CLINICAL STUDY

Changes in bioactive IGF-I and IGF-binding protein-1 during an oral glucose tolerance test in patients with liver cirrhosis

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Abstract

Objective: Liver cirrhosis is characterized by reduced circulating IGF-I and this has been linked to an adverse clinical outcome. Therefore, we investigated the dynamic changes in circulating total, free, and bioactive IGF-I, IGF-binding protein (IGFBP)-1, IGFBP-2, and IGFBP-1-bound IGF-I (binary complex) during an oral glucose tolerance test (OGTT) in patients with liver cirrhosis.

Methods: Seven Caucasian males with liver cirrhosis and seven healthy males matched for age (54.4 ± 3.2 vs 54.6 ± 4.4 years) and body mass index (25.3 ± 1.2 vs 25.9 ± 1.3 kg/m²) were studied. Blood samples were drawn at 0, 30, 60, 90, 120, 150, and 180 min for determination of serum total and free IGF-I, IGFBP-1, IGFBP-2, and binary complex, while bioactive IGF-I was measured at 0, 30, 60, 120, and 180 min.

Results: In comparison with healthy subjects, baseline levels of total (47%), free (36%), and bioactive IGF-I (51%) were lower, while IGFBP-1 (268%) was higher ($P < 0.05$). IGFBP-2 (172%) tended to be higher ($P > 0.05$), and the binary complex unchanged ($\sim 100\%$) in cirrhotic patients. Serum total and free IGF-I, and IGFBP-2 remained unchanged in both study groups during the OGTT. Bioactive IGF-I decreased by 29% from baseline to 60 min in cirrhotic patients and remained low at the end of the OGTT ($P < 0.05$). A similar tendency was observed in healthy controls ($P = 0.052$). Concomitantly, IGFBP-1, binary complex, and IGFBP-1 saturation index decreased significantly in both groups. The disappearance of the binary complex was about twofold faster than that of IGFBP-1 ($P < 0.05$).

Conclusion: Despite unchanged concentrations of total and free IGF-I, bioactive IGF-I declined significantly after an oral glucose load in patients with liver cirrhosis and the same tendency was observed in healthy subjects. We speculate that the reduction in bioactive IGF-I may be related to the higher levels of free IGFBP-1 and the faster disappearance of IGFBP-1-bound IGF-I.

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Introduction

The insulin-like growth factor (IGF) superfamily consists of two primary ligands, IGF-I and IGF-II, at least three different receptors, six specific high-affinity IGF-binding proteins (IGFBPs) and a non-IGF-binding peptide – the acid labile subunit (1). Due to its participation in numerous physiological and pathophysiological processes (2–4), IGF-I is considered the most important member of this superfamily. It is generally believed that IGF-I bioactivity is maintained primarily through free, unbound IGF-I (5, 6). Therefore, the inverse association between the circulating concentrations of free IGF-I and IGFBP-1, which has been demonstrated in healthy subjects (7, 8), suggests that IGFBP-1 is a potent inhibitor of IGF-I-mediated biological effects *in vivo*, most likely by regulating the levels of free IGF-I (1, 9).

Circulating IGF-I and IGFBP-1 are both synthesized predominantly in the liver under the control of various hormones (10–14), and accordingly, chronic liver disease has a profound impact on the plasma concentrations of IGF-I and IGFBP-1. Indeed, in comparison to healthy subjects, increased circulating levels of IGFBP-1, but lower total and free IGF-I, are characteristic features of liver cirrhosis (15–19). Furthermore, one of the major health problems in cirrhotic patients is malnutrition, which exerts a serious adverse effect on clinical outcome. Although the pathophysiology of malnutrition in cirrhosis is complex, there is a solid evidence that patients with cirrhosis suffer from both growth hormone (GH) resistance and a reduced ability to synthesize IGF-I, the major downstream effector peptide of GH (20, 21). It is very likely that these abnormalities may be related to and partly explain the relatively

high morbidity and mortality in patients with liver cirrhosis (22).

Moreover, it is well recognized that most cirrhotic patients are intolerant of oral glucose, even when their fasting blood glucose concentrations are normal (23, 24). This appears to be the result of impaired insulin secretion and insulin sensitivity (25). As a consequence of reduced hepatic IGF-I-producing capacity, elevated IGFBP-1, and lower insulin sensitivity, it may be anticipated that in patients with liver cirrhosis, the responsiveness of the IGF system to an oral glucose challenge may be different from that found in normal healthy subjects (19, 26). However, such studies have not yet been performed. Therefore, the objective of the present study was to compare the dynamic changes in circulating total, free, and bioactive IGF-I, and IGFBP-1 during an oral glucose tolerance test (OGTT) in patients with liver cirrhosis and healthy controls.

Subjects and methods

Subjects

The study protocol was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients and controls before initiating any trial-related activity.

Seven Caucasian males with liver cirrhosis and seven healthy males matched for age (54.4 ± 3.2 vs 54.6 ± 4.4 years) and body mass index (25.3 ± 1.2 vs 25.9 ± 1.3 kg/m²) were studied. Liver biopsies were performed in all cirrhotic subjects, who were class B according to the Child–Pugh classification. Six of the patients were diagnosed with alcoholic cirrhosis and one with primary biliary cirrhosis. A detailed biochemical description of these patients and their glucose homeostatic condition has been published previously (25). All patients receiving diuretics or other medications known to affect carbohydrate metabolism were instructed to discontinue the medication at least 48 h before the study. All study subjects were in good health, had normal blood pressure, and were at stable weight. None had a family history of diabetes among first-degree relatives. The subjects were instructed not to engage in vigorous exercise at least 3 days prior to study.

Experimental protocol

Following an overnight fast, study subjects were admitted to the general clinical research centre at 0800 h. An i.v. cannula was inserted into an ante-cubital vein for blood sampling. After ingestion of 75 g glucose over 1 min, blood samples were drawn at 0, 30, 60, 90, 120, 150, and 180 min for determination of serum concentrations of total and free IGF-I, IGFBP-1, IGFBP-2, and IGF-I:IGFBP-1 binary complex, while

bioactive IGF-1 was measured at 0, 30, 60, 120, and 180 min.

Assays

All blood samples were centrifuged immediately at 4 °C and stored at –20 °C until analysis.

Serum total (extractable) IGF-I was determined by an in-house time-resolved immunofluorometric assay (TR-IFMA) in acid-ethanol serum extracts, with within and between assay coefficient of variation (CV) values averaging less than 5 and 10% respectively (27). Serum free IGF-I was determined after ultrafiltration by centrifugation at conditions approaching those of *in vivo* (6). The within and between assay CV values averaged 15 and 20%.

Serum bioactive IGF-I was measured by an IGF-I kinase receptor activation assay (KIRA), which was based on human IGF-I receptor gene-transfected cells (human embryonic renal cells, EBNA 293; Invitrogen) (28). In brief, cultured cells were stimulated at 37 °C with either IGF-I standards (a serial dilution ranging from 0.3 to 5 µg/l of recombinant human IGF-I (Austral Biologicals, San Ramon, CA, USA)) or serum-diluted 1:10 in Krebs Ringer buffer. After 15 min, samples were removed and the cells lysed. Crude cell lysates were then transferred to an assay that detects the concentration of phosphorylated (i.e. activated) IGF-I receptors. This assay uses a MAB against the extracellular domain of the IGF-I receptor for coating and a europium-labeled monoclonal anti-phosphotyrosine antibody (PY20) as tracer. The IGF-I bioassay was sensitive (detection limit 0.08 µg/l), specific (cross-reactivity of insulin, rapid-acting insulin analogs, and pro-insulin were less than 1%; IGF-II cross-reactivity was 12%), and accurate (within and between assay CV values were less than 7 and 15% respectively).

Serum IGFBP-1 was assessed by an in-house RIA and IGFBP-2 by an in-house TR-IFMA as previously described (29). Mean within and between assay CV values were less than 6 and 12% respectively. IGFBP-1-associated IGF-I (binary complex of IGF-I and IGFBP-1) was determined by an in-house TR-IFMA using an IGFBP-1 antibody for coating, and a europium-labeled IGF-I antibody as tracer. The within and between assay CV values of the measurement were 5 and 15% respectively (9).

Statistical analysis

To improve the distribution of our data, all continuous variables were logarithmically (ln) transformed prior to the statistical analysis. At baseline (time 0), differences between cirrhotic patients and healthy controls were compared by Student's unpaired *t*-test. Linear regression analysis was also used to estimate correlations between parameters. During the OGTT, parameters were compared by ANOVA for repeated-measures or one-way

ANOVA followed by *post hoc* multiple comparisons (least significant difference), whenever necessary. Results were expressed as untransformed means $\pm$ S.E.M., except where stated. Furthermore, the area under the time-concentration curves (AUCs) have been calculated using the trapezoidal rule. The AUCs of the two groups were compared by Student's unpaired *t*-test. Statistical analyses were performed using SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). *P* values of 5% or less were considered significant.

Results

Glucose and insulin

The glucose-metabolic profile of the participants has been investigated thoroughly, and has been published elsewhere (25). In brief, the cirrhotic patients had a normal fasting glucose, but approximately twofold elevated fasting levels of insulin and C-peptide ($P < 0.05$). During the OGTT, cirrhotic patients were shown to be glucose intolerant (the 2-h value averaged 8.5 ± 1.0 vs 6.7 ± 0.9 mmol/l (patients versus controls), $P < 0.05$) and insulin resistant with higher levels of insulin and C-peptide throughout the OGTT as compared with controls (for further information, see reference (25)).

Baseline levels

At baseline, plasma concentrations of total, free, and bioactive IGF-I were significantly lower in patients with liver cirrhosis as compared with healthy subjects ($P < 0.05$, Table 1). In contrast, the circulating level of IGFBP-1 was higher in patients than in controls. Furthermore, the fasting levels of IGFBP-2 and IGF-I:IGFBP-1 binary complex were numerically higher in cirrhotic patients, but the differences did not reach statistical significance ($P = 0.07$ and 0.2 respectively, Table 1).

To evaluate the relationship between different parameters at baseline, a stepwise linear regression

analysis was employed with bioactive IGF-I as the dependent variable versus total and free IGF-I as the independent variables. This showed that bioactive IGF-I was significantly and positively predicated by the serum concentration of free IGF-I (slope = 2.65 ± 0.63 μ g/l, $r^2 = 0.59$, $P < 0.01$). This strong association remained significant after adjusting for IGFBP-1 and IGF-I:IGFBP-1 binary complex. The correlation between bioactive and free IGF-I was mainly driven by the patient group (cirrhotic patients: slope = 4.37 ± 0.85 μ g/l, $r^2 = 0.84$, $P < 0.01$; control subjects: slope = 3.28 ± 2.19 μ g/l, $r^2 = 0.31$, $P > 0.05$). Furthermore, free and bioactive IGF-I were negatively correlated with IGFBP-1 ($r^2 = 0.49$ and 0.31 respectively, $P < 0.05$). There was no statistically significant correlation between free or bioactive IGF-I and binary complex.

Changes during OGTT

In comparison with baseline, the plasma concentrations of total and free IGF-I did not change significantly in either cirrhotic patients or healthy controls after ingestion of 75 g glucose (Fig. 1A and B).

Serum IGF-I bioactivity decreased significantly by $24 \pm 0.1\%$ ($P < 0.05$) from 0.95 ± 0.24 μ g/l at baseline to 0.67 ± 0.13 μ g/l at 60 min in cirrhotic patients, and remained low at the end of the OGTT (180 min: 0.71 ± 0.22 μ g/l, reduced by $26 \pm 0.1\%$ from baseline; Fig. 1C and D). A gradual reduction in bioactive IGF-I was also observed in healthy controls, but did not reach the level of significance (baseline versus 180 min: 1.88 ± 0.33 vs 1.38 ± 0.29 μ g/l, $P = 0.052$; Fig. 1C and D).

Serum IGFBP-1 was significantly reduced by 21–32% ($P < 0.01$) from 91.6 ± 24.1 μ g/l at baseline to 72.3 ± 22.3 μ g/l at 90 min, 65.3 ± 22.0 μ g/l at 120 min, 63.9 ± 22.7 μ g/l at 150 min, and 62.3 ± 21.4 μ g/l at 180 min, in patients with liver cirrhosis (Fig. 2A). In healthy controls, the levels of IGFBP-1 were significantly decreased by 19–41% ($P < 0.01$) from 34.2 ± 2.9 μ g/l at baseline to 27.8 ± 2.5 μ g/l at 90 min, 23.7 ± 2.1 μ g/l at 120 min, 21.6 ± 1.9 μ g/l at 150 min, and 20.1 ± 2.7 μ g/l at 180 min respectively.

During the OGTT, serum levels of the IGF-I:IGFBP-1 binary complex decreased significantly by 44–59% in cirrhotic patients from 16.2 ± 3.1 μ g/l at baseline to 9.0 ± 1.8 μ g/l at 90 min, 6.9 ± 1.6 μ g/l at 120 min, 6.9 ± 1.5 μ g/l at 150 min, and 6.6 ± 1.3 μ g/l at 180 min (Fig. 2B). In healthy subjects, the reduction in binary complex from baseline (11.5 ± 2.8 μ g/l) appeared or tended to be statistically significant at 120 min (5.4 ± 0.9 μ g/l, $P < 0.01$), 150 min (4.5 ± 0.8 μ g/l, $P = 0.058$), and 180 min (3.8 ± 0.9 μ g/l, $P < 0.01$).

The IGFBP-1 saturation index was calculated as the molar ratio of IGF-I:IGFBP-1 binary complex to IGFBP-1 and it serves as an estimate of the fraction of 'free IGFBP-1' (9). In general, the index tended to be lower in patients with cirrhosis as compared with control subjects (Fig. 2C), but the differences were not

Table 1 Serum concentrations of total, free, and bioactive insulin-like growth factor-I (IGF-I), IGF-binding protein-I (IGFBP-1), IGF-I:IGFBP-1 binary complex and IGFBP-2 in liver cirrhotic patients and healthy controls at baseline. Results are expressed as means $\pm$ S.E.M.

	Cirrhotic patients	Healthy controls	<i>P</i> values
Total IGF-I (μ g/l)	55.7 ± 7.0	119.7 ± 8.4	< 0.001
Free IGF-I (μ g/l)	0.22 ± 0.04	0.61 ± 0.06	< 0.001
Bioactive IGF-I (μ g/l)	0.95 ± 0.24	1.88 ± 0.33	< 0.05
IGFBP-1 (μ g/l)	91.6 ± 24.1	34.2 ± 2.9	< 0.05
IGF-I:IGFBP-1 binary complex (μ g/l)	16.2 ± 3.1	11.5 ± 2.8	> 0.05
IGFBP-2 (μ g/l)	358.1 ± 78.8	208.6 ± 19.7	> 0.05

Figure 1 Changes in serum (A) total IGF-I, (B) free IGF-I, (C) bioactive IGF-I, and (D) relative reduction in bioactive IGF-I from baseline in patients with chronic liver cirrhosis (○) and health controls (●) during 75 g oral glucose tolerance test (OGTT). The results are expressed as mean $\pm$ S.E.M. The P value for each comparison of AUC between cirrhotic patients and controls is indicated in the individual figure.

statistically significant. In cirrhotic patients, the index was reduced from $24 \pm 6\%$ at baseline to $20 \pm 5\%$ at 30 min after the glucose challenge ($P > 0.05$). The reductions from baseline reached statistical significance ($P < 0.05$) at 90 min ($17 \pm 5\%$), 120 min ($14 \pm 4\%$), 150 min ($15 \pm 4\%$), and 180 min ($15 \pm 4\%$) respectively. In healthy subjects, the decrease in IGFBP-1 saturation index commenced at 90 min (baseline versus 90, 120, 150, and 180 min: 32 ± 6 vs 28 ± 3 , 22 ± 3 , 21 ± 4 , and $18 \pm 2\%$), being statistically significant at 120 and 180 min only ($P < 0.05$).

The time-concentration curves of IGFBP-1 and the binary complex (Fig. 2A and B) implied that the clearance of IGFBP-1-bound IGF-I might be faster than that of IGFBP-1. Therefore, the half-life ($T_{1/2}$) for each individual was calculated by linear regression analysis after ln-transformation of raw data, using the ratio between ln (2) and the slope of the regression line as an estimate of $T_{1/2}$. The $T_{1/2}$ of IGFBP-1 averaged 212 ± 13 min in healthy controls and 310 ± 99 min in cirrhotic patients. The larger variation in the patient group was caused by one subject ($T_{1/2} = 876$ min), who was omitted from the final analysis. The $T_{1/2}$ of IGFBP-1 was 216 ± 32 min in the remaining six patients, and this was similar to that of the controls ($P = 0.5$). In the control group, one of the subjects had levels of binary

complex below the detection limit, and therefore, this person was excluded from the calculations. In the remaining six subjects, the $T_{1/2}$ of the binary complex averaged 88 ± 3 min. The $T_{1/2}$ of the binary complex averaged 148 ± 29 min in cirrhotic patients. The difference in $T_{1/2}$ of the binary complex was not statistically significant between patients and controls ($P = 0.1$). However, the $T_{1/2}$ of the binary complex was significantly shorter than that of IGFBP-1 in both controls (2.5 ± 0.2 times, $P < 0.0001$) and cirrhotic patients (1.8 ± 0.3 times, $P < 0.005$).

The serum concentrations of IGFBP-2 did not change significantly during the OGTT in either cirrhotic patients or healthy controls (Fig. 2D).

Discussion

The primary aim of the present study was to investigate the dynamic changes in IGF-I (measured in four different forms, i.e., total, free, bioactive, and IGFBP-1-bound IGF-I), as well as IGFBP-1 and IGFBP-2 during an oral glucose challenge in patients with liver cirrhosis. In accordance with previous studies (15–18), we confirmed that fasting serum concentrations of total and free IGF-I were reduced and IGFBP-1 levels elevated, while IGF-I:IGFBP-1 binary complex and

Figure 2 Changes in serum (A) IGFBP-1, (B) IGF-I:IGFBP-1 binary complex, (C) ratio of binary complex and IGFBP-1, and (D) IGFBP-2 in patients with chronic liver cirrhosis (○) and health controls (●) during 75 g OGTT. The results are expressed as mean $\pm$ S.E.M. The *P* value for each comparison of AUC between cirrhotic patients and controls is indicated in the individual figure.

IGFBP-2 tended to be higher in cirrhotic patients, as compared with healthy controls. In addition, we showed that the circulating bioactivity of IGF-I was also subnormal in cirrhosis, and that levels, in contrast to total and free IGF-I, declined during the OGTT. This observation may be explained by the higher levels of unsaturated IGFBP-1 (i.e. 'free IGFBP-1') and faster disappearance of IGFBP-1-bound IGF-I, which may serve as a source of 'readily dissociable' and hence receptor-accessible IGF-I.

It is well known that circulating IGFBP-1, due to its inverse relationship with insulin, becomes suppressed after intake of meals and glucose (30–34). For this reason, serum IGFBP-1 has been employed as an estimate of β -cell function and insulin sensitivity in numerous clinical conditions, including liver cirrhosis (19, 35). In the present study, baseline levels of IGFBP-1 were elevated more than twofold in patients with cirrhosis as compared to healthy subjects, in keeping with the cirrhosis-related insulin resistance (19, 25). Nevertheless, following the 75 g glucose load (90–180 min), cirrhotic patients showed the same relative suppression of IGFBP-1 (21–32%) as that observed in healthy controls (19–41%), while IGFBP-2 remained unchanged during the OGTT in both study groups. Thus, despite the reduced hepatic insulin

sensitivity resulting in higher baseline levels, the hepatocytes were able to respond 'normally' to an increased insulin exposure.

The original IGF-I bioassay was based on measurement of cultured costal cartilage uptake of radio-labeled thymidine or sulfate. Later assays employed IGF-I-induced proliferation of BALB/c 3T3 fibroblast cells or MCF-7 human mammary adenocarcinoma cells as an estimate of IGF-I bioactivity (36–39). Although the early bioassays have contributed important information on factors controlling IGF-I bioactivity, they yield relatively unspecific signals. Therefore, modern bioassays detecting the initial commitment of a cell to respond (i.e. intracellular signals) following the ligand activation have been developed (40, 41). The IGF-I receptor belongs to the tyrosine kinase receptor superfamily (42, 43). The auto-phosphorylation of tyrosine residues after the stimulation of IGF-I receptor (ligand binding) is the first step of the intracellular signal cascade. Accordingly, we have recently developed a highly specific IGF-I KIRA, which has enabled us to determine the ability of serum to activate (i.e. phosphorylate) the IGF-I receptor *in vitro* (28). This measurement is most likely composed of the sum of two moieties: free, unbound IGF-I and IGF-I being dissociated from the IGFBPs during incubation of serum

with the IGF-I receptor gene-transfected cells. The latter, often referred to as 'readily dissociable IGF-I', has been suggested to be biologically active (44). Our recent studies have supported this hypothesis indirectly, since the level of bioactive IGF-I measured by the IGF-I KIRA has been higher than that of free IGF-I, but lower than total IGF-I (28, 45).

In the present study, the IGF-I bioactivity was significantly lower in cirrhotic patients than in healthy subjects. This observation is in accordance with old findings (46, 47). Furthermore, serum total IGF-I remained unchanged during the OGTT and the same was true for free IGF-I, despite the decrease in IGFBP-1 and rather stable IGFBP-2. Previous studies from our laboratory, as well as from others, have uniformly shown that serum-free IGF-I determined by either ultrafiltration or a commercial IRMA remains unchanged after oral glucose, as well as after meal intake (48). On the other hand, levels of bioactive IGF-I decreased significantly in cirrhotic patients and the same tendency was also observed in healthy controls during the OGTT ($P=0.052$). This finding argues against the observation by Bereket and co-workers (49), which indicated that in response to food (or glucose) intake, changes in IGFBP-1 concentrations within the physiological range would not sufficiently affect IGF-I bioactivity by limiting the availability of free IGF-I.

We have previously suggested that the IGFBP-1 saturation index reflects the fraction of IGFBP-1 being saturated with IGF-I (9). In keeping with this, a low IGFBP-1 saturation index implies that less IGFBP-1 is bound to IGF-I, i.e. more IGFBP-1 is present as unsaturated 'free IGFBP-1'. Since IGFBP-1 has a strong inhibitory effect on IGF-I bioactivity (1, 9), we speculate that during the OGTT, the gradual decrease in the IGFBP-1 saturation index, which equals an increase in the fraction of unsaturated IGFBP-1, may contribute to the reduction in bioactive IGF-I observed in the later part of the present experiment. However, the reduction in bioactive IGF-I during the OGTT may also reflect an increased trans-capillary transport of IGF-I bound to IGFBP-1 (please see below).

Although the present study was not designed to determine the half-lives of IGFBP-1 and the binary complex, the time-concentration curves implied that after the oral glucose load the disappearance of the binary complex was faster than that of IGFBP-1. Indeed, this was confirmed by the estimated half-lives, which showed that during the 180 min of study, the clearance of the binary complex was approximately twofold shorter than that of IGFBP-1.

Whether the faster clearance of IGF-I:IGFBP-1 binary complex is due to an increased degradation or an accelerated trans-capillary transport to the extravascular compartment remains to be determined. At the time of writing, there were only sparse data on the mechanisms controlling the trans-capillary transport of the circulating IGF-system. Payet *et al.* (50) did not

observe any effect of radio-labeled IGF-I on the transport of IGFBP-3 and IGFBP-5 across the cultured human umbilical vein endothelial monolayer. This was in agreement with findings by Lewitt *et al.* (51), who showed that in rats, the half-life in the serum of intravenously injected human IGFBP-1 was not influenced by co-administration of IGF-I. In contrast, we have indirect *in vivo* evidence supporting that in humans, IGFBP-1 may be cleared more rapidly in the presence of IGF-I. In an earlier study, healthy subjects received 3 days of s.c. infusion of either saline or IGF-I (10 µg/kg/h), after which a 300-min hyperinsulinemic clamp was performed. After 3 days of saline infusion, the hyperinsulinemic clamp reduced IGFBP-1 by about twofold. In comparison, after 3 days of IGF-I infusion, when all IGFBPs were likely to be fully saturated with IGF-I, the hyperinsulinemic clamp reduced IGFBP-1 by about fourfold (52). However, we acknowledge that further studies are required to confirm that the clearance of IGFBP-1 is dependent on IGF-I binding.

Cirrhotic patients were characterized by an abnormal glucose metabolism, which appears to be caused by insulin resistance in peripheral tissues (25). We believe these characteristic features are linked pathophysiologically to low levels of bioactive IGF-I. Accordingly, there is strong experimental evidence that low levels of circulating IGF-I result in peripheral insulin resistance. This appears to be mediated through two mechanisms: first, an inadequate IGF-I-mediated negative feedback at the level of the hypothalamus/pituitary leading to chronic GH hypersecretion and secondly, IGF-I has been suggested to exert a direct stimulating effect on insulin sensitivity in skeletal muscles (53). However, we acknowledge that these hypotheses remain to be tested.

In conclusion, we show that patients with liver cirrhosis suffer from markedly suppressed levels of bioactive IGF-I. Furthermore, we demonstrate that despite relatively unchanged circulating concentrations of total and free IGF-I, bioactive IGF-I declines significantly after an oral glucose load. We hypothesize that the reduction in bioactive IGF-I during the OGTT may be caused by the higher levels of unsaturated IGFBP-1 as well as the faster disappearance of 'readily dissociable IGF-I' present in IGF-I:IGFBP-1 binary complexes. Furthermore, our data imply that the clearance of IGFBP-1 may depend on its binding to IGF-I. However, this needs further investigation.

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References

- Jones JI & Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocrine Reviews* 1995 **16** 3–34.
- Bayes-Genis A, Conover CA & Schwartz RS. The insulin-like growth factor axis: a review of atherosclerosis and restenosis. *Circulation Research* 2000 **86** 125–130.
- Daughaday WH & Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocrine Reviews* 1989 **10** 68–91.
- Khandwala HM, McCutcheon IE, Flyvbjerg A & Friend KE. The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. *Endocrine Reviews* 2000 **21** 215–244.
- Cusi K & DeFronzo R. Recombinant human insulin-like growth factor I treatment for 1 week improves metabolic control in type 2 diabetes by ameliorating hepatic and muscle insulin resistance. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 3077–3084.
- Frystyk J, Skjaerbaek C, Dinesen B & Orskov H. Free insulin-like growth factors (IGF-I and IGF-II) in human serum. *FEBS Letters* 1994 **348** 185–191.
- Frystyk J, Vestbo E, Skjaerbaek C, Mogensen CE & Orskov H. Free insulin-like growth factors in human obesity. *Metabolism* 1995 **44** (10 Suppl 4) 37–44.
- Juul A, Flyvbjerg A, Frystyk J, Muller J & Skakkebaek NE. Serum concentrations of free and total insulin-like growth factor-I, IGF binding proteins -1 and -3 and IGFBP-3 protease activity in boys with normal or precocious puberty. *Clinical Endocrinology* 1996 **44** 515–523 (Oxford).
- Frystyk J, Hojlund K, Rasmussen KN, Jorgensen SP, Wildner-Christensen M & Orskov H. Development and clinical evaluation of a novel immunoassay for the binary complex of IGF-I and IGF-binding protein-1 in human serum. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 260–266.
- McConaghey P & Sledge CB. Production of 'sulphation factor' by the perfused liver. *Nature* 1970 **225** 1249–1250.
- Miller LL, Schalch DS & Draznin B. Role of the liver in regulating somatomedin activity: effects of streptozotocin diabetes and starvation on the synthesis and release of insulin-like growth factor and its carrier protein by the isolated perfused rat liver. *Endocrinology* 1981 **108** 1265–1271.
- Schwander JC, Hauri C, Zapf J & Froesch ER. Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. *Endocrinology* 1983 **113** 297–305.
- Arany E, Strain AJ, Hube MJ, Phillips ID & Hill DJ. Interactive effects of nutrients and hormones on the expression of insulin-like growth factor binding protein-1 (IGFBP-1) mRNA and peptide, and IGF I release from isolated adult rat hepatocytes. *Journal of Cellular Physiology* 1993 **155** 426–435.
- Villafuerte BC, Goldstein S, Murphy LJ & Phillips LS. Nutrition and somatomedin, XXV. Regulation of insulin like growth factor binding protein 1 in primary cultures of normal rat hepatocytes. *Diabetes* 1991 **40** 837–841.
- Moller S, Juul A, Becker U, Flyvbjerg A, Skakkebaek NE & Henriksen JH. Concentrations, release, and disposal of insulin-like growth factor (IGF)-binding proteins (IGFBP), IGF-I, and growth hormone in different vascular beds in patients with cirrhosis. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 1148–1157.
- Scharf JG, Schmitz F, Frystyk J, Skjaerbaek C, Moesius H, Blum WF, Ramadori G & Hartmann H. Insulin-like growth factor-I serum concentrations and patterns of insulin-like growth factor binding proteins in patients with chronic liver disease. *Journal of Hepatology* 1996 **25** 689–699.
- Wu JC, Daughaday WH, Lee SD, Hsiao TS, Chou CK, Lin HD, Tsai YT & Chiang BN. Radioimmunoassay of serum IGF-I and IGF-II in patients with chronic liver diseases and hepatocellular carcinoma with or without hypoglycemia. *Journal of Laboratory and Clinical Medicine* 1988 **112** 589–594.
- Zapf J, Morell B, Walter H, Laron Z & Froesch ER. Serum levels of insulin-like growth factor (IGF) and its carrier protein in various metabolic disorders. *Acta Endocrinologica (Copenhagen)* 1980 **95** 505–517.
- Shmueli E, Miell JP, Stewart M, Alberti KG & Record CO. High insulin-like growth factor binding protein 1 levels in cirrhosis: link with insulin resistance. *Hepatology* 1996 **24** 127–133.
- Donaghy A. Issues of malnutrition and bone disease in patients with cirrhosis. *Journal of Gastroenterology and Hepatology* 2002 **17** 462–466.
- Donaghy AJ, Delhanty PJ, Ho KK, Williams R & Baxter RC. Regulation of the growth hormone receptor/binding protein, insulin-like growth factor ternary complex system in human cirrhosis. *Journal of Hepatology* 2002 **36** 751–758.
- Talwalkar JA & Kamath PS. Influence of recent advances in medical management on clinical outcomes of cirrhosis. *Mayo Clinic Proceedings* 2005 **80** 1501–1508.
- Conn HO, Schreiber W & Elkington SG. Cirrhosis and diabetes. II. Association of impaired glucose tolerance with portal-systemic shunting in Laennec's cirrhosis. *American Journal of Digestive Diseases* 1971 **16** 227–239.
- Megyesi C, Samols E & Marks V. Glucose tolerance and diabetes in chronic liver disease. *Lancet* 1967 **2** 1051–1056.
- Nielsen MF, Caumo A, Aagaard NK, Chandramouli V, Schumann WC, Landau BR, Schmitz O & Vilstrup H. Contribution of defects in glucose uptake to carbohydrate intolerance in liver cirrhosis: assessment during physiological glucose and insulin concentrations. *American Journal of Physiology* 2005 **288** G1135–G1143.
- Frystyk J, Grofte T, Skjaerbaek C & Orskov H. The effect of oral glucose on serum free insulin-like growth factor-I and -II in health adults. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 3124–3127.
- Frystyk J, Dinesen B & Orskov H. Non-competitive time-resolved immunofluorometric assays for determination of human insulin-like growth factor I and II. *Growth Regulation* 1995 **5** 169–176.
- Chen JW, Ledet T, Orskov H, Jessen N, Lund S, Whittaker J, De Meyts P, Larsen MB, Christiansen JS & Frystyk J. A highly sensitive and specific assay for determination of IGF-I bioactivity in human serum. *American Journal of Physiology* 2003 **284** E1149–E1155.
- Krassas GE, Pontikides N, Kaltsas T, Dumas A, Frystyk J, Chen JW & Flyvbjerg A. Free and total insulin-like growth factor (IGF)-I, -II, and IGF binding protein-1, -2, and -3 serum levels in patients with active thyroid eye disease. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 132–135.
- Yeoh SI & Baxter RC. Metabolic regulation of the growth hormone independent insulin-like growth factor binding protein in human plasma. *Acta Endocrinologica (Copenhagen)* 1988 **119** 465–473.
- Panarelli M, Holloway CD, Mulatero P, Fraser R & Kenyon CJ. Inhibition of lysozyme synthesis by dexamethasone in human mononuclear leukocytes: an index of glucocorticoid sensitivity. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 872–877.
- Pao CI, Farmer PK, Begovic S, Villafuerte BC, Wu GJ, Robertson DG & Phillips LS. Regulation of insulin-like growth factor-I (IGF-I) and IGF-binding protein 1 gene transcription by hormones and provision of amino acids in rat hepatocytes. *Molecular Endocrinology* 1993 **7** 1561–1568.
- Powell DR, Suwanichkul A, Cubbage ML, DePaolis LA, Snuggs MB & Lee PD. Insulin inhibits transcription of the human gene for insulin-like growth factor-binding protein-1. *Journal of Biological Chemistry* 1991 **266** 18868–18876.
- Brismar K, Fernqvist-Forbes E, Wahren J & Hall K. Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 872–878.
- Clauson PG, Brismar K, Hall K, Linnarsson R & Grill V. Insulin-like growth factor-I and insulin-like growth factor binding protein-1 in a representative population of type 2 diabetic patients in Sweden. *Scandinavian Journal of Clinical and Laboratory Investigation* 1998 **58** 353–360.

- 36 Travali S, Reiss K, Ferber A, Petralia S, Mercer WE, Calabretta B & Baserga R. Constitutively expressed c-myc abrogates the requirement for insulinlike growth factor 1 in 3T3 fibroblasts. *Molecular and Cellular Biology* 1991 **11** 731–736.
- 37 Pietrzowski Z, Sell C, Lammers R, Ullrich A & Baserga R. Roles of insulin-like growth factor 1 (IGF-1) and the IGF-1 receptor in epidermal growth factor-stimulated growth of 3T3 cells. *Molecular and Cellular Biology* 1992 **12** 3883–3889.
- 38 Geier A, Weiss C, Beery R, Haimsohn M, Hemi R, Malik Z & Karasik A. Multiple pathways are involved in protection of MCF-7 cells against death due to protein synthesis inhibition. *Journal of Cellular Physiology* 1995 **163** 570–576.
- 39 Geier A, Hemi R, Haimson M & Beery R. Epidermal growth factor and insulin-like growth factor-1 preserve cell viability in the absence of protein synthesis. *In vitro Cellular & Developmental Biology* 1993 **29A** 231–234.
- 40 Rowland JE, Marshall NJ, Leung KC, Ho KK, Cotterill AM, Rowlinson SW & Waters MJ. A novel bioassay for human somatogenic activity in serum samples supports the clinical reliability of immunoassays. *Clinical Endocrinology* 2002 **56** 475–485 (Oxford).
- 41 Sadick MD, Intintoli A, Quarmby V, McCoy A, Canova-Davis E & Ling V. Kinase receptor activation (KIRA): a rapid and accurate alternative to end-point bioassays. *Journal of Pharmaceutical and Biomedical Analysis* 1999 **19** 883–891.
- 42 Hunter T & Cooper JA. Protein-tyrosine kinases. *Annual Review of Biochemistry* 1985 **54** 897–930.
- 43 Ullrich A & Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell* 1990 **61** 203–212.
- 44 Juul A, Holm K, Kastrup KW, Pedersen SA, Michaelsen KF, Scheike T, Rasmussen S, Muller J & Skakkebaek NE. Free insulin-like growth factor I serum levels in 1430 healthy children and adults, and its diagnostic value in patients suspected of growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2497–2502.
- 45 Chen JW, Hojlund K, Beck-Nielsen H, Sandahl CJ, Orskov H & Frystyk J. Free rather than total circulating insulin-like growth factor-I determines the feedback on growth hormone release in normal subjects. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 366–371.
- 46 Minuto F, Barreca A, Del Monte P, Buffa C, Bevevino R, Montale F, Torre G & Giordano G. *In vivo* and *in vitro* effect of liver on somatomedin generation. *Hormone and Metabolic Research* 1984 **16** 663–666.
- 47 Schimpff RM, Lebrec D, Dannadieu M & Repellin AM. Serum somatomedin activity measured as sulphation factor in peripheral, hepatic and renal veins of patients with alcoholic cirrhosis. *Acta Endocrinologica (Copenhagen)* 1978 **88** 729–736.
- 48 Frystyk J. Free insulin-like growth factors – measurements and relationships to growth hormone secretion and glucose homeostasis. *Growth Hormone and IGF Research* 2004 **14** 337–375.
- 49 Bereket A, Wilson TA, Blethen SL, Fan J, Frost RA, Gelato MC & Lang CH. Effect of short-term fasting on free/dissociable insulin-like growth factor I concentrations in normal human serum. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 4379–4384.
- 50 Payet LD, Firth SM & Baxter RC. The role of the acid-labile subunit in regulating insulin-like growth factor transport across human umbilical vein endothelial cell monolayers. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2382–2389.
- 51 Lewitt MS, Denyer GS, Cooney GJ & Baxter RC. Insulin-like growth factor-binding protein-1 modulates blood glucose levels. *Endocrinology* 1991 **129** 2254–2256.
- 52 Frystyk J, Hussain M, Skjaerbaek C, Schmitz O, Christiansen JS, Froesch ER & Orskov H. Serum free IGF-I during a hyperinsulinemic clamp following 3 days of administration of IGF-I vs. saline. *American Journal of Physiology* 1997 **273** E507–E513.
- 53 Yakar S, Liu JL, Fernandez AM, Wu Y, Schally AV, Frystyk J, Chernauek SD, Mejia W & Le Roith D. Liver-specific igf-1 gene deletion leads to muscle insulin insensitivity. *Diabetes* 2001 **50** 1110–1118.

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