CLINICAL STUDY

The IGF system after insertion of a transjugular intrahepatic porto-systemic shunt in patients with liver cirrhosis

Peter Holland-Fischer¹, Hendrik Vilstrup¹, Jan Frystyk², Dennis Tønner Nielsen³, Allan Flyvbjerg² and Henning Grønbaek¹

¹Department of Medicine V (Hepatology and Gastroenterology), ²Medical Department M (Diabetes and Endocrinology) and the Medical Research Laboratories, Clinical Institute and ³Department of Radiology R, Aarhus University Hospital, Noerrebrogade 44, 8000 Aarhus C, Denmark

(Correspondence should be addressed to P Holland-Fischer; Email: phf@svf.au.dk)

Abstract

Objective: Insertion of a transjugular intrahepatic porto-systemic shunt (TIPS) into patients with liver cirrhosis usually induces a gain in body cell mass. Changes in the IGF system in favor of anabolism may be involved. We, therefore measured blood concentrations of the components of the IGF system in cirrhosis patients before and after elective TIPS.

Design and methods: The study comprised 17 patients and 11 healthy controls. Patients were examined before and 1, 4, 12, and 52 weeks after TIPS. Biochemical analyses of the IGF system were compared with changes in body composition (bioimpedance analysis), glucose and insulin, and metabolic liver function (galactose elimination capacity).

Results: After TIPS, body cell mass rose by 3.2 kg (95% confidence interval (CI): 1.0–5.5) at 52 weeks, in correlation with baseline liver function ($r^2=0.22$; $P=0.03$). Peripheral blood concentrations of total IGF1 and 2, bioactive IGF1, and the IGF-binding proteins (IGFBP-1, -2, and -3) remained unchanged throughout the study period. There was no change in fasting glucose, whereas fasting insulin rose by 40% (CI: 11–77%) and glucagon by 58% (CI: 11–132%) from baseline to 52 weeks after TIPS.

Conclusion: Our data confirm that TIPS was associated with an increase in body cell mass in patients with liver cirrhosis, but without any change in the circulating IGF system. Thus, the results do not support the notion that effects on the circulating IGF system are involved in the anabolic effects of TIPS insertion.

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Introduction

Patients with liver cirrhosis often suffer from severe hepatic malnutrition with loss of body tissue that is difficult to treat and carries a sinister prognosis in terms of increased morbidity and mortality (1–3).

Since the introduction of transjugular intrahepatic porto-systemic shunt (TIPS) as a clinical procedure to reduce portal hypertension, it was observed that the procedure is frequently associated with marked nutritional improvement and gain in body cell mass (4, 5). The underlying mechanism is unknown, but it is important to identify in order to improve the pathophysiological understanding of the metabolic consequences of portal hypertension and because it may imply pharmacological treatment possibilities of malnutrition also in patients who are unable to receive a TIPS.

Patients with cirrhosis and portal hypertension are known to have significant changes in their insulin-like growth factor (IGF) system and its interaction with insulin that may play a role for their loss of body mass (6–9). Therefore, it is an obvious possibility that TIPS changes the prevailing IGF system and insulin levels in

a way that contributes towards improved nutrition (10–12). Still, the effects of TIPS on the IGF system have not been systematically described.

The IGF system comprises the peptides IGF1 and 2 in free and bound forms and their IGF-binding proteins (IGFBP-1 to -6). The small free-peptide fractions exert most of the growth-regulating effects, but also the IGFBPs are active, and regulation of them influences the IGF bioactivity. The peptide-free fractions and insulin interact in anabolism via an insulin effect on IGFBP-1 and -2 (13–15). The liver plays a central regulatory role for the system as a whole, both by synthesizing several of the components of the system, and by modifying portal-derived substances, including insulin (16–18).

Our *a priori* expectation was that insertion of TIPS in patients with cirrhosis would induce significant changes in the deranged IGF system, which are compatible with anabolism.

The primary purpose of our work, therefore, was to map the components of the circulating IGF system in cirrhosis patients before and after TIPS. The secondary purpose was to relate these findings to changes in body composition and liver function.

Methods

Subjects and ethics

Twenty-six patients with liver cirrhosis set to undergo an elective TIPS procedure were consecutively enrolled. Three never completed the baseline examination due to variceal bleeding demanding intensive care and immediate TIPS insertion. Out of the remaining 23 (15 males and eight females) patients, one underwent orthotopic liver transplantation, two died during follow-up, and three refused to participate in follow-up. Thus, 17 patients completed the study (Fig. 1). The diagnosis of cirrhosis was established by a combination of biochemical, clinical, and ultrasonographic findings and confirmed by liver biopsy in eight cases. The etiologies were alcohol intake ($n=13$), autoimmune hepatitis ($n=2$), primary sclerosing cholangitis ($n=1$), or unknown ($n=1$). Abstinence from alcohol was a goal during the study, and to our knowledge all save one adhered to that policy.

As controls, we studied 11 normal healthy volunteers recruited among hospital staff and matched for body mass index (BMI), sex, and age. All gave written informed consent to their participation in accordance with the Helsinki Declaration. The study was approved by the Research Ethics Committee of Aarhus.

Experimental design

The patients were studied within 2 weeks before TIPS insertion. Post-TIPS examinations were carried out 1, 4, 12, and 52 weeks after the procedure. All subjects were examined after an overnight fast. Seven patients with moderate or tense ascites underwent paracentesis prior to the pre-TIPS examination.

Figure 1 Flow chart. Number in boxes refers to number of patients. Grey horizontal bar indicates TIPS insertion and black horizontal line indicates exclusion of participants as indicated.

Nutritional therapy and assessment of energy intake

All patients received standard nutritional education and therapy according to European Society for Clinical Nutrition and Metabolism guidelines during TIPS evaluation and follow-up (19). The therapy was handled by a clinical dietician. Energy and protein intake were assessed by systematic dietary recall and food diary during hospital admission. All TIPS patients, except two, underwent a baseline assessment, and a random sample of eight patients was comprehensively assessed 12–26 weeks after TIPS insertion. Energy expenditure was calculated using Harris and Benedict's equation with the use of total body weight, in those with ascites after paracentesis (20).

TIPS procedure

Indications for TIPS insertion were refractory ascites (10 patients), secondary prevention of variceal rebleeding (five patients), or a combination (two patients). None had active variceal bleeding at the time for TIPS insertion. The TIPS was inserted using covered stents according to the method described by Rössle *et al.* (21). After insertion, clinical and ultrasonographic shunt controls were performed after 24 h, 4 weeks, and then every 12 weeks for the first year. There was no procedure-related complication. The porto-systemic pressure gradient was lowered from a median of 16 (range 10–29) to 6 (range 2–10) mmHg. Three patients needed stent revision during follow-up. Transient hepatic encephalopathy was observed in three patients and reverted on diet and laxatives without the need for shunt reduction.

Biochemical analyses

Blood samples were placed on ice, centrifuged at 5 °C, separated, and stored at –80 °C until analysis. Plasma glucose concentrations were determined after sampling (Beckman Instruments, Palo Alto, CA, USA). Serum insulin concentrations were measured by a two-site immunospecific insulin ELISA (22). Plasma glucagon concentrations were determined by RIA, as described by Orskov *et al.* (23). GH was measured using a commercial immunofluorometric assay (24). Serum total IGF1 and 2 were determined following acid ethanol extraction by in-house monoclonal time-resolved immunofluorometric assays (25). IGF1 bioactivity was determined by an in-house kinase receptor activation assay based on transfected cells (26). IGFBP-1 and -2 were determined by in-house immunoassays as previously described (27). IGFBP-3 was measured using a commercial IRMA (BioSource, Nivelles, Belgium). All measurements were performed in duplicates.

Liver function

Clinical status was assessed according to the model for end-stage liver disease score (MELD). The galactose elimination capacity (GEC) was used to quantitatively measure metabolic liver function. The GEC was determined from blood concentration decay curves corrected for urinary excretion, as described by Tygstrup (28).

Insulin resistance

Homeostasis model assessment (HOMA) insulin resistance (IR) index was calculated using simultaneous fasting insulin and glucose levels (29).

Body composition

Bioimpedance analysis (Quadscan 4000, Bodystat Ltd, Isle of Man, UK) was used to estimate body composition, in patients with ascites after paracentesis. The predictive equations are taken from Kushner *et al.* and Lautz *et al.* (30, 31). We focused on body cell mass, because this estimate constitutes metabolically active cells and is relatively independent on the degree of ascites during serial measurements (32).

Statistics analysis

Data analysis was performed using STATA 10 statistical software (StataCorp LP, College Station, TX, USA). Results are given as mean $\pm$ s.d. except for the IGF system in which results are presented as median (lower quartile–upper quartile). Changes from baseline were explored by ANOVA for repeated measurements. Due to non-systematic factors, blood samples were missing from eight examinations in six patients. For the statistical analyses, these missing values were replaced by the mean of adjacent values or, in case of missing 52 weeks values, the last observation was carried forward. Controls and the baseline groups were compared by unpaired Students *t*-test. The variables regarding the growth factor system, glucagon, and insulin depended on logarithmic transformation before parametric

methods could be applied. Correlation between variables was examined by linear regression at baseline and 52 weeks time points. A *P* value <0.05 was considered significant in a two-tailed test.

Results

Patient characteristics

Patients had a mean age of 56 (25–78) years and a BMI of 25 (17–37) kg/m², similar to controls with age 57 (35–64) years and BMI 24 (19–28) kg/m². After TIPS, one patient died because of alcohol abuse, one died, and one underwent liver transplantation due to liver failure.

At 52 weeks after TIPS, GEC had fallen from 59 to 52% of expected value (difference -7% , CI: -15 to -1). Mean MELD score was 8 (1–21) before TIPS and did not change (Table 1).

Body composition

At baseline, body cell mass was 6.7 kg (CI: 0.5–12.9) lower than controls. After TIPS, body cell mass rose on average by 2.6 kg (CI: 0.2–4.9) at 12 weeks and by 3.2 kg (CI: 1.0–5.5) at 52 weeks (Table 1). Thirteen patients increased their cell mass. The maximum change in body cell mass (BCM) correlated moderately with the baseline GEC ($r^2=0.25$, $P=0.03$).

IGFs and IGFBPs

Before TIPS insertion, the patients had reduced blood concentrations of total IGF1, bioactive IGF1, and IGFBP-3 compared with controls, whereas GH, IGFBP-1 and -2 were increased (Fig. 2). After TIPS, the concentrations remained unchanged during the 52-week study period. GEC correlated with bioactive IGF1 before ($r^2=0.30$, $P=0.02$) and 52 weeks after TIPS ($r^2=0.41$, $P=0.003$) and also with IGFBP-3 at both examinations (baseline: $r^2=0.20$, $P=0.04$; 52 weeks: $r^2=0.25$, $P=0.03$). Patients had reduced total IGF2 levels compared with controls (339 (298–592) vs

Table 1 Body composition and liver function.

	Controls <i>n</i> =11	TIPS baseline <i>n</i> =17	TIPS 1 week <i>n</i> =17	TIPS 4 weeks <i>n</i> =17	TIPS 12 weeks <i>n</i> =17	TIPS 52 weeks <i>n</i> =17	Equality of TIPS groups	Controls versus TIPS baseline
Weight (kg)	74.1 $\pm$ 14.6	76.6 $\pm$ 19.5	75.7 $\pm$ 16.7	79.1 $\pm$ 18.1	79.7 $\pm$ 18.1	80.9 $\pm$ 19.6	<i>P</i> =0.18	<i>P</i> =0.62
Body cell mass (kg)	31.9 $\pm$ 8.1 ^a	25.2 $\pm$ 8.4	26.5 $\pm$ 8.4	26.8 $\pm$ 8.4	27.8 $\pm$ 9.1 ^a	28.5 $\pm$ 9.6 ^a	<i>P</i> =0.03	<i>P</i> =0.034
GEC percentage of expected value	ND	59 $\pm$ 13	57 $\pm$ 10	55 $\pm$ 11	54 $\pm$ 10	52 $\pm$ 9 ^a	<i>P</i> =0.04	ND
MELD score	1 $\pm$ 0.2 ^a	8.3 $\pm$ 5.3	9.8 $\pm$ 4.5	9.9 $\pm$ 3.6	9.9 $\pm$ 4.3	10.3 $\pm$ 4.8	<i>P</i> =0.40	<i>P</i> <0.001

Data on body composition are determined by bioimpedance analysis. For each continuous variable, results are shown as mean $\pm$ s.d. GEC, galactose elimination capacity; *n*, number of non-missing values; ND, not done.

^aDenotes significant change from baseline.

Figure 2 Components of the growth factor system in controls and before (baseline) and after TIPS insertion (1, 4, 12, and 52 weeks respectively). Boxes display median with lower and upper quartile, whiskers indicates the 95% interval and circles represents outliers. The symbol * denotes $P < 0.001$ and ** $P < 0.01$ compared with baseline (ANOVA).

1076 (1001–1164) mg/l; $P < 0.001$), which did not change during follow-up.

Bioactive IGF1 correlated negatively with IGFBP-1 before TIPS ($r^2 = 0.33$; $P = 0.003$) and after 52 weeks ($r^2 = 0.25$, $P = 0.02$). There was a positive correlation with total IGF1 before TIPS ($r^2 = 0.25$, $P = 0.02$) and after 52 weeks ($r^2 = 0.24$, $P = 0.03$), as well as with IGFBP-3 before TIPS ($r^2 = 0.41$; $P = 0.001$) and after 52 weeks ($r^2 = 0.23$, $P = 0.03$). Bioactive IGF1 did not correlate with GH, IGFBP-2, or insulin levels.

IGFBP-1 and -2 showed a moderate negative correlation with insulin before TIPS (IGFBP-1: $r^2 = 0.33$, $P = 0.003$; IGFBP-2: $r^2 = 0.23$, $P = 0.04$) and also after 52 weeks (IGFBP-1: $r^2 = 0.33$, $P = 0.003$; IGFBP-2: $r^2 = 0.25$, $P = 0.02$).

Glucose, insulin, glucagon, and HOMA index

There was no difference in fasting glucose between the cirrhosis group before TIPS and controls, or in the cirrhosis group after TIPS. Fasting insulin was higher in the cirrhosis group before TIPS than in controls, and rose further by 40% (11–77%) 52 weeks after TIPS (Table 2). Correspondingly, the HOMA index of IR increased by 46% (11–93%) at 52 weeks. GH correlated negatively with insulin ($r^2 = 0.11$; $P = 0.015$), but no linear relationship was found with HOMA index of IR. The increase in insulin was paralleled by glucagon with levels being doubled at 52 weeks, and the two variables were positively associated both before and after TIPS insertion ($r^2 = 0.29$, $P < 0.001$).

Table 2 Glucose, insulin, glucagon, and homeostasis model assessment-insulin resistance (HOMA-IR).

	Controls <i>n</i> = 11	TIPS baseline <i>n</i> = 17	TIPS 1 week <i>n</i> = 16	TIPS 4 weeks <i>n</i> = 14	TIPS 12 weeks <i>n</i> = 17	TIPS 52 weeks <i>n</i> = 15	Equality of TIPS groups	Controls versus TIPS baseline
Glucose (mmol/l)	5.1 ± 0.5	5.9 ± 1.6	5.4 ± 0.9	6.0 ± 1.8	5.9 ± 1.7	6.0 ± 1.5	$P = 0.71$	$P = 0.22$
Insulin (pmol/l)	36 ± 17 ^a	125 ± 85	151 ± 87	183 ± 107 ^a	184 ± 116 ^a	172 ± 91 ^a	$P = 0.025$	$P = 0.002$
Insulin resistance	1.2 ± 0.7 ^a	4.7 ± 3.7	5.2 ± 3.6	7.3 ± 6.0	7.3 ± 5.8	7.7 ± 5.7 ^a	$P = 0.048$	$P = 0.005$
HOMA index								
Glucagon (pmol/l)	39 (30–75)	168 (89–375)	172 (125–332)	252 (112–441)	298 (145–354)	298 (108–659) ^a	$P < 0.001$	$P < 0.001$

Results are given as mean ± s.d. or median (lower quartile–upper quartile). Insulin resistance is calculated by the HOMA nomogram method where the value 1 represents the average healthy 35-year-old male. *n*, number of non-missing values.

^aDenotes significant change from baseline.

Energy and protein intake

Energy intake before TIPS was 7385 ± 1068 kJ/day equal to 80% of the predicted energy requirement of 9185 ± 1070 kJ/day ($P < 0.001$). Protein intake was 65 ± 12 g protein per day corresponding to 74% of predicted requirement. Eight patients with complete dietary record had an energy intake of 7215 ± 340 kJ/day before and 7630 ± 620 kJ/day after TIPS ($P = 0.56$) equal to 76% of predicted requirement.

High gainers versus low gainers

As an exploratory *post hoc* analysis, patients were divided into a body cell mass high-gain group ($n = 10$) and a low gain ($n = 7$) with an arbitrary discrimination value of 2.0 kg gain in BCM. There was no difference in either GEC, MELD score, or in bioactive IGF1 before TIPS between these groups, whereas HOMA calculated IR before TIPS tended to be higher among the low gainers (6.7 vs 2.8, $P = 0.06$).

Discussion

Our main finding was that TIPS neither acutely nor during follow-up changed the blood concentration of any component of the IGF system of the cirrhosis patients, even though their body cell mass rose. This finding is in contrast to our *a priori* expectation. The improvement in nutrition correlated positively with baseline liver function and marginally negatively with baseline IR.

Only total IGF1 has been studied previously in relationship to TIPS insertion, and as in our study its concentration did not change 1 month after the procedure (33). Both the malnutrition with low BCM in patients compared with controls and the observed increase after TIPS is in agreement with previous studies (4, 5, 34). Body weight only tended to increase which is explained by the resolution of ascites masking the non-water weight gain.

We expected that TIPS insertion with shunting of insulin-rich portal blood to the systemic circulation would increase the circulating blood concentration of bioactive IGF1 by decreasing levels of the inhibitory IGFBP-1 and -2 (13–15). Peripheral insulin levels did indeed increase markedly, implying that our data are not compatible with this mechanism. Also, there was no change in any other component of the IGF system, and there was no relationship between changes in body composition and the IGF system.

There may be several explanations for the lack of increase in activity of the IGF system after TIPS. The most likely one is related to the central function of the liver in both producing the key components of the system and being the main site for the interaction between them and insulin. Animal experiments show that the majority of the circulating IGF1 pool and IGFBPs originates from the liver (35, 36). The reduction

in metabolic liver function after TIPS, as measured by the decrease in GEC, may explain the lack of change in circulating IGF1 after TIPS (37). Another possible explanation is related to the fact that the production of key components of the growth factor system is also nutritionally regulated (38). We did not identify a consistent increase in energy intake after TIPS, and this might limit an increase in IGF1. Insulin is an important regulator of IGF1 by controlling the hepatic GH receptor expression and hence GH sensitivity. Furthermore, insulin inhibits the hepatic synthesis of IGFBP-1 and -2, thereby increasing IGF1 bioactivity (14). We found no increase in IGF1 bioactivity or any reductions in the two insulin-sensitive IGFBPs. This may reflect that the sinusoidal hepatocyte exposure to insulin remained unchanged or even reduced, despite the elevated peripheral blood insulin concentration. This could be due to insulin-rich portal bypassing the sinusoids via the TIPS, so that sinusoidal perfusion in that situation was mostly dependent on arterial and relatively insulin-depleted blood supply (39–41). Studies in dogs with shifting of insulin release from the portal to a peripheral vein immediately doubles peripheral blood insulin concentration, halves the sinusoidal concentration (42), and doubles the hepatic glucose output. This mechanism would partly explain the observed euglycemia despite the marked peripheral hyperinsulinemia.

Still, since many components of the IGF system are synthesized in non-hepatic tissues, we cannot exclude that some of the effects of TIPS on body composition are related to local (i.e. paracrine and/or autocrine) effects of the IGF system, not reflected by changes in the circulation.

The fasting euglycemia, the hyperinsulinemia, and the increased HOMA-calculated IR are in accordance with previous studies (33, 43). Others report no change in peripheral IR using the hyperinsulinemic clamp technique (43). It should be noted that GH, which is elevated in cirrhosis and associated with IR (44), did not change after TIPS. Baseline hyperglucagonemia aggravated after TIPS insertion. This observation has been made by others, and is often interpreted as a counter-regulatory response to hyperinsulinemia. This would be in agreement with our finding of a positive association between insulin and glucagon (45). It may, however, as well be a direct consequence of the porto-systemic shunting and reduced hepatic degradation. We found that the increase in insulin was not associated with the gain in body cell mass. The trend towards lower baseline HOMA-calculated IR among high gainers is likely to be interrelated with the better metabolic liver function. In any case, our findings of the possible importance of baseline metabolic state for the body cell mass gain after TIPS may have the implication that a relatively good clinical condition of the patient is important to obtain the favorable nutritional effect of TIPS – and more so than TIPS-related effects on the IGF system.

Insertion of a TIPS and the alleviation of portal hypertension have several effects that are likely to shift

the metabolic homeostasis from catabolic to anabolic. The frequency of stressful and catabolic events like esophageal bleeding, ascites, and bacterial peritonitis is effectively reduced.

Portal hypertension with ascites reduces dietary intake, by decreasing inducing nausea and promoting postprandial fullness (46). The impact of TIPS on energy intake has been addressed by Plauth *et al.* who found that protein and energy intakes were higher after TIPS, whereas Allard *et al.* like us, did not detect any consistent change (4, 5). However, precise determination of energy intake is difficult and uncertain and even small changes in daily energy intake result in large variation in body weight over time. Overall, we found an increase in energy intake by 415 kJ/day, which is in itself statistically non-significant, but nonetheless has the potential to induce a 3–4 kg weight gain if maintained stable during the first year.

Portal hypertension is also associated with intestinal malabsorption and the presence of bacterial products in the abdominal lymph nodes (47–49). Hence, TIPS probably leads to improved nutrient absorption and utilization and reduces the energy consuming low-grade inflammation (50).

One main limitation of this study is the low number of subjects; implying a risk of a type 2 error and preventing us from identifying baseline predictors for weight gain. As for the measurements of bioactive IGF, however, the variation of these analyses was so low (coefficient of variation <7%) (26) that our results safely preclude major or functionally important effects of TIPS. It was also not possible to include a comparable non-TIPS cirrhosis control group. Accumulation of fluids in patients with liver cirrhosis may produce inaccurate results when bioimpedance measurements are used to determine body cell mass. We used bioimpedance after reducing ascitic patients' body water component. Furthermore, when used during serial measurements on the same patient, the method is remarkably robust and produces stable results largely regardless of the degree of ascites (32). In the same way, the patients' fluid accumulation may also have interfered with our use of BMI to match patients and controls. This, however, would not be important since we used the controls mostly as a reference group for the hormone analyses, the temporal changes after TIPS being our primary goal.

In conclusion, the circulating IGF system did not change after TIPS even though body cell mass increased. Other mechanisms must be involved in the anabolic effect of TIPS, probably involving pre-TIPS metabolic status, portal pressure-related events, or unidentified nutritional effects.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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