats were randomly assigned to two groups: Group 1 received vehicle and Group 2 received rhIGF-1 2 μg·100 g $bw^{-1}·d^{-1}$ in two subcutaneous administrations. Control rats received vehicle. Body weight gain and food efficiency [FE=(g bw gain/g food intake)×100] were assessed at the end of the fifth day of refeeding. Control vs. Group 1: p=NS; Control vs. Group 2: p=NS; Group 1 vs. Group 2: p=0.01.*

TABLE 2

Food and water intake, feces weight and urinary volume in the three experimental groups of rats

| | Control ($n$=6) | Group 1 ($n$=6) | Group 2 ($n$=5) |
|---|---|---|---|
| Body weight (g) | 557±8 | 563±7 | 563±7 |
| Food intake ($g \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$) | 24.0±2.4 | 19.2±0.7* | 26.0±1.8 |
| Water intake ($ml \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$) | 27.2±2.8 | 20.0±0.7*# | 24.9±1.4 |
| Dry feces weight ($g \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$) | 6.4±0.6 | 4.4±0.2*# | 6.4±0.6 |
| Urine volume ($ml \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$) | 9.9±1.7 | 7.0±0.8 | 10.8±2.2 |

Values are means±SEM. *$p$<0.05 vs. Group 2; #$p$<0.05 vs. Control.

TABLE 3

Nitrogen intake, excretion, and absorption in the three experimental groups of rats

| | Control ($n$=6) | Group 1 ($n$=6) | Group 2 ($n$=5) |
|---|---|---|---|
| N intake ($g \cdot 5\ d^{-1}$) | 2.336±0.248 | 1.901±0.066* | 2.565±0.154 |
| [$g \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$] | [0.418±0.040] | [0.338±0.014*] | [0.457±0.033] |
| Feces N ($g \cdot 5\ d^{-1}$) | 0.609±0.038 | 0.492±0.028*# | 0.636±0.033 |
| [$g \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$] | [0.109±0.060] | [0.088±0.005]*# | [0.113±0.007] |
| Urinary N ($g \cdot 5\ d^{-1}$) | 1.220±0.158 | 1.077±0.025 | 1.284±0.096 |
| [$g \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$] | [0.218±0.026] | [0.192±0.006] | [0.228±0.017] |
| Absorbed N ($g \cdot 5\ d^{-1}$) | [1.727±0.213 | [1.409±0.046* | [1.929±0.130 |
| [$g \cdot 100\ g\ bw^{-1} \cdot 5\ d^{-1}$] | [0.308±0.035] | [0.251±0.001*] | [0.344±0.027] |

Values are means±SEM. * $p$<0.05 vs. Group 2; # $p$<0.05 vs. Control.

higher in Group 2 than in Group 1 ($p$<0.05). However, this does not imply that N absorption is impaired in Group 1 rats. The percent of ingested N which was absorbed averaged 73.3±0.01% in control rats, 74.3±0.01% in Group 1 and 74.4±0.01% in Group 2, indicating that N absorption was preserved in cirrhotic rats and was unmodified by rhIGF-I treatment. Differences in absolute values of N absorption reflect differences in food intake. Indeed, considering all animals as a whole there was a close direct and linear correlation between ingested and absorbed N ($r$=0.99; $p$<0.001) (Fig. 2).

Individual values of retained N revealed that every rat included in the study had had a positive N balance during the experimental period. However, N retention was lower (borderline statistical significance) in Group 1 than in controls, whereas the highest values were exhibited by rhIGF-I-treated cirrhotic rats, being significantly higher ($p$<0.05) than those in nontreated cirrhotic rats (Fig. 3).

Considering all the animals studied, retained N was directly and significantly correlated in a linear fashion with weight gain ($r$=0.80, $p$<0.001).

*Organ weight and total and dietary N content in the gastrocnemius muscle and the liver*

Table 4 shows mean values of gastrocnemius muscle weight and of total N content and dietary N content in the gastrocnemius muscle in the three groups of rats.

Absolute mean values of gastrocnemius weight were significantly lower in rats from Group 1 than in control rats ($p$<0.05) but there were no significant differences between Group 2 and controls. However, when gastrocnemius weight was corrected by 100 g

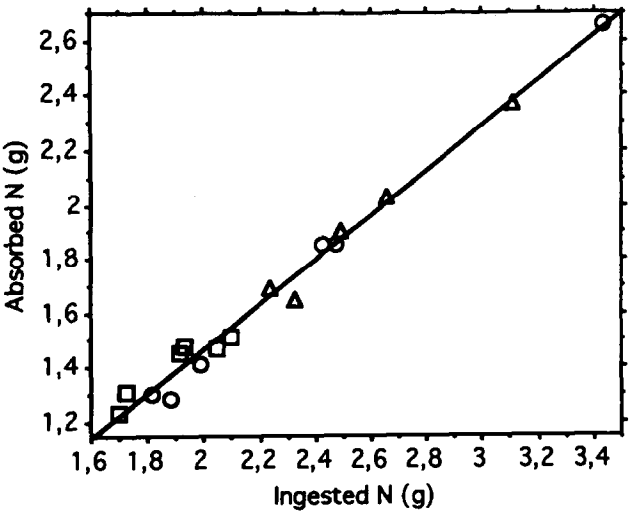


*Fig. 2. Correlation of absorbed vs. ingested nitrogen: r=0.99, p<0.001. Nitrogen intake and absorption were measured at the end of the five experimental days in the same three groups of rats of Fig. 1. Circles: Control rats. Squares: Group 1. Triangles: Group 2.*

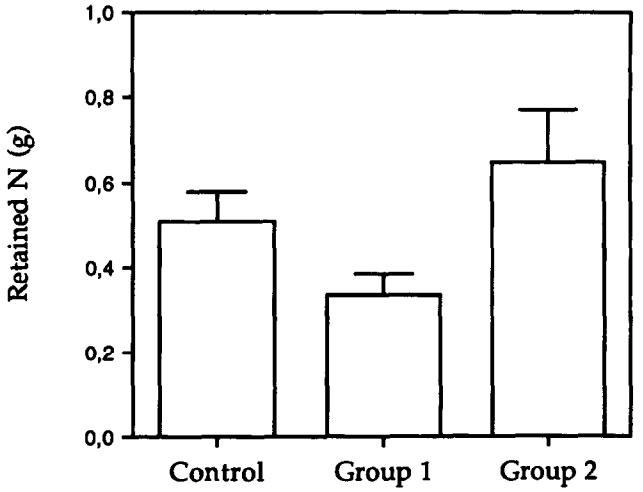


*Fig. 3. Retained nitrogen. Nitrogen balance [Retained N=N intake–(fecal N+urinary N)], was calculated at the end of 5 days of refeeding in the same three experimental groups of rats as in Fig. 1. Control vs. Group 1: p=NS; Control vs. Group 2: p=NS; Group 1 vs. Group 2: p<0.05.*

TABLE 4

Gastrocnemius muscle weight and nitrogen content

| | Control ($n$=6) | Group 1 ($n$=6) | Group 2 ($n$=5) |
|---|---|---|---|
| Muscle weight (g) | 3.318±0.128 | 2.918±0.077* | 3.063±0.107 |
| Muscle weight (g/100 g bw) | 0.627±0.018 | 0.565±0.009* | 0.576±0.014* |
| Total muscle N (mg) | 119±6 | 102±3* | 108±6 |
| Total muscle N (% muscle weight) | 3.6±0.1 | 3.5±0.1 | 3.6±0.1 |
| Muscle dietary N (mg) | 6.6±0.5 | 4.3±0.4* | 5.7±0.4 |
| Muscle dietary N (% total N) | 5.5±0.3 | 4.2±0.4*# | 5.4±0.4 |

Values are means±SEM. * $p$<0.05 vs. Control. # $p$<0.05 vs. Group 2. .

of rat bw, both Group 1 and Group 2 showed significantly lower values than those in the control group ($p$<0.05).

Absolute values of dietary N incorporated into the gastrocnemius muscle in Group 1 but not in Group 2 were significantly reduced as compared to control rats ($p$<0.05). When dietary N was expressed as percent of total gastrocnemius N, both the control group and Group 2 showed significantly higher values than Group 1 (Table 4). Nearly significant differences were observed when the dietary N content in the gastrocnemius was corrected per gram of gastrocnemius weight ($p$=0.068; Fig. 4).

Interestingly, we found that gastrocnemius dietary N showed a significant, linear and direct correlation with FE ($r$=0.76, $p$<0.001; Fig. 5).

Liver weight (expressed as g·100 g $bw^{-1}$) was significantly higher in Groups 1 and 2 (3.497±0.020 and 3.269±0.259, respectively) than in controls (2.575±0.037, $p$<0.05). However, liver N content estimated as percent of liver weight was significantly reduced in Groups 1 and 2 (3.4±0.1% and 3.4±0.1%) as compared to normal rats (3.6±0.1%, $p$<0.05), indicating a higher proportion of nonprotein components in cirrhotic livers. Total dietary N in the liver was not significantly different in the three groups of animals (control: 134±12 mg; Group 1: 142±7 mg; Group 2: 158±16 mg). However, when liver dietary N was corrected by gram of liver tissue, untreated cirrhotic rats showed less incorporation of exogenous N into the liver (8.3±0.2 mg·g $liver^{-1}$, $p$<0.05 in both cases) than control animals (9.8±0.6 mg·g $liver^{-1}$) and Group 2 animals (9.9±0.3 mg·g $liver^{-1}$).

*Serum levels of IGF-I and IGF-I binding proteins*

The mean values of IGF-I were not significantly different between control (754±36 ng/ml) and cirrhotic rats from Group 1 (753±63 ng/ml) and Group 2 (901±75 ng/ml), indicating that the synthesis of this hormone was still preserved in cirrhotic rats.

However, the serum IGFBPs pattern was markedly different among the three groups. As shown in Fig. 6, measurement of IGFBPs in two rats of each group disclosed that cirrhotic rats of both groups exhibited increased IGFBP 3 levels above that of control rats; but, in addition, an increase in IGFBPs 1–2 and 4 was observed in cirrhotic rats from Group 2 (those treated with rhIGF-I) as compared with nontreated cirrhotic

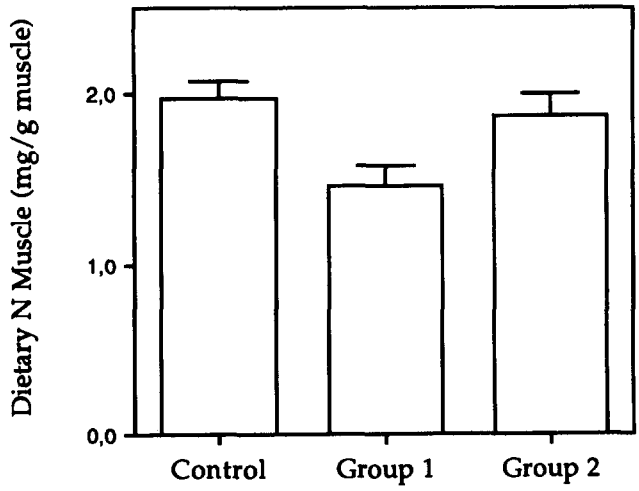


*Fig. 4. Incorporation rate of dietary nitrogen to gastrocnemius muscle. The same three groups of rats of Fig. 1 were allowed free access to $^{15}N$ enriched food during 5 days. Control vs. Group 1:* p=*0.01; Control vs. Group 2:* p=*NS; Group 1 vs. Group 2:* p=*0.068.*

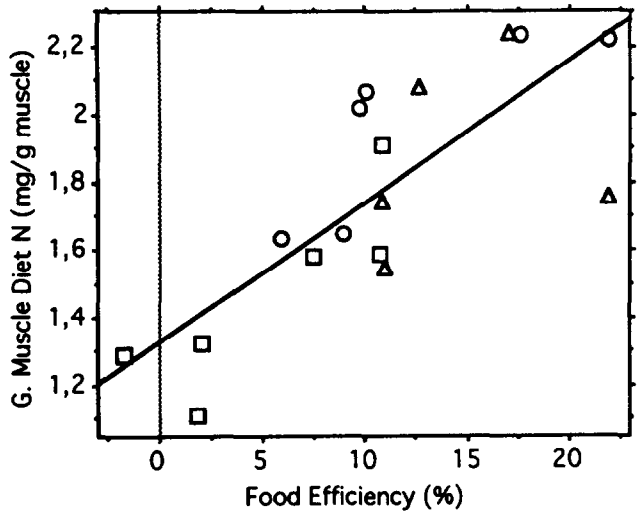


*Fig. 5. Scattergram showing the correlation between food efficiency (FE) and the absolute values of dietary N incorporated into gastrocnemius muscle (r=0.76;* p<*0.001) in the three experimental groups of rats. (Circles: control rats; Squares: Group 1; Triangles: Group 2).*

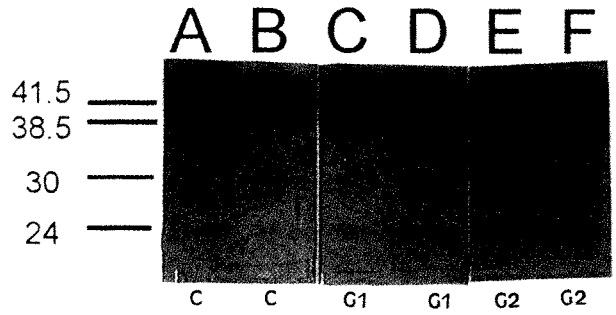


*Fig. 6. IGFBPs Western Blot. IGFBPs were determined by autoradiography after SDS-PAGE and electroblotting of rat plasma (3 µl) to a nitrocellulose filter and overnight incubation with $^{125}$I-IGF-I. Lanes A and B correspond to control rats, lanes C and D to cirrhotic rats (Group 1), and lanes E and F to rhIGF-I treated cirrhotic rats (Group 2). Numbers on the left indicate relative molecular mass kD (Mr ×1000). The triplet band at Mr 42000 corresponds to IGFBP 3, the band of about Mr 30000 to IGFBPs 1 and 2, and the band at Mr24000 to IGFBP.*

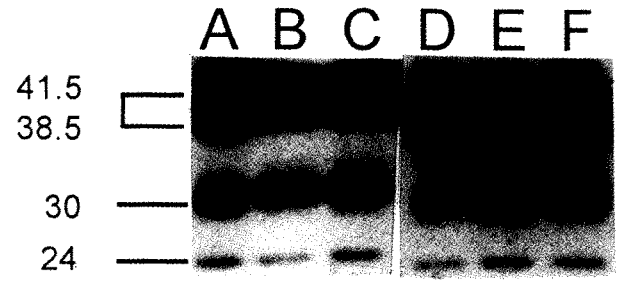


*Fig. 7. IGFBPs Western Blot. IGFBPs were determined in two additional cirrhotic rats after intravenous infusion of vehicle (lanes A, B, and C) or rhIGF-I (2 µg/100 g bw) (lanes D, E, F). Blood was withdrawn from the carotid artery before any infusion (lanes A and D), 90 min (lanes B and E), and 120 min (lanes C and F) after vehicle or rhIGF-I intravenous bolus, respectively. Numbers on the left indicate relative molecular mass kD (Mr ×1000). It shows that 90 and 120 min after IGF infusion there was a marked elevation in the levels of IGFBP 1–2 and 4, as compared with basal values. However, no changes were observed in the control rat with cirrhosis after vehicle infusion.*

rats. To assess whether the increased levels of IGFBPs 1–2 and 4 in Group 2 could be related to the administration of exogenous IGF-I, IGFBPs were measured in two additional rats which were subjected to an acute infusion of either rhIGF-I (2 µg/100 g bw) or vehicle. Fig. 7 shows that 90 min and 120 min after rhIGF-I infusion there was a marked elevation in the levels of IGFBP 1–2 and 4 as compared with basal values. No changes were observed in the control rat with cirrhosis after vehicle infusion.

## Discussion

This study shows that nonascitic cirrhotic rats exhibit diminished ability to gain weight during the refeeding period that follows 24 h fast. The deficient weight recovery observed in these animals is associated with diminished food intake and FE, decreased N retention and impaired incorporation of dietary N into muscle. Of importance, our results show that rhIGF-I administered at a low dose during the study period was able to reverse these nutritional alterations. The dose of rhIGF-I which we have used (20 $\mu g \cdot kg^{-1} \cdot day^{-1}$) is low compared with the doses usually administered in acute experiments (12,14), but it is comparable to those which can be given for several days without the risk of undesirable effects. Ebeling et al. (32) observed that 30 $\mu g \cdot kg^{-1} \cdot day^{-1}$ was effective in increasing bone turnover in postmenopausal women without producing side effects. Doses ranging from 60 to 180 $\mu g \cdot kg^{-1} \cdot day^{-1}$, although having a greater effect on bone turnover, were associated with a variety of undesirable side effects.

The cirrhotic rats used in this study had established cirrhosis with a mixed micro/macronodular pattern, a stage less advanced than the diffuse micronodular pattern, which represents the final histological phase of the carbon tetrachloride-induced cirrhosis (33,34). Contrary to the marked alterations in serum biochemistry found in rats with micronodular cirrhosis and massive ascites (35) very mild alterations in serum biochemical parameters were observed in our cirrhotic rats in accordance with the early stage liver cirrhosis exhibited by these animals.

While protein-calorie malnutrition with progressive muscle wasting occurs in patients with far-advanced cirrhosis and ascites, no apparent signs of malnutrition are observed in patients with compensated cirrhosis (36). In these patients most of the biochemical indicators of the nutritional status are normal. Despite these clinical findings, this study shows that mild but statistically significant muscle wasting is already present in nonascitic cirrhotic rats, suggesting that protein-calorie malnutrition might develop early in the evolution of cirrhosis independently of the occurrence of the common complications of advanced cirrhosis such as infections, hemorrhages, ascites, renal failure and hydromineral disturbances, which have an important impact on the nutritional status.

The marked reduction in body weight gain observed

in cirrhotic rats from Group 1 appears to be related to reduction in both food intake and FE (the ability to convert eaten food into body mass). Deficient food intake is believed to play a role in cirrhotic malnutrition (36) and it has been proposed that the liver might be involved in controlling food appetite (37). Mendenhall et al. (38), observed a direct correlation between the prevalence of malnutrition and daily calorie intake in patients with alcoholic chronic liver disease. In patients with mild liver disease the prevalence of clinical malnutrition was 4.7% and mean daily intake was 2015 kcal, while 72% of those severely ill were malnourished and their intake was reduced to 1552 kcal/day, anorexia being present in 87% of these patients (39). Interestingly, food intake and body weight gain were normal in rhIGF-I-treated cirrhotic rats from Group 2. Results of urine sodium output and sodium balance demonstrated that differences in sodium and fluid retention were not the cause of differences in body weight gain between untreated and treated cirrhotic rats. Although stimulation of food appetite is not a recognized biological effect of IGF-I, an increase in both food intake and FE appears to be one of the factors involved in the improvement of body weight gain observed in rhIGF-I-treated rats. On the other hand, this increase in food appetite does not appear to result from hypoglycemia, since unpublished data from our group have shown that rhIGF-I at the doses used in this study does not cause an hypoglycemic effect in rats.

The mechanisms which could be involved in the diminution of FE observed in cirrhotic rats include impaired absorption (40), altered disposal of substrates for energy generation, increased energy expenditure, or a combination of these factors. Preliminary data show that IGF-I-treatment restores sugar absorption in cirrhotic rats (40). In agreement with previous reports (41), our results show that N absorption (as percentage of N intake) was preserved in early cirrhotic rats. Carbohydrate and fat absorption, which has been found to be impaired in liver cirrhosis in some studies (40–42), were not assessed in our rats. However, dry feces weight and food intake were both reduced to a similar extent in rats from Group 1, making unlikely a substantial fecal loss of nutrients in these animals. Although not measured in this study, there is general agreement that resting energy expenditure is not increased in compensated cirrhosis (1,43–47), despite some reports showing increases in this parameter when absolute values were normalized by lean body mass (45–47). Glucose utilization, however, is impaired from the early stages of liver cirrhosis due to the existence of insulin resistance (48), which correlates with the impairment in muscle glycogen synthesis. As a result, there is a shift from glucose to free fatty acids as the main fuel in muscle (48). Gluconeogenesis is also stimulated, even in short-term fasting, leading to amino acid loss (38). Although the levels of glucose and insulin showed no significant alterations in our cirrhotic rats, these determinations were carried out in the fasting state and it is possible that biochemical abnormalities in the fed state could have gone unrecognized.

The beneficial effects of rhIGF-I in cirrhotic rats, increasing FE and N balance, could be due in part to improvement of glucose metabolism and secondary reduction in fat and protein utilization as fuels. In normal fasted rats the acute administration of pharmacological doses of IGF-I resulted in a decrease of insulin levels, enhancement of both glucose uptake and glycogen synthesis by liver and muscle and decreased levels of circulating amino acids due to a reduction in protein breakdown (49). These findings offer an explanation for the effects of rhIGF-I on N metabolism in cirrhotic rats since, in cirrhosis, insulin resistance stimulates gluconeogenesis to maintain blood glucose levels and this requires an increased supply of amino acids from muscle to liver (48).

The cumulative N balance after the 5 experimental days, which was reduced in untreated cirrhotic rats, returned to normal values in cirrhotic rats treated with rhIGF-I. This effect of the treatment might be due in part to the higher food intake in rats from Group 2. However, we also found that a major effect of rhIGF-I on muscle in this study was to enhance the incorporation of dietary N into the gastrocnemius, so that values of dietary N in muscle in rats from Group 2 and control rats were similar, and both values were significantly higher than in Group 1. This may be due to either increased synthesis or reduced breakdown of muscle protein. Acceleration of protein degradation has been suggested to occur in liver cirrhosis because of the finding of an increased urinary excretion of 3-methylhistidine (50,51), a marker of protein breakdown. It is well established that IGF-I inhibits muscle protein degradation in the fasting state (52), while stimulating protein synthesis in the fed state (53) and, recently, an inhibitory effect of IGF-I on the activity of a major muscle protease system, involved in myofibril degradation has been demonstrated (54). Thus, IGF-I could have several effects on cirrhotic rats, i.e. appetite enhancement, improved use of substrate for energy generation and improvement in muscle protein metabolism. Although both groups of cirrhotic rats exhibited a certain degree of muscle wasting, as reflected by the lower weight of the gastrocnemius as compared to control rats, it seems pos-

sible that a more prolonged treatment with rhIGF-I might restore the muscle mass in the cirrhotic rats. In fact, FE showed a good correlation with the dietary N content of the gastrocnemius muscle (Fig. 5).

Cirrhotic rats exhibited decreased liver content of total and dietary N when absolute values were corrected by gram of liver tissue. Dietary N was about 25% of total liver N in the three experimental groups of animals after 5 days with a diet enriched with the stable isotope, indicating a high turnover rate for liver proteins. Dietary N incorporation into liver tissue improved in the rhIGF-I-treated rats. However, data regarding rhIGF-I effects on the liver in our experimental model should be viewed cautiously, since the liver is the major target organ for carbon tetrachloride toxicity. Whether the differences observed between Group 1 and Group 2 in hepatic N metabolism reflect only the effects of rhIGF-I or are influenced by liver damage cannot be ascertained with data in this study.

It has been taken into account that the accuracy of measuring protein turnover through dietary $^{15}N$ incorporation into liver or muscle tissue depends on the achievement of a constant $^{15}N$/total N ratio, i.e. a steady state condition or the isotope incorporation increasing in a linear fashion (55). Such measurements have not been made in the present study. If there were differences in the turnover rates of tissue protein between groups, it would imply that different periods of time were necessary to achieve steady state, and therefore comparative differences would not be valid. Nevertheless, we do not think that these kinetic considerations would change the qualitative differences found in this study, since both cirrhotic rats and control animals were 16 weeks old and therefore the rate of weight gain was quite slow during the 5-day experimental period. It is likely that in both groups a steady state had been reached. In addition, animals with cirrhosis should have a higher turnover rate than controls, reaching a steady state sooner. Although quantitative differences may not be of relevance, qualitative comparisons do make sense.

Plasma total IGF-I levels measured 24 h after the last dose of rhIGF-I were not significantly different among groups, indicating that the hepatic synthesis of this hormone was preserved in cirrhotic rats. This is in agreement with findings in patients with cirrhosis in whom IGF-I plasma levels are reduced only in those with far-advanced disease but are normal in nonascitic patients (12,56–58). Therefore, rhIGF-I administration to cirrhotic rats from Group 2 in this study cannot be viewed as a true "replacement therapy". It should be recalled that this hormone circulates in blood largely bound to several IGF-I binding proteins (IGFBP), which act not merely as hormone carriers but as modulators of the delivery of IGF-I to target tissues (27,59). IGFBP 3 was increased in sera from cirrhotic rats compared with controls. According to current thinking, increased IGFBP 3 might reduce the tissue availability of IGF-I by preventing the interaction of the hormone with its tissue receptors. Of note, the levels of low-molecular weight IGFBPs, which are believed to facilitate the access of IGF-I to its target tissues, were markedly increased in rats treated with rhIGF-I. The modulating effect of IGF-I on the pattern of IGFBPs, which has been shown in other reports (27,59,60), was observed in the rats receiving the hormone for several days and was also noticed after a single injection of IGF-I (20 $\mu g \cdot kg^{-1}$). It seems, therefore, that changes in the tissue availability of IGF-I induced by treatment with this hormone might play a role in the metabolic effects observed in the present study.

In summary, a variety of nutritional alterations, including reduced FE and diminished incorporation of dietary nitrogen into the muscle, are present in early-stage cirrhosis. These changes, which are likely to represent the earliest and subclinical manifestations of the protein-calorie malnutrition present in far-advanced cirrhosis, revert with low doses of rhIGF-I. Further studies are needed to determine whether rhIGF-I might be useful as a treatment for malnutrition in liver cirrhosis.

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## References

1. Merli M, Riggio O, Romiti A, Ariosto F, Mango L, Pinto G, Savioli M, Capocaccia L. Basal energy production rate and substrate use in stable cirrhotic patients. Hepatology 1990; 12: 6–12.
2. McCullough AJ, Tavill AS. Disordered energy and protein metabolism in liver disease. Semin Liver Dis 1991; 11: 265–77.
3. Romijn JA, Endert E, Sauerwein HP. Glucose and fat metabolism during short term starvation in cirrhosis. Gastroenterology 1991; 100: 731–7.

4. Campillo B, Bories PN, Devanlay M, Sommer F, Wirquin E, Fouet P. The thermogenic and metabolic effects of food in liver cirrhosis: consequences on the storage of nutrients and the hormonal counterregulatory response. Metab Clin Exp 1992; 41: 476–82.
5. 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.
6. Mezey E. Liver disease and nutrition. Gastroenterology 1978; 74: 770–83.
7. Diehl AM. Nutrition, hormones, metabolism, and liver regeneration. Semin Liver Dis 1991; 11: 315–20.
8. Avgerinos A, Harris D, Bousboulas S, Theodossiadou E, Komesidou V, Pallikari A, Raptis S, McIntyre M. The effect of a eucaloric high carbohydrate diet on circulating levels of glucose, fructose and non-esterified fatty acids in patients with cirrhosis. J Hepatol 1992; 14: 78–87.
9. Owen OE, Trapp VE, Reichard GA, Mozzoli MA, Moctezuma J, P Paul, Skutches CL, Boden G. Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. J Clin Invest 1983; 72: 1821–32.
10. Petrides AS, Vogt C, Schulze-Berge D, Matthews D, Strohmeyer G. Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. Hepatology 1994; 19: 616–27.
11. Kondrup J., Nielsen K., Hamberg O. Nutritional therapy in patients with liver cirrhosis. Eur J Clin Nutr 1992; 46: 239–46.
12. Schimpf RM, Lebrec D, Donadieu M. Somatomedin production in normal adults and cirrhotic patients. Acta Endocrinol 1977; 86: 355–62.
13. Tomas FM, Knowles SE, Owens PC, Chandler CS, Francis GL, Read LC, Ballard FJ. Insulin-like growth factor-I (IGF-I) and especially IGF-I variants are anabolic in dexamethasone-treated rats. Biochem J 1992; 282: 91–7.
14. Clemmons DR, Underwood LE.. Clinical review. 59. Uses of human insulin-like growth factor-I in clinical conditions. J Clin Endocrinol Metab 1993; 79: 4–6.
15. Lieberman S, Butterfield GE, Harrison D, Hoffman AR. Anabolic effects of recombinant insulin-like growth factor-I in cachectic patients with the acquired immunodeficiency syndrome. J Clin Endocrinol Metab 1994; 78: 404–10.
16. Clemmons DR. Editorial: role of insulin-like growth factor-1 in reversing catabolism. J Clin Endocrinol Metab 1992; 75: 1183–5.
17. Bondy CA, Underwood LE, Clemmons DR, Guler HP, Bach MA, Skarulis M. Clinical uses of insulin-like growth factor-I. Ann Intern Med 1994; 120: 593–601.
18. Camps J, Sola, J, Arroyo V, Pérez-Ayuso RM, Gaya J, Rivera F, Rodés J. Temporal relationship between the impairment of free water excretion and antidiuretic hormone hypersecretion in rats with experimental cirrhosis. Gastroenterology 1987; 93: 498–505.
19. Bieri JG, Stoewsand GS, Briggs GM, Phillips RW, Woodard JC, Knapka JJ. Report of the American Institute of Nutrition. Ad hoc Committee on standards for nutritional studies. J Nutr 1977; 107: 1340–8.
20. Bieri JG. Second report of the Ad hoc Committee on standards for nutritional studies. J Nutr 1980; 110: 1726.
21. Oliveira AC, Sgarbieri VC. The influence of rat endogenous nitrogen excretion on the assessment of bean protein quality. J Nutr Sci Vitaminol 1986; 32: 425–36.
22. Wuzke A. A century of Kjeldahl's nitrogen determination. Z Med Laboratoriumsdiagn 1985; 26: 383–7.
23. Hauck RD. Nitrogen isotope ratio analysis. In: Page AL, Miller RH, Keeney DR, eds. Methods of Soil Analysis. Madison: A.S.A., 1982: 735–79.
24. Daughaday WH, Mariz IK, Blethen SL. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. J Clin Endocrinol Metab 1980; 51: 781–8.
25. Breier BH, Gallaher BW, Gluckman PD. Radioimmunoassay for insulin-like growth factor-I: solutions to some potential problems and pitfalls. J Endocrinol 1991; 128: 347–57.
26. Hossenlopp P, Seurin D, Segovia-Quinson B, Hardouin S, Binoux M. Analysis of serum insulin-like growth factor binding proteins using western blotting: use of the method for titration of the binding proteins and competitive binding studies. Anal Biochem 1986; 154: 138–43.
27. Hardouin S, Gourmelen M, Noguiez P, Seurin D, Roghani M, Le Bouc Y, Povoa G, Merimee JT, Hossenlopp P, Binoux M. Molecular forms of serum insulin-like growth factor (IGF)-binding proteins in man: relationships with growth hormone and IGFs and physiological significance. J Clin Endocrinol Metab 1989; 69: 1291–301.
28. Dawson CD, Jewell S, Druskell WJ. Liquid-chromatographic determination of total hydroxyproline in urine. Clin Chem 1988; 34: 1572–4.
29. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248–54.
30. Fujiwara K, Ogata I, Otha Y, Hayashi S, Mishiro S, Takatsuki K, Sato Y, Yamada S, Hirata K, Oka H, Oda T, Kawaji H, Matsuda S, Niyama Y, Tsukuda R. Decreased collagen accumulation by a prolyl hydroxylase inhibitor in pig serum-induced fibrotic rat liver. Hepatology 1988; 8: 804–7.
31. Smanik EJ, Mullen KD, Giroski WG, McCullough AJ. The influence of portocaval anastomosis on gonadal and anterior pituitary hormones in a rat model standardized for gender, food intake, and time after surgery. Steroids 1991; 56(6): 237–41.
32. 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.
33. Proctor E, Chatamra K. Standardized micronodular cirrhosis in the rat. Eur Surg Res 1984; 16: 182–6.
34. Ariosto F, Riggio O, Cantafora A, Colucci S, Gaudio E, Machelli C, Merli M. Carbon tetrachloride-induced experimental cirrhosis in the rat. A reappraisal of the model. Eur Surg Res 1989; 21: 280–6.
35. Blond-Cynober F, Plassart F, Rey C, Coudray-Lucas C, Moukarbel N, Poupon R, Giboudeau J, Cynober L. Assessment of the carbon tetrachloride-induced cirrhosis model for studies of nitrogen metabolism in chronic liver disease. Ann Nutr Metab 1994; 38: 238–48.
36. McCullough A. Disorders of nutrition and intermediary metabolism in cirrhosis. In: Rector WG, ed. Complications of Chronic Liver Disease. St. Louis: Mosby Year Book, 1992: 182–211.
37. Novin D, Robinson K, Culbreth L, Tordoff MG. Is there a role for the liver in the control of food intake? Am J Clin Nutr 1985; 42: 1050–62.

38. Mendenhall CL, Anderson S, Weesner RE, Goldberg SJ, Crolic KA. Protein-calorie malnutrition associated with alcoholic hepatitis. Am J Med 1984; 76: 211–22.
39. Eriksson LS, Persson A, Wahren J. Branched chain amino acids in the treatment of chronic hepatic encephalopathy. Gut 1982; 23: 801–6.
40. Castilla-Cortázar I, Urdaneta E, Núñez M, Zugasti A, Muguerza B, Pascual M, Cemborain A, Quiroga J, Prieto J, Santidrián S. The reduced intestinal transport of D-galactose exhibited by liver cirrhotic rats is normalized by low doses of IGF-I. FASEB J 1996; 10: A2200.
41. Miura S, Asakura H, Munakata Y, Kobayashi K, Yoshioka M, Morishita T, Tsuchiya M. Lymphatic role in the pathogenesis of fat malabsorption in liver cirrhosis in rats. Dig Dis Sci 1982; 27: 1030–6.
42. Sarfeh IJ, Aaronson S, Lombino D, Rypins EB, Mason GR, Dadufalza L, Hollander D. Selective impairment of nutrient absorption from intestines with chronic venous hypertension. Surgery (St. Louis) 1986; 99: 166–9.
43. Heymsfield SB, Waki M, Reinus J. Are patients with chronic liver disease hypermetabolic? Hepatology 1990; 11: 502–4.
44. Mullen KD, Denne SC, McCullough AJ, SM Savini, D Bruno, AS Tavill, SC Kalhan. Leucine metabolism in stable cirrhosis. Hepatology 1986; 6: 622–30.
45. Schneeweiss B, Graninger W, Ferenci P, Eichinger S, Grimm GF, Schneider B, Laggner AN, Lenz K, Kleinberger G. Energy metabolism in patients with acute and chronic liver disease. Hepatology 1990; 11: 387–93.
46. John WJ, Phillips R, Ott L, Adams LJ, McLain CJ. Resting energy expenditure in patients with alcoholic hepatitis. J Parenter Enter Nutr 1989; 13: 124–7.
47. Shanbhogue RLK, Bistrian BR, Jenkins RL, Jones C, Benotti P, Blackburn. Resting energy expenditure in patients with end-stage liver disease and in normal population. J Parenter Enter Nutr 1987; 11: 305–8.
48. Petrides AS, Defronzo RA. Glucose metabolism in cirrhosis: A review with some perspectives for the future. Diabetes Metab Rev 1989; 5: 691–709.
49. Tomas FM, Knowles SE, Chandler CS, Francis GL, Owens PC, Ballard FJ. Anabolic effects of insulin-like growth factor-I (IGF-I) and an IGF-I variant in normal female rats. J Endocrinol 1993; 137: 413–21.
50. Zoli M, Marchesini G, Dondi C, Pisi E. Myofibrilar protein catabolic rates in cirrhotic patients with and without muscle wasting. Clin Sci 1982; 62: 683–6.
51. Marchesini G, Zoli M, Dondi C, Pisi E. Anticatabolic effect of branched-chain amino acid-enriched solutions in patients with liver cirrhosis. Hepatology 1982; 2: 420–5.
52. Jacob R, Barrett E, Plewe G, Fagin KD, Sherwin RS. Acute effects of insulin-like growth factor I on glucose and amino acid metabolism in the awake fasted rat. Comparison with insulin. J Clin Invest 1989; 83: 1717–23.
53. Russell-Jones DL, Umpleby AM, Hennessy TR, Bowes SB, Shojaee-Moradie F, Hopkins KD, Jackson NC, Kelly JM, Jones RH, Sonksen PH. Use of leucine clamp to demonstrate that IGF-I actively stimulates protein synthesis in normal humans. Am J Physiol 1994; 267: E591-8.
54. Hong D, Forsberg EN. Effects of serum and insulin-like growth factor I on protein degradation and protease gene expression in rat L8 myotubes. J Anim Sci 1994; 72: 2279–88.
55. Waterlow JC, Garlick PJ, Millward DJ. In: Protein Turnover in Mammalian Tissues and in the Whole Body. Amsterdam: North Holland, 1980.
56. Hattori N, Kurahachi H, Ikekubo K, Ishihara T, Moridera K, Hino M, Saiki Y, Imura H. 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.
57. Moller S, Gronbaek M, Main K, Becker U, Skakkebaek NE.. Urinary growth hormone (U-GH) excretion and serum insulin-like growth factor 1 (IGF-1) in patients with alcoholic cirrhosis. J Hepatol 1993; 17: 315–20.
58. Caufriez A, Reding P, Urbain D, Goldstein J, Copinschi G. Insulin-like growth factor-I: a good indicator of functional hepatocelular capacity in alcoholic liver cirrhosis. J Endocrinol Invest 1991; 14: 317–21.
59. Sara VR, Hall K. Insulin-like growth factors and their binding proteins. Physiol Rev 1990; 70: 591–614.
60. Young SCJ, Clemmons DR. Changes in insulin-like growth factor (IGF)-binding proteins after IGF-I injections in non-insulin dependent diabetics. J Clin Endocrinol Metab 1994; 78: 609–14.