Serum Insulin-Like Growth Factors and Their Binding Proteins in Patients With Hepatic Failure and After Liver Transplantation

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The liver is the major source of circulating insulin-like growth factor-I and -II (IGF-I and IGF-II) and several of their binding proteins (BPs). This study examined the effects of end-stage liver disease (ESLD) and subsequent liver transplantation (LT) on serum levels of these growth factors and their BPs in four children and six adults for up to 2 years. Serum IGF-I and IGF-II were quantified by radioimmunoassay (RIA), IGFBP-3 by immunoradiometric assay (IRMA), and changes in IGFBP-1, -2, -3, and -4 were estimated by Western ligand blotting (WLB). In severe hepatic disease, serum concentrations of IGF-I (10 ± 5 ng/mL) and IGF-II (126 ± 32 ng/mL) were significantly ($P < .01$) less than in normal controls (170 ± 37 and 590 ± 41 ng/mL, respectively). One year following LT, the mean levels of IGF-I (344 ± 55 ng/mL) and IGF-II (627 ± 38 ng/mL) were within normal limits and remained so for the duration of the study. Patients exhibited considerable variation not only in the rate of achieving normal IGF-I and IGF-II concentrations, but also in the ultimate height and stability of these peptide levels. Serum IGFBP-3 in hepatic failure (580 ± 140 ng/mL) was significantly ($P < .05$) lower than in controls (2,900 ± 220 ng/mL) and increased to normal levels (3,650 ± 360 ng/mL) 2 to 14 weeks after LT. Serum levels of IGFBP-1, -2, and -4 before and after LT were variable but usually remained within normal limits compared with control sera. The decreases observed in IGF-I, IGF-II, and IGFBP-3 in patients with hepatic failure and their subsequent restoration after LT probably result primarily from the reduced number of functional hepatocytes in ESLD and their subsequent replacement by healthy hepatic tissue. These changes may also result from hormonal alterations and nutritional deficiencies known to exist in patients with severe liver dysfunction, which are corrected by LT. We conclude that LT in patients with severe hepatic insufficiency enhances the potential for normal cell growth and replication by restoring serum IGF-I, IGF-II, and IGFBP-3 concentrations to normal concomitantly with the improvement in hormonal and nutritional status.

THE LIVER IS THE PRINCIPAL SITE for the synthesis and secretion of circulating insulin-like growth factor-I and -II (IGF-I and IGF-II) and several of their binding proteins (BPs).[1-6] Consequently, one would expect hepatic dysfunction to be associated with some alteration in the concentration of these proteins in the circulation. Indeed, several investigators have reported reduced serum levels of IGF, or somatomedin, both in animals and in humans with severe hepatic insufficiency.[7-13] Moreover, when hepatic failure occurs in children, it is usually associated with severe impairment of growth.[14-16] The successful transplantation of cadaveric livers into human recipients has provided the opportunity to study not only the effects of severe hepatic disease on serum IGF-I, IGF-II, and IGFBPs, but also what changes, if any, occur after liver transplantation (LT). We have evaluated 10 patients (four children and six adults) for up to 2 years after LT, noting that the markedly reduced preoperative serum levels of IGF-I, IGF-II, and IGFBP-3 significantly increase following transplantation. Some of these data have already been presented in abstract form.[17,18]

SUBJECTS AND METHODS

Subjects

The study was approved by the Institutional Review Board of the University of Wisconsin Medical School, and all 10 patients (or their parents) provided informed consent to participate in the investigation. These subjects (five females and five males aged 4 months to 63 years) were referred to the Transplant Service of the Department of Surgery because of end-stage liver disease (ESLD). Causes of hepatic failure included biliary atresia (n = 3), posthepatitis cirrhosis (n = 2), polycystic liver disease (n = 1), primary biliary cirrhosis (n = 1), Laennec's cirrhosis (n = 1), cholangiolitic carcinoma (n = 1), and cystic fibrosis (n = 1) (Table 1). Following baseline studies, orthotopic LT was performed on all subjects (patient no. 2 received a second liver after failure of the first). Postoperatively, all patients were evaluated at weekly, biweekly, and then monthly intervals until they were clinically and biochemically stable. Blood samples were drawn in the morning 2 to 3 hours postprandially for routine pretransplant and posttransplant chemistry analyses that included, among other things, hepatic enzymes, albumin, bilirubin, prothrombin time, hematocrit, and creatinine; in addition, the sera were kept frozen at −70°C to be assayed later for IGF-I, IGF-II, and IGFBPs. After LT, all patients were placed on a standard immunosuppressive protocol consisting of azathioprine 1 to 2 mg/kg/d, cyclosporine 4 to 6 mg/kg/d, and prednisone 10 mg/d. Weaning of prednisone to every other day was attempted in all children to encourage growth. Rejection episodes were treated with methylprednisolone 1 g/d for 2 days, followed by a rapid taper. Cyclosporine levels were measured with a polyclonal polarized immunofluorescence assay, with target levels of 200 to 400 ng/mL. Each subject served as his or her own control, since blood samples were obtained before and at 1- to 2-month intervals up to 22 months after LT, at which time all laboratory determinations were relatively stable. In addition, 16 normal individuals (aged 23 to 62 years) served as controls for serum concentrations of IGF-I, IGF-II, and IGFBPs in adult patients. Although we did not include sera from normal children as controls for our four youngest patients, previous studies of serum IGF-I and IGF-II in normal and diabetic subjects in our laboratory were used for comparison.[19] The mean IGF-I level of age-matched controls for the two younger children and the two older children is approximately one third and half, respectively, the level of normal adults. We used commercial IGFBP-3 immunoradiometric assay (IRMA) kit age-related levels as controls for this BP in children.

Assays

Routine blood chemistry analyses were performed by the hospital clinical laboratory using established methods. Serum IGF-I and IGF-II

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Table 1. Clinical Characteristics of the ESLD Patients Undergoing LT

Patient No.	Age*/Sex	Diagnosis
1	4 mo/M	Biliary atresia
2	8 mo/F	Biliary atresia
3	6 yr/M	Biliary atresia
4	7 yr/M	Cystic fibrosis
5	33 yr/M	Cholangiolitic carcinoma
6	51 yr/F	Primary biliary cirrhosis
7	52 yr/F	Posthepatitic cirrhosis
8	53 yr/F	Laennec's cirrhosis
9	62 yr/F	Polycystic liver
10	63 yr/M	Posthepatitic cirrhosis

*Age at time of LT.

levels were measured by double-antibody radioimmunoassay (RIA) after separating these peptides from their BPs by high-performance liquid chromatography (HPLC) as previously described.[20] Briefly, 75 μL serum was added to 450 μL sample preparation buffer (0.1 mol/L trimethylamine hydrochloride and 0.2 mol/L acetic acid) and acidified to pH 2.8 with 3.3 μL 2.0-mol/L HCl. The sample was incubated for 30 minutes at 4°C and centrifuged for 10 minutes at 3,000 rpm, and 250 μL supernatant was injected into a 10-mL HPLC protein purification column (Protein-pak 125; Waters, Milford, MA) using degassed sample preparation buffer as the mobile phase. Serum IGF-I and IGF-II levels were measured in duplicate by specific RIAs that have been previously described in detail,[21,22] using recombinant human IGF-I (Chiron, Emeryville, CA) and IGF-II (Eli Lilly, Indianapolis, IN) for standards. Intraassay and interassay coefficients of variation for these assays are 5.0% and 10.5%, respectively. Serum levels of IGFBP-3 were determined by a specific IRMA purchased from Diagnostic Systems Laboratories (Webster, TX), using recombinant DNA–derived nonglycosylated human IGFBP-3 as the standard. It is reported to have less than 0.01% cross-reactivity with IGFBP-2 and -4,[12] although it may measure some immunoreactive proteolytic fragments of IGFBP-3. The intraassay coefficient of variation is 6.0%.

Western Ligand Blotting

IGFBP concentrations were estimated by a modification[23] of the Western ligand blotting (WLB) method originally described by Hossenlopp et al.[24] Briefly, 2 μL serum or standard were electrophoresed in Laemmli buffer first in a 4% polyacrylamide stacking gel for approximately 1.5 hours at 100 V before entering a 7.5% to 15% gradient sodium dodecyl sulfate–polyacrylamide separation gel for approximately 5 hours at 200 V. The protein bands were then transferred to nitrocellulose (NC) paper using an electroblot box with 150 mA constant current for 12 to 14 hours. The NC was air-dried, prehybridized in saline buffer containing 1 mol/L Tris, 0.5% sodium azide, and 0.1% Tween 20 (pH 7.4) for 30 minutes, and hybridized overnight in 10 mL buffered saline containing ^{125}I-IGF-I (~400,000 cpm). The filter paper was then washed, blotted dry, and exposed to Kodak X-Omat (Eastman Kodak, Rochester, NY) film placed between two intensifying screens for 7 to 14 days at −70°C. Using this method, the following IGFBPs (and their apparent kd) are detectable: IGFBP-1 (30 kd), IGFBP-2 (34 kd), IGFBP-3 (39-42 kd), and IGFBP-4 (24 kd).

Statistical Analysis

Results are expressed as the mean ± SEM both when the data for all 10 patients are summarized and when data for the four children and six adults are analyzed separately. Student's *t* test was used to compare mean serum levels of IGF-I, IGF-II, and IGFBP-3 before and after LT, with significance defined as *P* less than .05.

RESULTS

All patients were in hepatic failure at the time of LT, with a range for disease duration of 4 months in one child with biliary atresia to approximately 15 years in an adult with polycystic liver disease. Results reported in this study are for the period from 1 to 8 weeks before and 1 to 2 years after LT. A summary of pretransplant and posttransplant biochemical data for all 10 patients shows that the mean values markedly improved and often normalized after LT (Table 2). Despite this overall successful outcome, hepatic function did decrease in several patients during transient periods of rejection, although these episodes were reversible and the patients usually responded promptly to adjustments in immunosuppressive therapy.

In general, the marked improvement in serum hepatic enzyme concentrations following LT was associated with significant increases in serum IGF-I and IGF-II levels. These data for the four children and six adults are summarized separately in Fig 1. Approximately 2 months after LT, the mean serum IGF-I (222 ± 42 ng/mL) in all 10 patients was increased more than 20-fold over the pretransplant concentration (10 ± 5 ng/mL), which was only 5.9% of the normal control levels (170 ± 37 ng/mL). By the end of 1 year, the mean serum IGF-I (344 ± 55 ng/mL) remained relatively constant at approximately twice the control value, although this difference did not

Table 2. Biochemical Data for the ESLD Patients Undergoing LT

Parameter	Normal Range	Pre-LT (N = 10)	Post-LT (mo) 2	12	24
Albumin (g/dL)	3.0-5.0	2.9 ± 0.2	3.1 ± 0.2	3.5 ± 0.2	3.6 ± 0.2
Prothrombin time (s)	11-13	13.2 ± 0.4	12.1 ± 0.5	11.6 ± 0.3	11.9 ± 0.2
Total bilirubin (mg/dL)	0-1.4	7.8 ± 2.2	1.2 ± 0.3	0.8 ± 0.2	0.7 ± 0.1
Alkaline phosphatase (U/L)	75-375*	428 ± 117	280 ± 104	361 ± 54	324 ± 84
	35-130†	243 ± 86	141 ± 34	133 ± 34	152 ± 37
Aspartate aminotransferase (U/L)	0-45	116 ± 29	56 ± 17	78 ± 30	45 ± 6.9
Alanine aminotransferase (U/L)	0-35	84 ± 31	156 ± 54	133 ± 51	53 ± 13
Gamma glutamyl transferase (U/L)	0-55	419 ± 176	318 ± 156	276 ± 124	163 ± 72
Lactate dehydrogenase (U/L)	200-325*	316 ± 86	209 ± 29	386 ± 119	203 ± 61
	90-200†	568 ± 146	241 ± 37	233 ± 25	205 ± 33
Hematocrit (mL/dL)	35-45	38.3 ± 9.0	33.2 ± 1.1	34.9 ± 1.0	35.0 ± 1.2
Creatinine (mg/dL)	0.4-1.2	1.7 ± 0.5	1.4 ± 0.2	1.6 ± 0.1	1.3 ± 0.2

NOTE. Results are the mean ± SEM for all 10 subjects except when normal and patient data are expressed separately for children (n = 4)* and adults (n = 6)†.

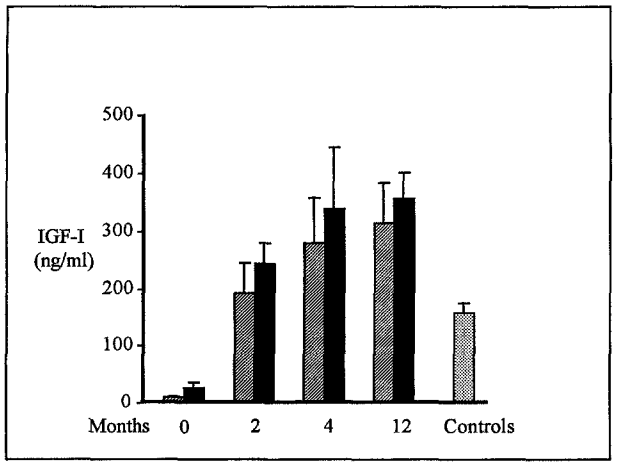

Fig 1. Serum IGF-I levels in 10 patients before and after LT. Mean ± SEM values for 4 children (▨) and 6 adults (■) are shown separately and compared against values in 16 control subjects (▩). Blood samples were drawn before (time 0) and 2, 4, and 12 months after LT.

reach statistical significance because of considerable variation. The mean IGF-I levels in the four children were lower than in the six adults at all time points. Serum IGF-II in all patients, summarized separately for the four children and six adults in Fig 2, increased after LT more slowly than IGF-I, and at approximately 2 months the mean level (441 ± 66 ng/mL) was significantly less ($P < .05$) than for the normal controls (590 ± 41 ng/mL). This represented less than a fourfold increase over the pretransplant level (126 ± 32 ng/mL), which was 21.4% of the level in normal subjects. By the end of 1 year, the mean serum IGF-II (627 ± 38 ng/mL) reached and subsequently remained within the normal range. Again, mean levels for the four children were less than for the six adults at all time points. Although the mean posttransplant level of both IGF-I and IGF-II increased significantly in all patients, considerable variation occurred between subjects during the period of study.

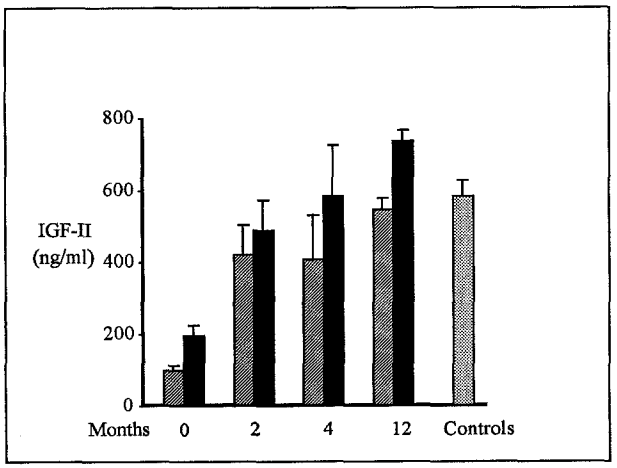

Fig 2. Serum IGF-II levels in 10 patients before and after LT. Mean ± SEM values for 4 children (▨) and 6 adults (■) are shown separately and compared against values in 16 control subjects (▩). Blood samples were drawn before (time 0) and 2, 4, and 12 months after LT.

These values in individual subjects are shown in Fig 3 for children and Fig 4 for adults.

Serum concentrations of IGFBP-3 measured by IRMA were markedly reduced in ESLD (580 ± 140 ng/mL, n = 10), being only 20% of the control levels (2,900 ± 220 ng/mL). This BP increased significantly ($P < .05$) during the 6 months after LT to a mean level (3,650 ± 360 ng/mL) that was indistinguishable from normal. The variability in individual serum levels for seven of 10 patients is shown in Fig 5. These findings were also confirmed by WLB, which showed marked differences in both the concentration and rate of change in serum IGFBP-3 in these patients. Estimations of the relative change in the levels of IGFBP-1, -2, -3, and -4 in sequential serum samples can best be made by WLB, and representative blots for two adults (Fig 6) and two children (Fig 7) are presented. A normal concentration was seen as soon as day 14 in one adult (Fig 6), but not until day 201 in one child (Fig 7). In a few patients, a marked but transient decrease in the concentration of most or all IGFBPs was exhibited in the fourth week after LT (Figs 6 and 7). Of note, serum IGFBP-1 was normal or, in some cases, slightly elevated in our patients with hepatic failure, and remained normal or even slightly decreased following transplantation. Two of four children before LT and the youngest (age 23 years) of the control subjects had clearly higher serum IGFBP-2 concentrations than the other normal controls. Pretransplant serum IGFBP-4 levels were mainly diminished or, in some cases, normal, usually demonstrating a slight and variable decrease after LT, mirroring the changes in IGFBP-1.

DISCUSSION

The liver plays a key role in many metabolic processes and is in part hormonally regulated. It is recognized as an essential organ in synthesizing numerous constitutive and secretory proteins, reversibly converting glucose to glycogen under

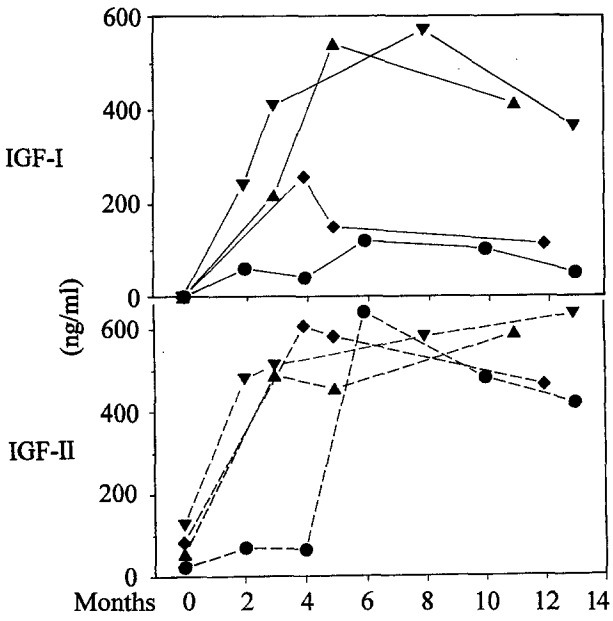

Fig 3. Individual serum IGF-I and IGF-II levels in 4 children before and after LT. Blood samples were drawn before (time 0) and ≤13 months after LT.

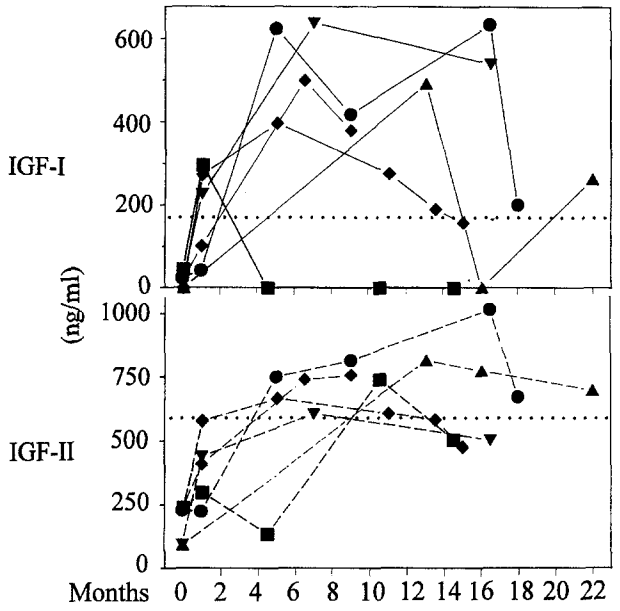

Fig 4. Individual serum IGF-I and IGF-II levels in 6 adults before and after LT. Blood samples were drawn before (time 0) and ≤22 months after LT. (-----) Mean IGF-I and IGF-II levels for 16 normal controls.

hormonal regulation, and serving an integral role in lipid metabolism. When it was discovered that the liver, primarily under growth hormone (GH) control, was the major site for the production of circulating IGF-I and IGF-II, it assumed a new role as an endocrine organ as well.[1-4] Understandably, since IGF-I and IGF-II mediate many of the growth-promoting effects of GH, the liver occupies a vital place in regulating tissue growth and repair. It follows therefore that hepatic dysfunction may lead to disordered growth through a variety of mechanisms

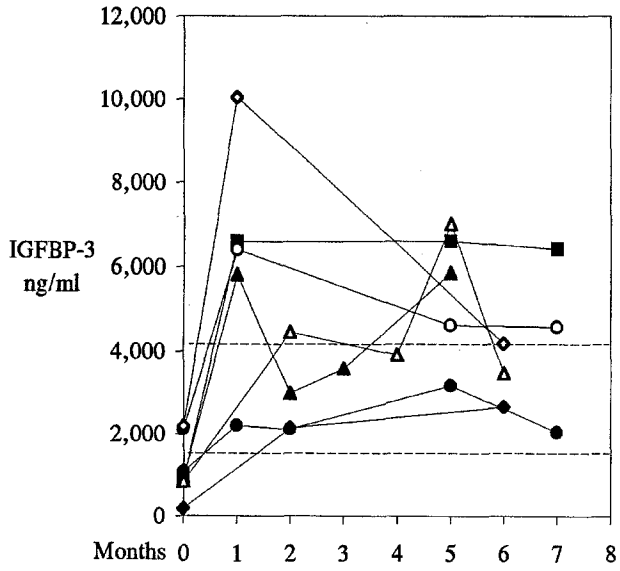

Fig 5. Individual serum IGFBP-3 levels in 4 children and 3 adults before and after LT. Blood samples were drawn before (time 0) and ≤7 months after LT. Serum IGFBP-3 concentrations in children (solid symbols) and adults (open symbols) were measured by IRMA. (-----) Mean ± 2 SD IGFBP-3 levels for 8 normal adults.

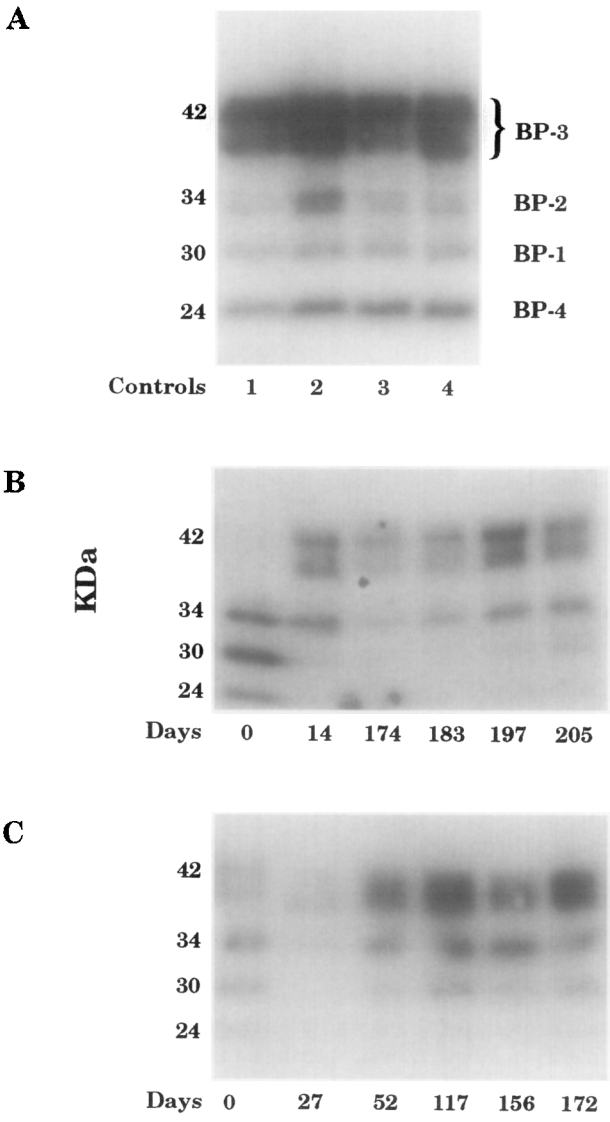

Fig 6. Serum IGFBPs in control subjects and 2 adults before and after LT. WLB was used to estimate the relative concentration of IGFBPs in 4 representative control subjects (A) and 2 adults before and after LT (B and C).

including, among others, a reduction in the production of IGF-I and IGF-II and, as recently shown, changes in some of the IGFBPs.[9-12,15]

This study examines the effects of hepatic failure on these important growth regulators in relation to alterations in the common biochemical indicators of liver function. Well-known changes in the latter prevailed in all 10 subjects before transplantation, and marked improvements, if not complete normalization, followed LT. As might be expected, hepatic failure was associated with a marked reduction in serum levels of IGF-I, IGF-II, and their predominant BP, namely IGFBP-3. It seems more likely that these decreased levels result from diminished production rather than increased degradation. These changes probably reflect primarily a reduction in the mass of functional hepatic parenchyma, but may also result from altered GH, insulin, and/or cortisol effects on the liver, illness-imposed

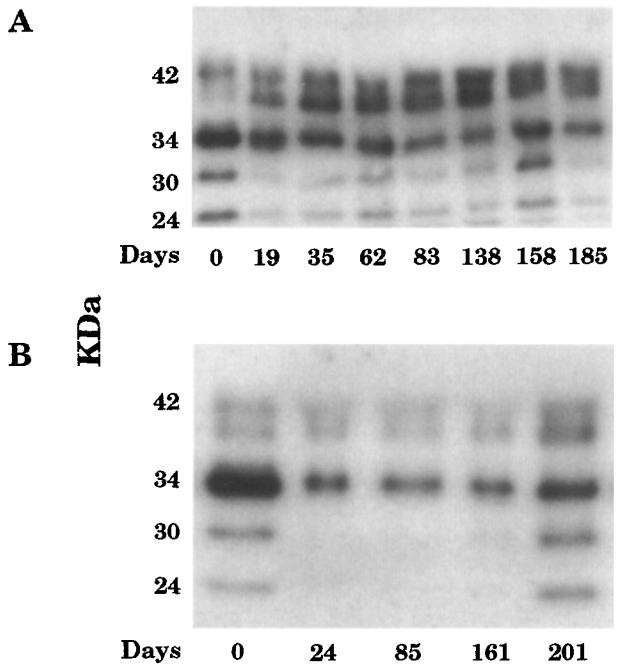

Fig 7. Serum IGFBPs in 2 children before and after LT. WLB was used to estimate the relative concentration of IGFBPs in 2 representative children before and after LT (A and B). Compare with control subjects in Fig 5.

nutritional deficiencies, or a combination of hormonal and nutritional factors, all of which might be associated with a generalized reduction in anabolic activity occurring in chronic illness.

Clearly, the marked improvement in the clinical status of these patients after LT was associated with significant hormonal, biochemical, and nutritional changes reflecting complex interrelationships. Although we did not measure serum insulin, GH, or cortisol levels in these subjects, previous studies have shown that patients with hepatic failure due to cirrhosis usually exhibit fasting hyperinsulinemia, insulin resistance, and a reduced clearance rate for this hormone.[25-27] Since insulin is known to enhance the synthesis of IGF-I and IGF-II,[22,28] hepatic production might actually be expected to be augmented by hyperinsulinemia under more favorable conditions. Basal serum GH, like insulin, also has been reported to be elevated in hepatic insufficiency,[12,29-31] and this is probably due, at least in part, to reduced negative feedback regulation resulting from decreased IGF-I and IGF-II levels. Despite significant increases in both insulin and GH in patients with hepatic insufficiency, the very low serum IGF-I and IGF-II concentrations seen in our patients suggest that the hormonal milieu favoring their production is surely less important than the reduced mass of functional hepatocytes in these patients.

Serum cortisol levels are usually normal[32] or slightly elevated[33] in patients with hepatic insufficiency, probably secondary to a prolongation of the $t_{1/2}$ of serum cortisol.[34] Corticosteroids have been shown to enhance hepatic IGF-I but not IGF-II production,[35] while actually decreasing IGF bioactivity.[36,37] Since all patients received 10 mg prednisone daily after LT for immunosuppression, it was not possible to compare the effects of steroids in the pretransplant and posttransplant periods. However, a recent study[38] reported that serum levels of IGF-I and IGF-II were normal in children treated with prednisone following renal transplantation. In any clinical disorder, hormonal levels per se do not permit one to predict their effects on target organs or tissues without knowing whether there is a change in the number of specific cell receptors or in the postreceptor pathways. As mentioned, before LT, most of our patients consumed somewhat nutritionally deficient diets. Relevant to this, Maes et al[39] and Clemmons et al[40] have shown that reduced serum IGF-I levels in malnourished rats are due to both a decrease in hepatic GH receptor number and a postreceptor defect. Likewise, Scheiwiller et al[41] have demonstrated that GH has little effect in stimulating hepatic IGF-I production in undernourished rats with poorly controlled diabetes. Clearly, the regulation of IGF-I and IGF-II production is multifactorial, and after LT, the marked increase in the serum level of these growth factors in our patients is a reflection of the restoration of hepatic function. Our observations confirm the recent data from Holt et al[12] in children with hepatic failure who underwent LT.

Emerging data indicate that IGFBPs play an important role in regulating the biologic actions of IGF-I and IGF-II.[42] Thus, the effects of hepatic dysfunction and subsequent LT on these BPs may significantly influence the growth-promoting properties of these peptides. The most consistent finding in our patients with hepatic failure was a reduction in IGFBP-3, the most abundant serum BP binding IGF-I and IGF-II. This is also in agreement with the data from studies of children with ESLD by Holt et al.[12] These investigators noted no difference in protease activity before and after LT, suggesting that the reduced serum IGFBP-3 in liver failure was due to decreased production rather than increased degradation. Since the mean percent reduction of IGFBP-3 before LT was considerably less than that of IGF-I (although almost identical to that of IGF-II), the increase in the IGFBP-3/IGF-I + IGF-II molar ratio would serve to further decrease the biological effects of these two mitogenic peptides in patients with hepatic insufficiency. Following LT, the mean IGFBP-3 concentration in our patients usually increased to the high-normal range, consistent with the elevated levels posttransplant reported by others.[12,15] While the marked increase in this BP after LT is undoubtedly mainly due to an increase in functional hepatic tissue, it is well known that GH, IGF-I, and glucocorticoids all increase serum levels of IGFBP-3.[33,40,43]

Serum IGFBP-1 was usually normal or only slightly elevated in patients with hepatic insufficiency, and it often decreased, as did IGFBP-4, after LT. Using a specific RIA for IGFBP-1, others[12,44] have noted a 2.5-fold to fourfold increase in this BP in patients with hepatic failure before LT, but the patients had fasted for 4 hours or longer before blood sampling. Fasting is known to markedly increase serum IGFBP-1 levels, while insulin decreases them.[45-47] On the other hand, glucocorticoids appear to increase IGFBP-1 synthesis,[48] and this probably occurs at the level of gene transcription.[49] Two of the four children we studied exhibited increased IGFBP-2 levels before LT, as did the youngest control subject. Although the reasons for this finding are unknown, Pucilowska et al[50] reported that in undernourished children IGFBP-2 levels were twice the values in control subjects. From our data and the results reported by other groups, it is obvious that the regulation of the synthesis

and secretion, and perhaps also the degradation, of IGFBPs is complex and is linked, at least in part, to the regulation of IGF-I and IGF-II production, as well.

In summary, our data serve to indicate what the serum levels of IGF-I, IGF-II, and IGFBP-1, -2, -3, and -4 are before and after LT in patients with ESLD, although they do not reveal the mechanism(s) that mediate the changes observed. Further studies must be performed to determine the precise causes and their relative importance for the diminished production of these important growth factors and BPs during hepatic insufficiency, and their restoration after LT. These changes probably result from a reduced number of hepatocytes per se. However, they undoubtedly also result from impaired signal transduction mediating the actions of GH, insulin, and/or cortisol, and perhaps from nutritional deficiencies concomitant with severe hepatic insufficiency.

REFERENCES

1. Uthne K, Uthne T: Influence of liver resection and regeneration on somatomedin (sulfation factor) activity in sera from normal and hypophysectomized rats. Acta Endocrinol (Copenh) 71:255-264, 1972
2. Hintz RL, Clemmons DR, Van Wyk JJ: Growth hormone–induced somatomedin-like activity from liver. Pediatr Res 6:353, 1972 (abstr)
3. Daughaday WH, Phillips LS, Mueller MC: The effects of insulin and growth hormone on the release of somatomedin by the isolated rat liver. Endocrinology 98:1214-1219, 1976
4. Schalch DS, Heinrich UE, Draznin B, et al: Role of the liver in regulating somatomedin activity: Hormonal effects on the synthesis and release of insulin-like growth factor and its carrier protein by the isolated perfused rat liver. Endocrinology 104:1143-1151, 1979
5. Emler CA, Schalch DS: Nutritionally induced changes in hepatic insulin-like growth factor I (IGF-I) gene expression in rats. Endocrinology 120:832-834, 1987
6. Rechler MM: Insulin-like growth factor binding proteins. Vitam Horm 47:1-114, 1993
7. Zapf J, Morell B, Walter H, et al: Serum levels of insulin-like growth factor (IGF) and its carrier protein in various metabolic disorders. Acta Endocrinol (Copenh) 95:505-517, 1980
8. 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 108:1265-1271, 1981
9. Sheppard MS, Minuk GY, Bhaumick B, et al: Insulin-like growth factors (IGF) in liver disease: Differential changes of IGF-I and IGF-II. Clin Invest Med 10:49-53, 1987
10. Wu JC, Daughaday WH, Lee SD, et al: Radioimmunoassay of serum IGF-I and IGF-II in patients with chronic liver diseases and hepatocellular carcinoma with or without hypoglycemia. J Lab Clin Med 112:589-594, 1988
11. Donaghy A, Ross F, Gimson A, et al: Growth hormone, insulinlike growth factor-1, and insulinlike growth factor binding proteins 1 and 3 in chronic liver disease. Hepatology 21:680-688, 1995
12. Holt RI, Jones JS, Stone NM, et al: Sequential changes in insulin-like growth factor I (IGF-I) and IGF-binding proteins in children with end-stage liver disease before and after successful orthotopic liver transplantation. J Clin Endocrinol Metab 81:160-168, 1996
13. Sarna S, Sipila I, Vihervuori E, et al: Growth delay after liver transplantation in childhood: Studies of underlying mechanisms. Pediatr Res 38:366-372, 1995
14. Bucuvalas JC, Cutfield W, Horn J, et al: Resistance to the growth-promoting and metabolic effects of growth hormone in children with chronic liver disease. J Pediatr 117:397-402, 1990
15. Codoner-Franch P, Bernard O, Alvarez F: Long-term follow-up of growth in height after successful liver transplantation. J Pediatr 124:368-373, 1994
16. Cardona J, Houssin D, Gauthier F, et al: Liver transplantation in children with Alagille syndrome—A study of twelve cases. Transplantation 60:339-342, 1995
17. Schalch DS, Kalayoglu M, Yang H, et al: Serum IGF-I and IGF-II concentrations in patients with hepatic failure: Effects of transplantation. Prog Endocr Soc 1988 (abstr 876)
18. Schalch D, Kalayoglu M, Scheff A, Pirsch J, Yang H: Serum IGF-I and IGF-II levels in patients undergoing liver transplantation: Functional assessment by long-term follow-up. Prog Endocr Soc 1990 (abstr 210)
19. Dills DG, Allen C, Palta M, et al: Insulin-like growth factor-I is related to glycemic control in children and adolescents with newly diagnosed insulin-dependent diabetes. J Clin Endocrinol Metab 80:2139-2143, 1995
20. Yang H, Ney DM: Insulin-like growth factor-I (IGF-I) responses in rats maintained with intravenous or intragastric infusion of total parenteral nutrition solutions containing medium- or long-chain triglyceride emulsions. Am J Clin Nutr 59:1403-1408, 1994
21. Furlanetto RW, Underwood LE, Van Wyk JJ, et al: Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay. J Clin Invest 60:648-657, 1977
22. Yang H, Scheff AJ, Schalch DS: Effects of streptozotocin-induced diabetes on growth and hepatic insulin-like growth factor I gene expression in the rat. Metabolism 39:295-301, 1990
23. McCusker RH, Camplon DR, Jones WK, et al: The insulin-like growth factor–binding proteins of porcine serum: Endocrine and nutritional regulation. Endocrinology 125:501-509, 1989
24. Hossenlopp P, Seurin D, Segovia-Quinson B, et al: Analysis of serum insulin-like growth factor binding proteins using Western blotting: Use of the method for titration of the binding proteins and competitive binding studies. Anal Biochem 154:138-143, 1986
25. Petrides AS, Groop LC, DeFronzo RA: Effect of physiologic hyperinsulinemia on glucose and lipid metabolism in cirrhosis. J Clin Invest 88:561-570, 1991
26. Kruszynska YT, Harry DS, Bergman RN, et al: Insulin sensitivity, insulin secretion and glucose effectiveness in diabetic and non-diabetic cirrhotic patients. Diabetologia 36:121-128, 1993
27. Barzilai N, Cohen P, Karnieli E, et al: In vivo insulin action in hepatocellular and cholestatic liver cirrhosis. J Endocrinol Invest 14:727-735, 1991
28. Eigenmann JE, Becker M, Kammermann B, et al: Decrease of nonsuppressible insulin-like activity after pancreatectomy and normalization by insulin therapy. Acta Endocrinol (Copenh) 85:818-822, 1977
29. Samaan NA, Stone DB, Eckhardt RD: Serum glucose, insulin, and growth hormone in chronic hepatic cirrhosis. Arch Intern Med 124:149-152, 1969
30. Conn HO, Daughaday WH: Cirrhosis and diabetes. V. Serum human growth hormone levels in Laennec's cirrhosis. J Lab Clin Med 76:678-688, 1970
31. Buzzelli G, Dattolo P, Pinzani M, et al: Circulating growth hormone and insulin-like growth factor-I in nonalcoholic liver cirrhosis with or without superimposed hepatocarcinoma: Evidence of an altered circadian rhythm. Am J Gastroenterol 88:1744-1748, 1993
32. Kley HK, Nieschlag E, Wiegelmann W, et al: Steroid hormones and their binding in plasma of male patients with fatty liver, chronic hepatitis and liver cirrhosis. Acta Endocrinol (Copenh) 79:275-285, 1975
33. Yamamoto M, Ariyoshi Y, Matsui M: The serum concentrations of unbound, transcortin bound and albumin bound cortisol in patients with dysproteinemia. Endocrinol Jpn 29:639-646, 1982
34. Kawai S, Ichikawa Y, Homma M: Differences in metabolic

properties among cortisol, prednisolone, and dexamethasone in liver and renal diseases: Accelerated metabolism of dexamethasone in renal failure. J Clin Endocrinol Metab 60:848-854, 1985

35. Miell JP, Taylor AM, Jones J, et al: The effects of dexamethasone treatment on immunoreactive and bioactive insulin-like growth factors (IGFs) and IGF-binding proteins in normal male volunteers. J Endocrinol 136:525-533, 1993

36. Elders MJ, Wingfield BS, McNatt ML, et al: Glucocorticoid therapy in children: Effect on somatomedin secretion. Am J Dis Child 129:1393-1396, 1975

37. Unterman TG, Phillips LS: Glucocorticoid effects on somatomedins and somatomedin inhibitors. J Clin Endocrinol Metab 61:618-626, 1985

38. Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, et al: Levels of growth hormone, insulin-like growth factor-I (IGF-I) and -II, IGF-binding protein-1 and -3, and cortisol in prednisone-treated children with growth retardation after renal transplantation. J Clin Endocrinol Metab 77:932-938, 1993

39. Maes M, Amand Y, Underwood LE, et al: Decreased serum insulin-like growth factor I response to growth hormone in hypophysectomized rats fed a low protein diet: Evidence for a postreceptor defect. Acta Endocrinol 117:320-326, 1988

40. Clemmons DR, Thissen JP, Maes M, et al: Insulin-like growth factor-I (IGF-I) infusion into hypophysectomized or protein-deprived rats induces specific IGF-binding proteins in serum. Endocrinology 125:2967-2972, 1989

41. Scheiwiller E, Guler HP, Merryweather J, et al: Growth restoration of insulin-deficient diabetic rats by recombinant human insulin-like growth factor I. Nature 323:169-171, 1986

42. Baxter RC: Insulin-like growth factor (IGF) binding proteins: The role of serum IGFBPs in regulating IGF availability. Acta Paediatr Scand Suppl 372:107-114, 1991

43. Zapf J, Hauri C, Waldvogel M, et al: Recombinant human insulin-like growth factor I induces its own specific carrier protein in hypophysectomized and diabetic rats. Proc Natl Acad Sci USA 86:3813-3817, 1989

44. Ross RJM, Rodriguez-Arnao J, Donaghy A, et al: Expression of IGFBP-1 in normal and cirrhotic human livers. J Endocrinol 141:377-382, 1994

45. Suikkari AM, Koivisto VA, Rutanen E-M, et al: Insulin regulates serum levels of low molecular weight insulin-like growth factor–binding protein. J Clin Endocrinol Metab 66:266-274, 1988

46. Cotterill AM, Cowell CT, Baxter RC, et al: Regulation of growth hormone–independent insulin-like growth factor binding protein in children. J Clin Endocrinol Metab 67:882-887, 1988

47. Hall K, Brismar K, Grissom F, et al: IGFBP-1. Production and control mechanisms. Acta Endocrinol (Copenh) 124:48-54, 1991

48. Conover CA, Divertie GD, Lee PD: Cortisol increases plasma insulin-like growth factor binding protein-1 in humans. Acta Endocrinol (Copenh) 128:140-143, 1993

49. Goswami R, Lacson R, Yang E, et al: Functional analysis of glucocorticoid and insulin response sequences in the rat insulin-like growth factor–binding protein-1 promoter. Endocrinology 134:736-743, 1994

50. Pucilowska JB, Davenport ML, Kabir I, et al: The effect of dietary protein supplementation on insulin-like growth factors (IGFs) and IGF-binding proteins in children with shigellosis. J Clin Endocrinol Metab 77:1516-1521, 1993