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; Hattori 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 acid uptake in vitro were improved in LC by treatment with human recombinant IGF-I (Leyba et al. 2002). These results suggest that IGF-I could also have a beneficial effect on the intestinal transport of D-galactose in vivo. These findings are of great interest for the treatment of liver cirrhosis, which is one of the main causes of morbidity and mortality worldwide.

**Abbreviations:** BW, body weight; CO, control group; CI, cirrhotic group; IGF, insulin-like growth factor; LC, liver cirrhosis; Phen, phenobarbital.

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acids shown by LC rats was corrected by IGF-I administra-
tion (Castilla et al. 1997b; Pascual et al. 2000). Since
IGF-I levels correlate well with the degree of LC and nutri-
tional 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 ± 2°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 CCl₄ (Mercck, Madrid, Spain) inhalation.
The organic solvent was administered two times per
week, with a progressively increasing exposure time (ran-
A small number of 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-jejunal
loop was hooked between two glass cannulas and con-
ected 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, KHCO₃ 10, KH₂PO₄ 0·4, K₂HPO₄ 2·4,
CaCl₂ 1·2, MgCl₂, 1·2) at 37°C. For the entire experimental
period, animals were kept in a glass chamber where tempera-
ture was maintained at 25–30°C with the help of elec-
tric 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 3·70 kBq
D-[1-¹⁴C]galactose/ml (1184–1331 MBq/mmol; Amer-
sham International) through a tube connected to a peristal-
tic 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 col-
llected separately and concentration of D-galactose
absorbed was calculated as the difference between the
amount of sugar in the solution before and after the per-
fusion 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 (Boerhringer-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 pro-
jection 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-M⁹; Amer-
sham 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-M⁹), triiodothyronine

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-jejunal
loop was hooked between two glass cannulas and con-
ected to a perfusion system via a constant flow electric
pump (Microperpex model 2123; LKB, Stockholm,
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fusion 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.
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, ×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 (×10 and ×20 magnification). A score of one to three points was given when fibroblasts or penetration into nodules was less pronounced. Fibrous septa width, calculated at ×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, proteininaemia, 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 macromiconodular 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 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 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 proteininaemia, albuminaemia, alkaline phosphatase, cholesterol 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) μmol 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)

<table>
<thead>
<tr>
<th>Variable</th>
<th>CO (n 12)</th>
<th>CO - Phen (n 12)‡</th>
<th>CO + IGF-I (n 11)§</th>
<th>CI (n 10)</th>
<th>CI + IGF-I (n 11)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>545 14</td>
<td>543 13</td>
<td>539 18</td>
<td>460*** 12</td>
<td>458*** 9</td>
</tr>
<tr>
<td>Liver</td>
<td>29·9 0·7</td>
<td>35·7*** 0·7</td>
<td>28·3 0·5</td>
<td>35·9 1·5</td>
<td>35·0 1·8</td>
</tr>
<tr>
<td>Spleen</td>
<td>1·5 0·1</td>
<td>1·5 0·1</td>
<td>1·5 0·1</td>
<td>3·2*** 0·4</td>
<td>3·0*** 0·3</td>
</tr>
<tr>
<td>Jejunum</td>
<td>5·9 0·2</td>
<td>5·4 0·3</td>
<td>6·1 0·3</td>
<td>6·1 0·2</td>
<td>6·5 0·3</td>
</tr>
<tr>
<td>Right kidney</td>
<td>2·4 0·1</td>
<td>2·3 0·1</td>
<td>2·4 0·1</td>
<td>2·7 0·1</td>
<td>2·7 0·1</td>
</tr>
<tr>
<td>Right testicle</td>
<td>3·6 0·1</td>
<td>3·6 0·1</td>
<td>3·4 0·1</td>
<td>3·5 0·1</td>
<td>3·6 0·1</td>
</tr>
<tr>
<td>Right femur</td>
<td>2·5 0·1</td>
<td>2·4 0·1</td>
<td>2·4 0·1</td>
<td>2·2 0·2</td>
<td>2·5 0·1</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>5·3 0·1</td>
<td>5·5 0·2</td>
<td>5·4 0·2</td>
<td>5·9** 0·2</td>
<td>5·8* 0·1</td>
</tr>
<tr>
<td>Soleus</td>
<td>0·6 0·2</td>
<td>0·4 0·1</td>
<td>0·5 0·1</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
</tr>
<tr>
<td>EDL</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
</tr>
<tr>
<td>Liver (g/kg body wt)</td>
<td>276 11</td>
<td>293 8</td>
<td>264 11</td>
<td>283 8</td>
<td>311* 9</td>
</tr>
<tr>
<td>Water intake (ml/kg body wt)</td>
<td>439 17</td>
<td>460 21</td>
<td>381 30</td>
<td>481 35</td>
<td>415 39</td>
</tr>
<tr>
<td>Liver wt (g/kg body wt)</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>0·99 0·01</td>
<td>1·03 0·01</td>
<td>1·02 0·04</td>
</tr>
<tr>
<td>N balance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N retained (g)</td>
<td>1·37 0·10</td>
<td>0·98* 0·06</td>
<td>1·40 0·10</td>
<td>0·85* 0·10</td>
<td>1·19 0·11</td>
</tr>
<tr>
<td>N retained (g/kg body wt)</td>
<td>2·5 0·2</td>
<td>1·9 0·1</td>
<td>2·6 0·2</td>
<td>1·8* 0·2</td>
<td>2·4 0·2</td>
</tr>
<tr>
<td>TDC (N absorbed) (100/N ingested)</td>
<td>83·20 0·49</td>
<td>81·16*** 0·41</td>
<td>84·80** 0·37</td>
<td>81·08** 0·39</td>
<td>82·30± 0·37</td>
</tr>
<tr>
<td>TBV (N retained/N absorbed)</td>
<td>41·20 2·31</td>
<td>31·05* 1·41</td>
<td>43·90 2·65</td>
<td>32·02* 3·95</td>
<td>36·37 3·47</td>
</tr>
<tr>
<td>NPU (TDC × TBV/100)</td>
<td>34·35 2·04</td>
<td>25·21* 1·17</td>
<td>37·27 2·35</td>
<td>26·02* 3·26</td>
<td>29·91 2·84</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>CO (n 12)</th>
<th>CO + Phen (n 12)‖</th>
<th>CO + IGF-I (n 12)¶</th>
<th>CI (n 10)</th>
<th>CI + IGF-I (n 11)¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein retention (g/kg body wt)</td>
<td>2·8 0·7</td>
<td>1·7 0·6</td>
<td>2·8 0·7</td>
<td>1·7 0·6</td>
<td>2·8 0·7</td>
</tr>
<tr>
<td>Plasma IGF-I (μg/ml)</td>
<td>29·9 1·8</td>
<td>35·7*** 1·7</td>
<td>29·9 1·8</td>
<td>35·7*** 1·7</td>
<td>29·9 1·8</td>
</tr>
<tr>
<td>Plasma TSH (μg/ml)</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
<td>0·4 0·1</td>
</tr>
<tr>
<td>Plasma fT4 (ng/ml)</td>
<td>0·6 0·1</td>
<td>0·6 0·1</td>
<td>0·6 0·1</td>
<td>0·6 0·1</td>
<td>0·6 0·1</td>
</tr>
<tr>
<td>Plasma T4 (μg/dl)</td>
<td>1·3 0·1</td>
<td>1·3 0·1</td>
<td>1·3 0·1</td>
<td>1·3 0·1</td>
<td>1·3 0·1</td>
</tr>
<tr>
<td>Brain weight (g)</td>
<td>15·9 0·3</td>
<td>15·9 0·3</td>
<td>15·9 0·3</td>
<td>15·9 0·3</td>
<td>15·9 0·3</td>
</tr>
<tr>
<td>Brain wt (g/kg body wt)</td>
<td>0·30 0·01</td>
<td>0·30 0·01</td>
<td>0·30 0·01</td>
<td>0·30 0·01</td>
<td>0·30 0·01</td>
</tr>
<tr>
<td>Liver wt (g/kg body wt)</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
</tr>
<tr>
<td>Liver wt (g/kg body wt)</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
</tr>
<tr>
<td>Liver wt (g/kg body wt)</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
</tr>
<tr>
<td>Liver wt (g/kg body wt)</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
</tr>
<tr>
<td>Liver wt (g/kg body wt)</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
<td>1·02 0·01</td>
</tr>
</tbody>
</table>

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Mean values were significantly different from those of the CO: *P<0·05, **P<0·01, ***P<0·001.
† 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.

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.

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.

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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.
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†

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<thead>
<tr>
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<th>CO (n 12)</th>
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<th>CO + IGF-I (n 10)¶</th>
<th>CI (n 10)</th>
<th>CI + IGF-I (n 11)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/l)</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>368</td>
<td>33</td>
<td>362</td>
<td>39</td>
<td>375</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>194</td>
<td>13</td>
<td>215</td>
<td>19</td>
<td>214</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>64·9</td>
<td>0·8</td>
<td>67·1</td>
<td>0·9</td>
<td>65·4</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>34·1</td>
<td>0·3</td>
<td>34·5</td>
<td>0·6</td>
<td>34·6</td>
</tr>
<tr>
<td>AP (IU/ml)</td>
<td>283</td>
<td>17</td>
<td>239</td>
<td>10</td>
<td>263</td>
</tr>
<tr>
<td>Bilirubin (mg/l)</td>
<td>4·0</td>
<td>0·8</td>
<td>4·8</td>
<td>0·4</td>
<td>3·1</td>
</tr>
<tr>
<td>Cholesterol (mg/l)</td>
<td>826</td>
<td>41</td>
<td>894</td>
<td>46</td>
<td>743</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>1920</td>
<td>70</td>
<td>1640</td>
<td>80</td>
<td>1920</td>
</tr>
<tr>
<td>Urea (g/l)</td>
<td>33·4</td>
<td>1·3</td>
<td>32·6</td>
<td>1·1</td>
<td>36·2</td>
</tr>
<tr>
<td>Creatinine (mg/l)</td>
<td>0·56</td>
<td>0·03</td>
<td>0·47**</td>
<td>0·01</td>
<td>0·54</td>
</tr>
<tr>
<td>Na⁺ (mmol/l)</td>
<td>140·9</td>
<td>0·6</td>
<td>139·1</td>
<td>0·7</td>
<td>141·2</td>
</tr>
<tr>
<td>K⁺ (mmol/l)</td>
<td>4·8</td>
<td>0·15</td>
<td>4·8</td>
<td>0·15</td>
<td>4·9</td>
</tr>
<tr>
<td>Cl⁻ (mmol/l)</td>
<td>107·6</td>
<td>0·6</td>
<td>105·4</td>
<td>0·7</td>
<td>106·8</td>
</tr>
<tr>
<td>Ca²⁺ (mmol/l)</td>
<td>2·46</td>
<td>0·03</td>
<td>2·42</td>
<td>0·02</td>
<td>2·44</td>
</tr>
</tbody>
</table>

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.
§ Groups 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†

<table>
<thead>
<tr>
<th>Variable</th>
<th>CO (n 12)</th>
<th>CO + Phen (n 12)§</th>
<th>CO + IGF-I (n 10)¶</th>
<th>CI (n 10)</th>
<th>CI + IGF-I (n 11)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces weight</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Total (g/6 d)</td>
<td>22·2</td>
<td>0·9</td>
<td>22·7</td>
<td>1·2</td>
<td>19·7</td>
</tr>
<tr>
<td>g/kg body wt</td>
<td>40·5</td>
<td>1·7</td>
<td>44·4</td>
<td>1·6</td>
<td>35·6</td>
</tr>
<tr>
<td>Fat in faeces %</td>
<td>0·11</td>
<td>0·008</td>
<td>0·137</td>
<td>0·01</td>
<td>0·093</td>
</tr>
<tr>
<td>g/g</td>
<td>3·07</td>
<td>0·16</td>
<td>3·57</td>
<td>0·20</td>
<td>3·57</td>
</tr>
</tbody>
</table>

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; Krahnenbuhl 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. 2000).
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. 1993), 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 hypoalbuninaemia, 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 in 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 creatininuria. 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 (Müller, 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 aminoacidemia (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

**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‡**

| Variable          | CO               | Mean | SEM  | CO + Phen§ | Mean | SEM  | CI      | Mean | SEM  | CI + IGF-|| | 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 |      |
| T3 (nmol/l)       | 1·84             | 0·09 |      | 1·83       | 0·21 |      | 1·66    | 0·21 |      | 1·19*      | 0·16 |      |
| T4 (nmol/l)       | 160·3***         | 7·0  |      | 105·7††    | 10·7 |      | 88·5††  | 4·5  |      |            |      |      |

CO, control group; Phen, Phenobarbitali; 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.

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

For details of procedures, see p. 930.

Group received 20 μg IGF-I/kg body weight per d subcutaneously for 14 d.
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 thyronine 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 & Lopez, 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 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 \(V_{\text{max}}\) 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 \(\mu\)g 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.

**References**


Corkins MR, Park JH, Davis DV, Slentz DH & MacDonald RG (1999) Regulation of the insulin like growth factor axis 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 \(V_{\text{max}}\) 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.

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