

Effect of insulin-like growth factor-I on nitrogen balance and intestinal galactose transport in rats with moderate liver cirrhosis

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The malnutrition caused by liver cirrhosis (LC) often worsens the course of the disease. Patients affected by LC often have a low bioavailability of the anabolic liver peptide insulin-like growth factor (IGF)-I. The present study was undertaken to investigate the effect of low doses of IGF-I on the nutritional status and *in vivo* jejunal transport of D-galactose in anatomically, pathologically and biochemically confirmed moderate, non-ascitic, cirrhotic rats. LC was experimentally induced in growing rats by inhalation of CCl₄ and addition of phenobarbital to drinking water. Both the nutritional status, as evaluated by N balance, and *in vivo* intestinal transport of D-galactose, were significantly impaired in cirrhotic rats. As compared with healthy rats, administration of 20 µg human recombinant IGF-I/kg body weight for 14 d to cirrhotic rats significantly improved N balance variables and restored *in vivo* intestinal transport of the sugar. However, IGF-I had no effect on the steatorrhoea associated with LC. These results suggest that low doses of IGF-I may have beneficial effects on the malnutrition associated with moderate LC.

Cirrhosis: Insulin-like growth factor-I: Nitrogen balance: Intestinal absorption

It is well documented that patients affected by liver cirrhosis (LC) often have malnutrition, which further exacerbates liver disease (McCullough & Tavill, 1991; Muñoz, 1991; Merli *et al.* 1992). The ascitis associated with advanced LC, mainly due to reduced hepatic albumin synthesis as well as to portal hypertension, resembles that of protein malnutrition (kwashiorkor) (Cabre & Gassull, 1999). Moreover, patients with LC may have reduced concentrations of lecithins and bile salts, and thus reduced ability to absorb lipids (Greco *et al.* 1998). As a consequence, the nutritional and metabolic disturbances associated with malnutrition trigger some of the pathophysiological events linked to LC (Mezey, 1978) and increases morbidity in patients with LC (O'Keefe *et al.* 1980; Abad *et al.* 1987; McCullough, 1992), but the physiological events related to nutritional status in patients with LC are poorly understood (Kondrup *et al.* 1992).

On the other hand, plasma levels and bioavailability of insulin-like growth factor (IGF)-I, an anabolic peptide synthesized mainly in the liver upon the active stimulation by somatotropin, are reduced in patients with LC (Schimpff *et al.* 1977; Hattori *et al.* 1992; Moller *et al.* 1993; Donaghy *et al.* 1995). It is well known that growth hormone activates Jak2, which leads to stimulation of growth, a specific effect mediated mainly by stimulation of IGF-I transcription. In addition, ligand binding to the

growth hormone receptor is known to have insulin-like metabolic effects (Butler *et al.* 2002). There is a strong negative correlation between the degree of LC and plasma concentrations of IGF-I (Donaghy *et al.* 1995; Moller *et al.* 1995). The lower IGF-I plasma levels, the higher the severity of the hepatopathy (Scholmerich *et al.* 1987; Canfriez *et al.* 1991; Donaghy *et al.* 1995). It has been shown that IGF-I bioavailability depends not only upon plasma levels of the free peptide, but also on its affinity to plasma binding proteins, especially binding protein-3 (Imai *et al.* 2000). While hepatocytes have a reduced IGF-I receptor population, enterocytes express them abundantly (Corkins *et al.* 1999; MacDonald, 1999) and therefore plasma free IGF-I levels may influence intestinal physiology and be involved in the malnutrition related to LC through impairment of nutrient absorption.

In addition, the nutritional status influences the expression of both IGF-I and IGF-I-binding protein (Bang & Hall, 1992), with reduced levels of plasma IGF-I found in undernourished animals and human subjects (Holt *et al.* 1998). These levels are commonly used as sensitive markers of nutritional status (Imai *et al.* 2000). Very recently, some authors (Carro *et al.* 2002) have shown that IGF-I is a physiological regulator of brain amyloid levels.

We recently showed that in intestinal brush border membrane vesicles, the reduced transport of galactose and amino

acids shown by LC rats was corrected by IGF-I administration (Castilla *et al.* 1997b; Pascual *et al.* 2000). Since IGF-I levels correlate well with the degree of LC and nutritional status, the aim of the present study was to further investigate whether the administration of the anabolic peptide to cirrhotic rats was capable of improving N balance and nutritional performance of laboratory animals.

Materials and methods

Animals and experimental design

All the experimental procedures were performed in accordance with *The Guiding Principles for Research Involving Animals* (National Academy of Sciences, 1991). Male Wistar rats (*n* 60), body weight (BW) approximately 160–170 g, were housed in suspended plastic-bottomed mesh cages (four or five animals per cage) in an air-conditioned room at $22 \pm 2^\circ\text{C}$ on a 12 h light–dark cycle (lights on at 06.00 hours). Animals were fed a standard commercial diet (Purina diet; Purina, Valladolid, Spain). LC was induced by CCl_4 (Merck, Madrid, Spain) inhalation. The organic solvent was administered two times per week, with a progressively increasing exposure time (ranging from 1 to 5 min) during the 11 weeks. To accelerate development of LC, phenobarbital (Phen) (Luminal[®]; Bayer, Leverkusen, Germany) was added to the drinking water (400 mg/l) throughout the entire period of cirrhosis induction, beginning 1 week before the first CCl_4 exposure (Chatamra & Proctor, 1981; Ariosto *et al.* 1989).

The study protocol was approved by the Committee of Animal Care of the Centro de Investigación de Farmacología Aplicada (CIFA) of the University of Navarra and was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Beginning on day 0 of the experimental period, two groups of CCl_4 -induced cirrhotic rats received either saline (9 g NaCl/l; group CI) or subcutaneous human recombinant IGF-I (20 $\mu\text{g}/\text{kg}$ BW per d; group CI + IGF-I) for 2 weeks. Three other groups of healthy rats (*n* 12 per group) were used as control animals; one group received saline (9 g NaCl/l) during the last 2 weeks of the study (group CO). The other two groups were set up to further explore the effect of IGF-I and Phen on healthy rats: one received IGF-I (20 $\mu\text{g}/\text{kg}$ BW per d; CO + IGF-I), and the other only Phen throughout the entire experimental period (CO + Phen). Excluding the cirrhosis induction, these three CO groups were subjected to the same protocol as CI rats.

At the beginning and the end of the experiment, blood (about 3 ml) was collected from the retroocular plexus in all rats for biochemical and hormonal determinations. After 14 d of peptide administration and without interrupting IGF-I injections, seven rats per group were housed in metabolism cages (Tecniplast 3701MO-000; Panlab, Barcelona, Spain) for 6 d so that food, urine and faeces could be collected in order to carry out the N balance study (Maxvold *et al.* 2000). Determination of total fat (total diethyl ether extract) in faeces was carried out (Longwell & Stiles, 1968) as an indirect approach to assess intestinal lipid absorption. Once the metabolic study was over, lightly

anaesthetized rats were killed by decapitation. The jejunum, liver, testicles, several muscles, femur and spleen were carefully dissected out and weighed. Liver samples were processed for histological examination and sections of jejunum were taken for functional and histological studies.

In vivo intestinal absorption of D-galactose

The *in vivo* absorption of D-galactose by jejunal loops was assessed by the validated method of Ponz *et al.* (1979). Once the metabolic study period was over, animals (*n* 5) were fasted overnight with free access to water. Rats were anaesthetized with subcutaneous injections of sodium thiopental (40 mg/kg BW). A 200 mm mid-jejunum loop was hooked between two glass cannulas and connected to a perfusion system via a constant flow electric pump (Microperpex model 2123; LKB, Stockholm, Sweden). The jejunal loop was replaced in the abdomen and rinsed free of intestinal contents with Ringer's solution (mm: NaCl 140, KHCO_3 10, KH_2PO_4 0.4, K_2HPO_4 2.4, CaCl_2 1.2, MgCl_2 , 1.2) at 37°C . For the entire experimental period, animals were kept in a glass chamber where temperature was maintained at $25\text{--}30^\circ\text{C}$ with the help of electric blankets and all perfusions were made at 37°C . After the initial wash, the loop was perfused for 15 min with Ringer's solution + 2.0 mm-D-galactose and 3.700 kBq D-[1- ^{14}C]galactose/ml (1184–1331 MBq/mmol; Amersham International) through a tube connected to a peristaltic pump (Microperpex; LKB) at a flow rate of 2 ml/min. At the end of this time, the intestine was flushed out with saline (9 g NaCl/l) for 5 min. Both effluents were collected separately and concentration of D-galactose absorbed was calculated as the difference between the amount of sugar in the solution before and after the perfusion period plus the substrate remaining in the final washing solution. Radioactive samples were counted in a Hisafe LKB liquid scintillator in a β counter (Wallac 1409/11; LSC, Pharmacia, Finland). Results were expressed as μmol D-galactose/15 min per mm jejunum.

Laboratory studies and histological procedures

Serum levels of albumin, total protein, glucose, cholesterol, bilirubin, alkaline phosphatase and aminotransferases (aspartate aminotransferase and alanine aminotransferase) were determined by routine laboratory methods using a Hitachi 747 autoanalyser (Boehringer-Mannheim, Lever Russeu, Germany) at the beginning and at the end of the experimental period. Conventional histological techniques for optical microscopy were carried out using a light projection microscope (Micro Promar, Wetzlar, Germany). Four or five segments of jejunum were evaluated in each animal by two different observers. In addition, insulin (Insik-5[®]; Sorin Biomedica, Stockholm, Sweden), IGF-I (DSL-2900 Rat IGF-I Radioimmunoassay; Sorin Biomedica), growth hormone (Rat GH assay with Amerlex-M[™]; Amersham Biosciences, London, UK) (inter- and intra-assay CV (%) for these assays were: insulin 3.2, IGF-I 5.4, growth hormone 2.4), thyroid-stimulating hormone (Rat TSH assay system with Amerlex-M[™]), triiodothyronine

and thyroxine (Coat-A-Count[®] Total T₃ DPC and T₄-CTRIA; CIS Bio International, San Francisco, CA, USA) were assessed in the samples of blood taken at the end of the N balance study.

Histological scoring of liver fibrosis

In liver sections stained with Masson's Trichrome, semi-quantitative assessment of fibrosis was performed blindly by using a numerical scoring system based on the number, length and thickness of fibrous septa, each one scored from one to four points depending on their severity (light projection microscope, $\times 4$ magnification; Micro Promar Leitz GmbH, Wetzlar, Germany) (Fujiwara *et al.* 1988). The minimal degree of fibrosis (score one point) was ascribed to rats that showed a mild fibrosis in the periportal space and around the central veins. Maximum length of the septa (four points) was considered as greatest when there was confluence between the portal tracts and around the central veins. LC was scored as two or three when intermediate lengths of septa were observed. On the other hand, the number of collagen fibres was scored between one and four as well. A score of four points was recorded when the septa extended into the nodules surrounding one or two hepatocytes ($\times 10$ and $\times 20$ magnification). A score of one to three points was given when fibre confluence or penetration into nodules was less pronounced. Fibrous septa width, calculated at $\times 10$ magnification, was scored highest (four) when the degree of thickness observed was maximum. For each preparation, four fields were observed twice by two different and independent observers. The arithmetical mean value of these scores was taken as the final score.

Statistical analyses

Results were expressed as mean values with their standard errors. Statistical analysis was done by one-way ANOVA in combination with Tukey's test. Any *P* value < 0.05 was considered to be statistically significant. Calculations were performed with the SPSSW program, version 6.0 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows that the procedure followed to induce liver cirrhosis by CCl₄ inhalation and Phen added to drinking water was efficient. Besides the significantly lower growth in CI rats, plasma transaminases, alkaline phosphatase, bilirubin and total cholesterol were markedly increased in CI animals as compared with healthy untreated rats. Glycaemia, proteinaemia, and urinary urea were significantly reduced in CI rats. No changes were noted in urinary creatinine or plasma non-esterified fatty acids.

Observation of the animals revealed no sign of ascitis in any of the CI rats. In addition, the histological observation of liver revealed the presence of micronodular or macromicronodular cirrhosis. The histological score of fibrosis was significantly lower ($P < 0.01$) in CI + IGF-I than in CI animals, with both groups showing higher scores than CO rats. The electron microscopy of jejunum sections showed

Table 1. Body weight and plasma liver function variables in rats at the start of the experimental period†
(Mean values with their standard errors)

Variable	CO (n 40)		CI (n 40)	
	Mean	SEM	Mean	SEM
Weight (g)	533.0	17.0	442.0***	11.0
AST (IU/l)	549	52	2966***	491
ALT (IU/l)	259	24	2735***	494
Glucose (mmol/l)	2210	150	1170*	60
Total protein (g/l)	68.6	0.6	63.9*	1.8
Total cholesterol (mg/l)	816	39	1155***	49
Alkaline phosphatase (IU/ml)	310.0	43.0	701.0**	146.0
Bilirubin (mg/l)	4.4	0.4	121**	3.4
Urea (g/l)	39.6	1.7	28.2**	2.7
Creatinine (mg/l)	5.9	0.3	5.0	0.2
Non-esterified fatty acids (mmol/l)	0.48	0.03	0.46	0.06

CO, control group; CI, CCl₄-induced cirrhotic group; AST, aspartate amino-transferase; ALT, alanine aminotransferase.

Mean values were significantly different from those of the CO: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details of procedures, see p. 930.

microvilli enlargement. All present histological observations were similar to those previously reported by our group in recent publications (Castilla *et al.* 1997a, 2000).

The effects of IGF-I on growth, relative organ and muscles weights, and N balance variables in CI rats, as well as those related to the effect of Phen and IGF-I on CO rats are shown in Tables 2 and 3. No changes were observed in organ or muscles weights, with the exception of characteristic splenomegaly in both CI and CI + IGF-I rats and liver enlargement caused by Phen administration (Table 2). No major differences in growth were noted as a consequence of the different treatments. N balance data showed that N retention, expressed per kg BW, was significantly reduced in CI rats as compared with either CO group (Table 3).

Administration of IGF-I to CI rats raised ($P < 0.05$) N retention to the value of healthy CO animals. A similar pattern was observed with regard to digestibility (true digestibility coefficient), true biological value and net protein utilization. Administration of Phen to CO rats caused a decrease in digestibility, biological value and net protein utilization (Table 3). Administration of IGF-I to control rats had almost no effect on any of the variables assessed.

On the other hand, the effects of IGF-I on liver function variables in CI rats, as well as those related to the effect of Phen and IGF-I on CO rats are shown in Table 4. Administration of IGF-I to CI rats significantly improved proteinaemia, albuminaemia, alkaline phosphatase, cholesterolaemia and glycaemia. While the administration of IGF-I to CO rats had no effect on liver function variables, a hypoglycaemic effect was observed in CO rats treated with Phen.

The intestinal lipid absorption, as measured by total lipid faeces content (Table 5) shows that the significant ($P < 0.01$) steatorrhoea observed in CI rats was not affected by IGF-I.

The markedly reduced *in vivo* intestinal capacity to actively transport galactose by jejunum in CI rats (0.025 (SEM 0.001) $\mu\text{mol D-galactose/15 min per mm jejunum}$)

Table 2. Effect of treatment with insulin-like growth factor-I on body weight (g) and organ weights (g/kg body weight) in healthy control rats and carbon tetrachloride-induced cirrhotic rats at the end of the 6 d N-balance period†
(Mean values with their standard errors)

Variable	CO (n 12)		CO + Phen (n 12)‡		CO + IGF-I (n 11)§		CI (n 10)		CI + IGF-I (n 11)§	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body weight	545	14	543	13	539	18	460***	12	458***	9
Liver	29.9	0.7	35.7***	0.7	28.3	0.5	35.9	1.5	35.0	1.8
Spleen	1.5	0.1	1.5	0.1	1.5	0.1	3.2***	0.4	3.0***	0.3
Jejunum	5.9	0.2	5.4	0.3	6.1	0.3	6.1	0.2	6.5	0.3
Right kidney	2.4	0.1	2.3	0.1	2.4	0.1	2.7	0.1	2.7	0.1
Right testicle	3.6	0.1	3.6	0.1	3.4	0.1	3.5	0.1	3.6	0.1
Right femur	2.5	0.1	2.4	0.1	2.4	0.1	2.2	0.2	2.5	0.1
Gastrocnemius	5.3	0.1	5.5	0.2	5.4	0.2	5.9**	0.2	5.8*	0.1
Soleus	0.6	0.2	0.4	0.1	0.5	0.1	0.4	0.1	0.4	0.1
EDL	0.4	0.1	0.4	0.1	0.4	0.1	0.4	0.1	0.4	0.1

CO, control group; Phen, phenobarbital; IGF, insulin-like growth factor; CI, CCl₄-induced cirrhotic group; EDL, extensor digitorum longus. Mean values were significantly different from those of the CO: **P*<0.05, ***P*<0.01, ****P*<0.001.

† For details of procedures, see p. 930.

‡ Group received 400 mg Phen/l drinking water throughout the experimental period.

§ Groups received 20 µg IGF-I/kg body weight per d subcutaneously for 14 d.

Table 3. Effect of treatment with insulin-like growth factor-I on nutritional variables in healthy control rats and carbon tetrachloride-induced cirrhotic rats at the end of the 6 d N-balance period§
(Mean values with their standard errors)

Variable	CO (n 12)		CO + phen (n 12)		CO + IGF-I (n 12)¶		CI (n 10)		CI + IGF-I (n 11)¶	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Initial wt (g)	533	17	504	11	523	13	442***	11	448***	8
Final wt (g)	543	14	517	12	534	17	457***	11	459***	8
Weight gain (g/15 d)	9.2	6.4	12.7	2.6	-2.8	0.7	14.9	1.7	11.3	1.8
Body wt (g)	540	14	509	11	535	16	448†††	11	453††	8
Food intake (g/kg body wt)	276	11	293	8	264	11	283	8	311*	9
Water intake (ml/kg body wt)	439	17	460	21	381	30	481	35	415	39
BGI (initial wt/final wt)	1.02	0.01	1.02	0.01	0.99	0.01	1.03	0.01	1.02	0.04
N balance										
N retained (g)	1.37	0.10	0.98*	0.06	1.40	0.10	0.85*	0.10	1.19	0.11
N retained (g/kg body wt)	2.5	0.2	1.9	0.1	2.6	0.2	1.8*	0.2	2.4	0.2
TDC (N absorbed × 100/N ingested)	83.20	0.49	81.16**	0.41	84.80**	0.37	81.08**	0.39	82.30‡	0.37
TBV (N retained/N absorbed)	41.20	2.31	31.05*	1.41	43.90	2.65	32.02*	3.95	36.37	3.47
NPU (TDC × TBV/100)	34.35	2.04	25.21*	1.17	37.27	2.35	26.02*	3.26	29.91	2.84

CO, control group; Phen, phenobarbital; IGF, insulin-like growth factor; CI, CCl₄-induced cirrhotic group; BGI, body growth index; TDC, true digestibility coefficient; TBV, true biological value.

Mean values were significantly different from those of the CO: **P*<0.05, ***P*<0.01, ****P*<0.001.

Mean values were significantly different from those of the CO + Phen: ††*P*<0.01, †††*P*<0.001

Mean value was significantly different from that of the CI: ‡*P*<0.05.

§ For details of procedures, see p. 930.

|| Group received 400 mg Phen/l drinking water throughout the experimental period.

¶ Groups received 20 µg IGF-I/kg body weight per d subcutaneously for 14 d.

was completely restored (*P*<0.001) to normal values by the administration of IGF-I (0.035 (SEM 0.001) µmol D-galactose/15 min per mm jejunum).

Finally, Table 6 shows that CI rats did not have reduced plasma IGF-I levels, regardless of treatment, although reduced growth and thyroid-related hormones levels were observed in these animals.

Discussion

The results of the present study demonstrate that the administration of 20 µg IGF-I/kg BW had no effect on

liver function in healthy CO rats, supporting the fact that hepatocytes are insensitive to their own IGF-I (Ross *et al.* 1996). The only effect of the peptide in these animals was a dietary independent increase in true digestibility coefficient, an effect that could be related to an increase in the intestinal transport of nutrients, as will be pointed out later.

Since LC induction involved the ingestion of Phen through drinking water, it seemed convenient to assess to what extent the barbiturate itself might affect the nutritional status of experimental animals. Our findings showed that the effects of Phen were twofold. First, there

Table 4. Effect of treatment with insulin-like growth factor-I on plasma liver function variables in healthy control rats and carbon tetrachloride-induced cirrhotic rats at the end of the 6 d N-balance period‡
(Mean values with their standard errors)

Variable	CO (n 12)		CO + Phen (n 12)§		CO + IGF-I (n 10)		CI (n 10)		CI + IGF-I (n 11)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
AST (IU/l)	368	33	362	39	375	2.0	797	146	843	189
ALT (IU/l)	194	13	215	19	214	14	401***	44	411***	59
Total protein (g/l)	64.9	0.8	67.1	0.9	65.4	0.9	61.7*	0.9	65.4	0.9
Albumin (g/l)	34.1	0.3	34.5	0.6	34.6	0.3	29.1***	0.4	31.1****†	0.9
AP (IU/ml)	283	17	239	10	263	14	489***	66	398†	30
Bilirubin (mg/l)	4.0	0.8	4.8	0.4	3.1	0.4	9.9*	2.8	5.6	1.4
Cholesterol (mg/l)	826	41	894	46	743	48	1471*	320	1141	69
Glucose (mmol/l)	1920	70	1640**	80	1920	100	1610**	60	1900†	110
Non-esterified fatty acids (mmol/l)	0.37	0.03	0.37	0.03	0.36	0.03	0.45	0.04	0.36	0.04
Urea (g/l)	33.4	1.3	32.6	1.1	36.2	1.2	29.0**	1.3	31.9	1.7
Creatinine (mg/l)	0.56	0.03	0.47**	0.01	0.54	0.01	0.43***	0.01	0.44***	0.02
Na ⁺ (mmol/l)	140.9	0.6	139.1*	0.7	141.2	0.2	139.3	0.4	139.9	0.4
K ⁺ (mmol/l)	4.8	0.15	4.8	0.15	4.9	0.14	4.4	0.18	4.5	0.14
Cl ⁻ (mmol/l)	107.6	0.6	105.4*	0.7	106.8	0.6	106.4	0.6	106.9	0.6
Ca ²⁺ (mmol/l)	2.46	0.03	2.42	0.02	2.44	0.21	2.34	0.02†	2.39	0.02

CO, control group; Phen, Phenobarbital; IGF, insulin-like growth factor; CI, CCl₄-induced cirrhotic group; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase.

Mean values were significantly different from those of the CO: **P*<0.05, ***P*<0.01, ****P*<0.001.

Mean values were significantly different from those of the CI: †*P*<0.05.

‡ For details of procedures, see p. 930.

§ Group received 400 mg Phen/l drinking water throughout the experimental period.

|| Groups received 20 µg IGF-I/kg body weight per d subcutaneously for 14 d.

Table 5. Effect of treatment with insulin-like growth factor-I on faecal variables in healthy control rats and carbon tetrachloride-induced cirrhotic rats at the end of the 6 d N-balance period‡
(Mean values with their standard errors)

Variable	CO (n 12)		CO + Phen (n 12)‡		CO + IGF-I (n 12)§		CI (n 10)		CI + IGF-I (n 11)§	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Faeces weight										
Total (g/6 d)	22.1	0.9	22.7	1.2	19.7	1.1	20.5	0.6	20.2	0.7
g/kg body wt	40.5	1.7	44.4	1.6	35.6*	1.6	45.0	0.1	44.6	1.6
Fat in faeces										
g/d	0.11	0.008	0.137	0.01	0.093	0.003	0.136	0.009	0.136	0.009
%	3.07	0.16	3.57	0.20	3.57	0.20	3.98**	0.27	4.04**	0.23

CO, control group; Phen, Phenobarbital; IGF, insulin-like growth factor; CI, CCl₄-induced cirrhotic group.

Mean values were significantly different from those of the CO: **P*<0.05, ***P*<0.01.

‡ For details of procedures, see p. 930.

‡ Groups received 400 µg Phen/l drinking water throughout the experimental period.

§ Groups received 20 µg IGF-I/kg body weight per d subcutaneously for 14 d.

was reduced digestibility and biological value of the ingested protein: this could be associated with the depressive action of Phen on the hypothalamic centres that control appetite (Johnston & File, 1989), as well as with a reduction in the general enzymatic capacity of the liver (Dube *et al.* 1991; Krähenbuhl *et al.* 1991). Second, there was a reduction of plasma glucose levels, which may be related to the effect of Phen on the activity of hepatic glycolytic or gluconeogenic enzymes.

The use of CCl₄ inhalation with oral Phen is a well-validated method used by our group and by other workers to induce the metabolic, haemodynamic and biochemical alterations of LC in rats, resembling that caused by alcohol in human subjects (Epstein, 1990; Plaa & Charbonneau, 1994). In the present study, a mixed micronodular pattern

of LC accompanied by mild portal hypertension, and thereby mild signs of ascitis, was established. Most of the biochemical and pathological criteria related to tissue damage and cholestasis featuring LC were fulfilled in the present study (Mullen & McCullough, 1989). The choice of the dose of 20 µg IGF-I/kg BW was based on previous observations (Castilla *et al.* 1997b; Picardi *et al.* 1997) suggesting that this is the optimal dose to counteract many of the adverse effects caused by experimental LC (Castilla *et al.* 1997a). In this, as well as previous studies, plasma levels of IGF-I in CI rats were similar to those of CO animals. Liver IGF-I synthesis diminished only when the hepatic disease is advanced (Donaghy *et al.* 1995; Castilla *et al.* 2000). Higher doses of IGF-I have been used to treat osteopaenia in postmenopausal women (Ebeling *et al.*

Table 6. Effect of treatment with insulin-like growth factor-I on plasma hormone levels in healthy control rats and carbon tetrachloride-induced cirrhotic rats at the end of the 6 d N-balance period‡
(Mean values with their standard errors)

Variable	CO		CO + Phen§		CI		CI + IGF-I	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
IGF-I (ng/ml)	2577	62	3590**	221	3232**	260	2826	226
GH (ng/ml)	38.30	1.17	36.40**	1.49	33.63**	0.90	21.64††	0.64
Insulin (mU/l)	41.92	2.46	40.44	4.56	45.33	3.45	37.48	3.82
TSH (ng/ml)	6.93	3.11	2.26	0.95	3.97	1.71	1.95*	1.22
T ₃ (nmol/l)	1.84	0.09	1.63	0.21	1.66	0.21	1.19*	0.16
T ₄ (nmol/l)	108.1	0.52	160.3***	7.0	105.7††	10.7	88.5††	4.5

CO, control group; Phen, Phenobarbital; IGF, insulin-like growth factor; CI, CCl₄-induced cirrhotic group, GH, growth hormone; TSH, thyroid-stimulating hormone; T₃, triiodothyronine; T₄, thyroxine.

Mean values were significantly different from those of the CO: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Mean values were significantly different from those of the CO-Phen: †† $P < 0.01$.

* For details of procedures, see p. 930.

§ Group received 400 mg Phen/l drinking water throughout the experimental period.

|| Group received 20 µg IGF-I/kg body weight per d subcutaneously for 14 d.

1993). Interestingly, although moderate LC does not affect plasma IGF-I levels, it may have some influence on IGF-I-binding proteins levels (Smith *et al.* 1995), and therefore on IGF-I bioavailability. However, low IGF-I levels are associated with adverse clinical outcomes in cirrhotic patients (Donaghy *et al.* 2002), as the liver is the primary source of circulating IGF-I. Measurement of IGF-I in the tissues of the growth hormone receptor knockout model (in which IGF-I mRNA expression is not reduced) will be needed to confirm that the post-transcriptional steps of IGF-I synthesis have been not affected (Lupu *et al.* 2001).

In human subjects, it is quite common to observe signs of protein–energy malnutrition only in advanced stages of LC, but not in moderate, compensated LC (Campillo *et al.* 1992; Müller *et al.* 1992). As compared with CO animals, cirrhotic rats had no differences in food intake and relative weights of bone, muscle, testicle and kidney. This emphasizes the fact that a moderate, compensated liver fibrosis was established in cirrhotic rats. Nevertheless, these animals exhibited a state of malnutrition characterized by decreased weight gain, hypoglycaemia, hypoproteinaemia, especially hypoalbuminaemia, and reduced N balance. In addition, total digestibility coefficient, total biological value and net protein utilization were reduced as well. As mentioned earlier, advanced LC is usually associated with skeletal muscle wasting (McCullough & Tavill, 1991), with the urinary output of *N*-methylhistidine (an indicator of myofibrillar protein breakdown) increased in cases of LC (Marchesini *et al.* 1982; Zoli *et al.* 1982). On the other hand, it has been reported that IGF-I may increase synthesis (Russell-Jones *et al.* 1994) and reduce breakdown of muscle proteins (Jacob *et al.* 1989). However, the degree of LC in the present study was not severe enough to cause changes in either soleus, a red, oxidative muscle with slow-twitch fibres, or in the extensor digitorum longus, a white, glycolytic muscle with fast-twitch fibres. The paradoxical increase of G weight, a mixed muscle composed of both red and white fibres, found in both CI and CI + IGF-I rats agrees with previously reported results (Picardi *et al.* 1997) and correlates with the reduced creatinuria. This finding may be related to

the special effect of LC on reducing adipose tissue in skeletal muscle (Hobler *et al.* 1998) or to a specific target action of the peptide on this big postural muscle.

The most relevant effect of the administration of IGF-I found in the present study was the significant improvement in all the nutritional variables assessed, such as food intake, plasma albumin, N balance, total digestibility coefficient, true biological value and net protein utilization. All of these measurements, reduced in CI rats, were partly or completely matched to control values in CI + IGF-I. In addition, cholestasis, as judged by plasma alkaline phosphatase, bilirubin and cholesterol values, was significantly improved in treated cirrhotic rats, but not to the point of correcting the steatorrhoea associated with cirrhotic rats (Salvioli *et al.* 1990; Merli *et al.* 1992). Several mechanisms may explain, at least partly, the beneficial effects of IGF-I. First, as has been reported by Castilla *et al.* (1997a), Holt *et al.* (1998) and Schalah *et al.* (1998), IGF-I caused an outstanding improvement in liver function. It has to be emphasized that fibrogenesis was reduced in CI rats receiving the dose of IGF-I used in the present study in accordance with previous findings (Muguerza *et al.* 1995; Castilla *et al.* 1997a). Second, a reduced food intake seems to play a role in the undernutrition observed in cirrhotic subjects (Muller, 1998). Although this extreme was not seen in our present study, the administration of IGF-I to CI rats caused an increase in food intake that might have contributed to improving the nutritional status, in accordance with results from previous studies (Picardi *et al.* 1997). It is accepted that the liver plays a central role in controlling appetite through the regulation of both glycaemia and aminoacidaemia (Felber & Galay, 1995). Moreover, a possible direct effect of IGF-I on the hypothalamic centres that control the sensation of hunger and satiety cannot be discarded. In any case, the increased food intake observed in CI + IGF rats was not associated with hypoglycaemic conditions, since plasma glucose levels were elevated in the treated animals. The low plasma level of glucose in CI rats is related to the insulin resistance, a well-documented characteristic of cirrhotic subjects (Petrides & DeFronzo, 1989). As a result, there

is a reduced muscle glycogen synthesis and a shift to fatty acid utilization and gluconeogenesis, at the expense of muscular amino acids, arising from reduced muscle protein synthesis or muscle protein increased breakdown (Mendenhall *et al.* 1984).

Despite the increased food intake and N retention, no body-weight gain was observed as a consequence of the treatment. This is in agreement with the finding of other workers (Picardi *et al.* 1997), and was not due to alterations in body water or Na balance.

Unlike findings of our previous studies (Picardi *et al.* 1997), the N bioavailability was reduced in CI rats and completely restored by the peptide. This difference is most probably explained by the degree of LC, severe enough in the study of Picardi *et al.* (1997) to significantly affect the N metabolism and amino acid intestinal transport. In more recent studies, we have shown that LC rats exhibited reduced intestinal transport of leucine, proline, glutamate and cysteine, accompanied by gut morphological disturbances characterized by microvilli enlargement (Pascual *et al.* 2000). Both functional and morphological alterations were restored by administering IGF-I at the same dose used in the present study. Therefore, the improvement in the intestinal amino acid transport seems to play a central role in the nutritional improvement caused by IGF-I in CI rats.

With regard to the reduced thyroid-stimulating hormone, thyroxine and triiodothyronine levels observed in both treated and untreated CI rats, it has been reported that there is an inverse correlation between plasma levels of thyroid hormones and of total cholesterol, LDL-cholesterol, hepatic synthesis of apolipoprotein B-100 and bile excretion, hepatic excretion of LDL (VLDL)-cholesterol and activity of LDL receptors and hydroxymethylglutaryl-CoA reductase (Potter, 1995). In addition, the decrease of triiodothyronine might be associated to a blunted thyroxine deiodinase type I activity, responsible for most of the conversion of thyroxine into triiodothyronine. This enzyme depends on the trace elements Zn and Se, and bioavailability of trace elements is impaired in liver disease (Scholmerich *et al.* 1987; Navarro-Alarcon & López, 2000).

The beneficial effect on dietary N bioavailability in CI rats caused by IGF-I treatment could be related to an improvement in monosaccharide metabolism caused by the peptide. Our present study showed that the *in vivo* absorption of D-galactose in jejunal loops was markedly decreased in CI rats and recovered to a large extent when those animals were treated with IGF-I for 5 d. As mentioned earlier, CI rats exhibited a marked microvilli enlargement that could alter the spatial insertion of the Na⁺-glucose transporter-1 within the microvilli lipid layer, thereby impairing the affinity of the protein to couple both Na⁺ and galactose (or glucose). This agrees with our previously published work in which specific Na⁺-glucose transporter-1 antibodies were used to stain the transporter in the microvilli (Castilla *et al.* 1997b) and may explain the modifications of both K_m and V_{max} found in the present study. Since IGF-I is capable of restoring microvilli structure, it seems feasible that, as a consequence, the transporter insertion in the membrane returns

to its physiological position, allowing recovery of the physiological capacity of the brush border to transport monosaccharides.

In conclusion, the administration of 20 µg human recombinant IGF-I/kg BW to moderately cirrhotic rats for 14 d significantly improved N balance variables and restored *in vivo* intestinal transport of sugars, while having no effect on the steatorrhoea caused by the disease. These results suggest that low doses of IGF-I may have beneficial effects on the malnutrition associated with moderate LC.

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