The Contribution of Proinsulin and Des-31,32 Proinsulin to the Hyperinsulinemia of Diabetic and Nondiabetic Cirrhotic Patients

Yolanta T. Kruszynska, David S. Harry, Vidya Mohamed-Ali, Philip D. Home, John S. Yudkin, and Neil McIntyre

We used specific, monoclonal antibody–based, two-site immunoradiometric assays to test the hypothesis that serum levels of proinsulin and des-31,32 proinsulin would be increased in cirrhosis, particularly in those with overt diabetes. A 75-g oral glucose tolerance test was performed after an overnight fast in eight cirrhotic patients with diabetes (fasting blood glucose, 7.8 ± 2.2 [SE] mmol/L), seven nondiabetic cirrhotic patients, and eight normal control subjects. Fasting serum immunoreactive insulin levels were approximately six times higher in cirrhotics than in controls, but were not different between diabetic and nondiabetic cirrhotic patients. After oral glucose, the incremental area under the serum insulin concentration curve was 3,475 ± 1,009 pmol · L^{-1} · h in nondiabetic cirrhotic patients, significantly higher than in controls (761 ± 48, $P < .001$) or diabetic cirrhotic patients (881 ± 186, $P < .05$). Fasting serum proinsulin levels in diabetic cirrhotic patients (24.0 ± 5.7 pmol/L) were higher than in controls (2.3 ± 0.5, $P < .001$) or nondiabetic cirrhotic patients (4.4 ± 0.8, $P < .005$). Fasting serum levels of des-31,32 proinsulin were also much higher in diabetic cirrhotic patients than in nondiabetic cirrhotic patients or controls ($P < .02$ and $P < .005$, respectively). Fasting proinsulin plus des-31,32 proinsulin constituted 12.5% ± 1.4% of serum immunoreactive insulin in diabetic cirrhotics, higher than in nondiabetic cirrhotics (3.7% ± 0.5%, $P < .001$) and normal controls (7.8% ± 1.5%, $P = .035$). The fasting proinsulin to C-peptide molar ratio was significantly higher in diabetic cirrhotic patients (25.1 ± 8.6) than in controls (6.3 ± 1.4) or nondiabetic cirrhotic subjects (4.9 ± 1.4; $P < .05$ for both). In diabetic cirrhotic patients, proinsulin correlated with fasting blood glucose levels ($r = .95$, $P < .001$), as did des-31,32 proinsulin ($r = .87$, $P < .01$), proinsulin as a proportion of immunoreactive insulin ($r = .82$, $P < .02$), and the proinsulin to C-peptide molar ratio ($r = .87$, $P < .005$). Proinsulin levels were increased in both diabetic and nondiabetic cirrhotic patients, but a disproportionate elevation relative to insulin and C-peptide was seen only in diabetic patients. Compared with findings in type II diabetes, the changes in proinsulin as a proportion of total immunoreactive insulin were small (<15%) because insulin clearance is impaired in cirrhosis.

IMPAIRED ORAL GLUCOSE tolerance is found in most patients with cirrhosis,[1,2] and they have a prevalence of overt diabetes two to four times that of the general population.[2] Impaired glucose tolerance is mainly due to insulin insensitivity of peripheral tissues.[3,4] Overt diabetes occurs in those cirrhotic patients who have a marked impairment of insulin secretion and insulin insensitivity[4]; however, serum insulin levels, both fasting and after oral or intravenous glucose, are high in diabetic and nondiabetic cirrhotic patients.[4] The clearance of insulin decreases in cirrhosis due to hepatocellular dysfunction and/or portal-systemic shunting.[3,5,6] Reduced clearance is the major determinant of increased insulin levels in cirrhosis, although increased insulin secretion also plays a part in nondiabetic cirrhotic patients.[3,7] The reduction of the insulin metabolic clearance rate in cirrhosis is much greater for secreted insulin than for insulin infused into the systemic circulation,[3,7] suggesting that reduced first-pass hepatic uptake of endogenous insulin due to portal-systemic shunting plays a major role. However, most insulin assays are not specific. They show a high degree of cross-reactivity with proinsulin and its partially cleaved products.[8] Because the half-lives of intact and split proinsulins are five to seven times that of insulin,[9,10] an increased contribution of proinsulin and its derivatives to circulating "insulin" concentrations in cirrhosis could also help to explain the greater reduction in the metabolic clearance rate of secreted "insulin" as compared with exogenous insulin.[3,7]

Many patients with type II diabetes have high fasting and postprandial serum insulin levels. Recent studies using highly specific, monoclonal antibody–based, two-site immunoradiometric assays for proinsulin and its derivatives have shown that up to 50% of circulating immunoreactive insulin in type II diabetes may be due to proinsulin and its major intermediate conversion product, des-31,32 proinsulin[11]; thus, the concentration of true insulin in type II diabetes may be overestimated with standard insulin assays.[12]

If proinsulin levels increase as a consequence of insulin insensitivity and/or hyperglycemia increasing the demand for insulin and leading to depletion of mature islet β-cell granules with subsequent release of proinsulin-rich immature granules,[13,14] one might expect proinsulin levels to be increased in cirrhotics, particularly those with overt diabetes mellitus. However, reports of serum proinsulin levels in cirrhosis are conflicting.[15,16] Increased circulating proinsulin levels could contribute to the delayed onset of insulin action seen after glucose ingestion in cirrhosis,[17] because the effect of proinsulin to promote tissue glucose uptake is delayed compared with that of insulin.[18] An increased contribution of proinsulin to immunoreactive insulin would also lead to an overestimation of the degree of insulin insensitivity by methods that rely on endogenously secreted insulin such as Bergman's minimal model method,[4,19] since the in vivo potency of proinsulin is only approximately 10% that of insulin.[10,18] We have therefore used specific, mono-

From the Department of Medicine, Royal Postgraduate Medical School, London; the Department of Medicine, Whittington Hospital, London; and the Department of Medicine, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, UK.

Submitted January 21, 1994; accepted April 14, 1994.

Supported by the British Diabetic Association and the University of London Central Research Fund.

Address reprint requests to Yolanta T. Kruszynska, PhD, MRCP, Department of Metabolic Medicine, Royal Postgraduate Medical School, Du Cane Road, London W12 ONN, UK.

0026-0495/95/4402-0021$03.00/0

clonal antibody–based, two-site immunoradiometric assays[20] to test the hypothesis that circulating levels of intact proinsulin and des-31,32 proinsulin would be increased in cirrhosis, particularly in those with overt diabetes.

SUBJECTS AND METHODS

Fifteen stable, biopsy-proven, alcoholic cirrhotic patients with and without overt diabetes mellitus were recruited. The nondiabetic cirrhotic group (n = 7) had a normal fasting blood glucose. Diabetic cirrhotic patients (n = 8) were on treatment with oral hypoglycemic agents (Table 1) and a weight-maintaining diet in which at least 50% of energy was derived from carbohydrate (~ 250 g/d). They had previously been documented to have a fasting blood glucose off treatment of greater than 6.7 mmol/L, consistent with a diagnosis of diabetes according to World Health Organization criteria.[21] At the time of study, all were outpatients. Marked insulin insensitivity had been documented in six of the nondiabetic cirrhotic patients and seven of the diabetic cirrhotic patients by the euglycemic clamp and minimal model methods.[3,4] Three nondiabetic cirrhotic patients and four diabetic cirrhotic patients were taking spironolactone, but none had ascites at the time of study. Eight normal control subjects (laboratory staff or relatives of patients) were also studied. Clinical characteristics of patients and controls are presented in Table 1. None of the subjects had a family history of diabetes mellitus, and apart from oral hypoglycemic agents (see above), they were not on treatment known to affect glucose tolerance. Twelve patients had esophageal varices on endoscopy; eight had previously bled from varices, but not in the 3 months before study. Patients with alcoholic cirrhosis had abstained from alcohol for at least 2 months before study. Diabetic patients were asked to continue with their usual diet but to stop taking their oral hypoglycemic agents 3 days before study. Control and nondiabetic cirrhotic subjects consumed a diet containing at least 200 g carbohydrate/d. The study was approved by the local ethics committee.

Oral Glucose Tolerance Tests

All studies were performed in the morning after an overnight fast. For blood sampling, a venous cannula was inserted retrogradely in a hand vein, with the hand being maintained in a hand warmer at 60°C. After each blood sample, the cannula was flushed with 0.15 mol/L NaCl in water. Two basal blood samples were taken for estimation of blood glucose, serum insulin, proinsulin, and des-31,32 proinsulin concentrations. Subjects then ingested 75 g glucose in 390 mL water. The glucose was divided into five aliquots and administered at minute intervals. Blood samples for glucose and insulin determinations were taken at 15-minute intervals until +60 minutes and then at 30-minute intervals until +180 minutes. Blood samples for proinsulin and des-31,32 proinsulin determinations were taken at 60, 120, and 180 minutes after glucose ingestion.

Table 1. Clinical Characteristics of Cirrhotic and Control Subjects

	Controls	Nondiabetic Cirrhotics	Diabetic Cirrhotics
n	8	7	8
Age (yr)	47 ± 9	54 ± 8	56 ± 7
Weight (kg)	66 ± 12	71 ± 15	75 ± 11
Body mass index (kg/m²)	24.5 ± 3.2	26.0 ± 4.2	25.4 ± 3.5
Esophageal varices (n)	—	6	6
Oral hypoglycemic agents (n)			
Tolbutamide			7
Glipizide	—	—	1
Serum albumin (g/L)	44 ± 2	38 ± 7	40 ± 3
Serum bilirubin (μmol/L)	10 ± 3	25 ± 11	40 ± 43
Prothrombin time (s)	12 ± 1	16 ± 2	16 ± 2

NOTE. Results are the mean ± SD.

Analyses

Blood glucose levels were measured by a glucose oxidase method (Yellow Springs glucose analyzer, Clandon Scientific, London, UK). Serum insulin levels were measured using a double-antibody technique[22]; intraassay and interassay coefficients of variation were 6.8% and 7.9%, respectively. The assay measures immunoreactive insulin levels and shows 100% cross-reactivity with the proinsulin-like molecules. Serum C-peptide levels were measured by ethanol precipitation radioimmunoassay[23] using the M1221 antibody and a synthetic standard (Novo Research Institute, Bagsvaerd, Denmark). The cross-reactivity of the M1221 antibody with proinsulin is approximately 80%. Proinsulin and des-31,32 proinsulin levels were measured using monoclonal antibody–based, two-site immunoradiometric assays.[20] Monoclonal antibodies to insulin and proinsulin were obtained from Serono Diagnostics (Woking, UK), and anti–C-peptide antibody (PEP001) was from Novo (Copenhagen, Denmark). Intact, des-31,32, and des-64,65 proinsulin standards were supplied by Eli Lilly & Co. (Indianapolis, IN). Intact proinsulin and des-31,32 proinsulin were captured with 3BI immobilized on microtiter plates (Immunol 4; Dynatech, Billinghurst, Sussex, UK) and detected with iodinated antibody and PEP001, respectively. Intraassay and interassay coefficients of variation were 6.3% and 9.8% for intact proinsulin and 8.6% and 12.6% for des-31,32 proinsulin. Detection limits (mean ± 3 SD of zero signal) for intact and des-31,32 proinsulin were 0.25 and 0.125 pmol/L, respectively. There was less than 1% cross-reactivity with insulin in either assay. In the intact proinsulin assay, there was 78% cross-reactivity with des-64,65 proinsulin; in the des-31,32 proinsulin assay, there was 59% cross-reactivity with intact proinsulin and 54% cross-reactivity with des-64,65 proinsulin. In all circumstances, competition curves were parallel to those of the specific hormone. Recovery of intact or des-31,32 proinsulin added to human plasma (3 to 33 pmol/L) was 93% (range, 83% to 101%) and 89% (range, 84% to 99%), respectively. The des-31,32 proinsulin concentration was calculated by subtracting the cross-reactivity of measured intact proinsulin.

Statistical Analysis

Results are expressed as the mean ± SEM unless otherwise indicated. Areas under the glucose and insulin concentration curves were calculated by the trapezoidal rule. The significance of differences within groups was tested by Student's paired t test and between groups by ANOVA followed by Tukey's multiple comparison test. Correlations were sought by Pearson's least-squares method. A P value of less than .05 was considered statistically significant.

RESULTS

Oral Glucose Tolerance Tests

Fasting blood glucose concentrations did not differ statistically between nondiabetic cirrhotic patients and control subjects (4.9 ± 0.2 v 4.5 ± 0.1 mmol/L), but were higher in diabetic cirrhotic patients (7.8 ± 2.2, $P < .001$ v both control and nondiabetic cirrhotic patients). Following the oral glucose load, blood glucose concentrations were significantly higher in both cirrhotic groups than in controls, and were much higher in diabetic cirrhotic patients than in nondiabetic cirrhotic patients (Fig 1).

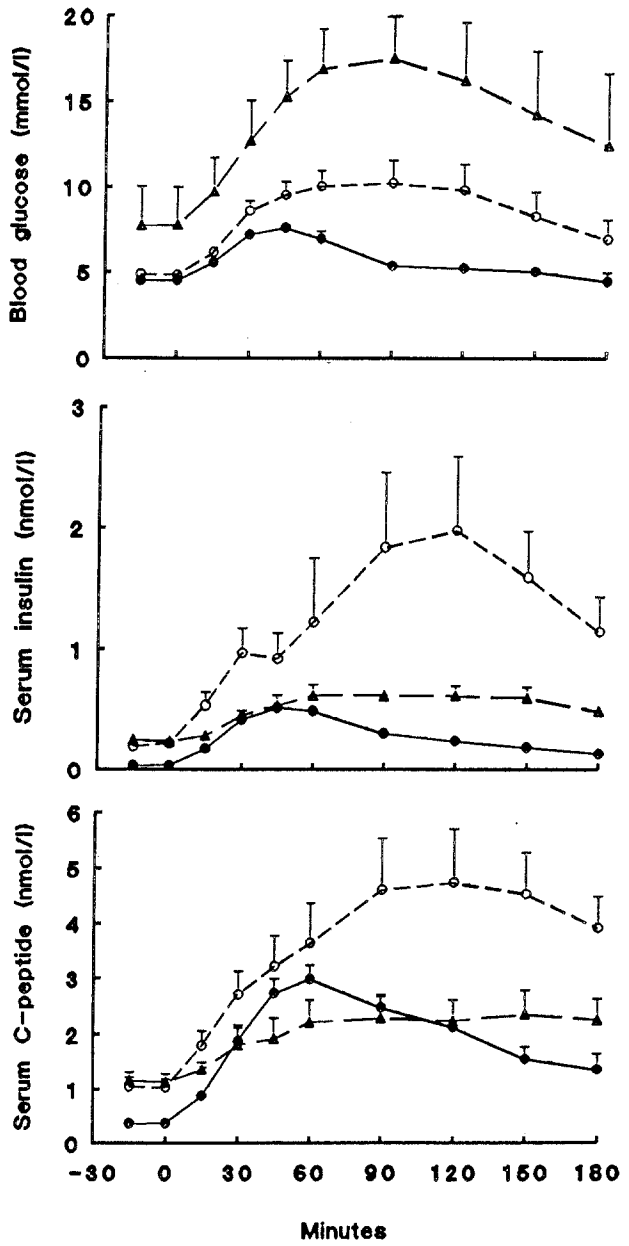

Fig 1. Blood glucose, serum insulin, and C-peptide levels after an overnight fast and after ingestion of 75 g oral glucose at t = 0 minutes in seven nondiabetic cirrhotic patients (○), eight diabetic cirrhotic patients (▲), and eight normal control subjects (●). Mean ± SEM.

Fasting serum insulin levels were higher in both cirrhotic groups (nondiabetic cirrhotics, 209 ± 48 pmol/L [35.1 ± 8.0 mU/L]; diabetic cirrhotics, 247 ± 39 pmol/L [41.4 ± 6.6 mU/L]) as compared with controls (36 ± 7 pmol/L [6.0 ± 1.1 mU/L], P = .006 and P < .001, respectively), but were not significantly different between diabetic and nondiabetic cirrhotic patients. After the oral glucose load, serum insulin levels were markedly increased in nondiabetic cirrhotics and remained elevated until +180 minutes (Fig 1). In diabetic cirrhotics the incremental area under the 3-hour serum insulin concentration curve (881 ± 186 pmol · L^{-1} · h) was markedly reduced compared with that of nondiabetic cirrhotic patients (3,475 ± 1,009, P < .05), but it was not significantly different from that of controls (761 ± 48).

Fasting serum C-peptide levels were higher in both cirrhotic groups (nondiabetic cirrhotics, 1.04 ± 0.16; diabetic cirrhotics, 1.15 ± 0.15 nmol/L) as compared with controls (0.37 ± 0.04, P = .006 and P = .002, respectively). However, after glucose ingestion, diabetic cirrhotic patients had a very blunted C-peptide response (Fig 1). The incremental area under their 3-hour serum C-peptide concentration curve was only one third of that in nondiabetic cirrhotic patients (2.78 ± 0.77 v 8.23 ± 1.56 nmol · L^{-1} · h, P = .002). The incremental area under the serum C-peptide concentration curve was greater in nondiabetic cirrhotics than in controls (4.86 ± 0.31 nmol · L^{-1} · h, P < .05).

Fasting levels of intact proinsulin and des-31,32 proinsulin tended to be a little higher in nondiabetic cirrhotics than in controls, but the differences did not reach statistical significance (Fig 2). Fasting serum proinsulin levels in diabetic cirrhotic patients (24.0 ± 5.7 pmol/L) were markedly higher than in control subjects (2.3 ± 0.5, P < .001) or nondiabetic cirrhotic patients (4.4 ± 0.8, P < .005). Fasting serum levels of des-31,32 proinsulin were also much higher in diabetic cirrhotic patients (8.8 ± 2.4 pmol/L)

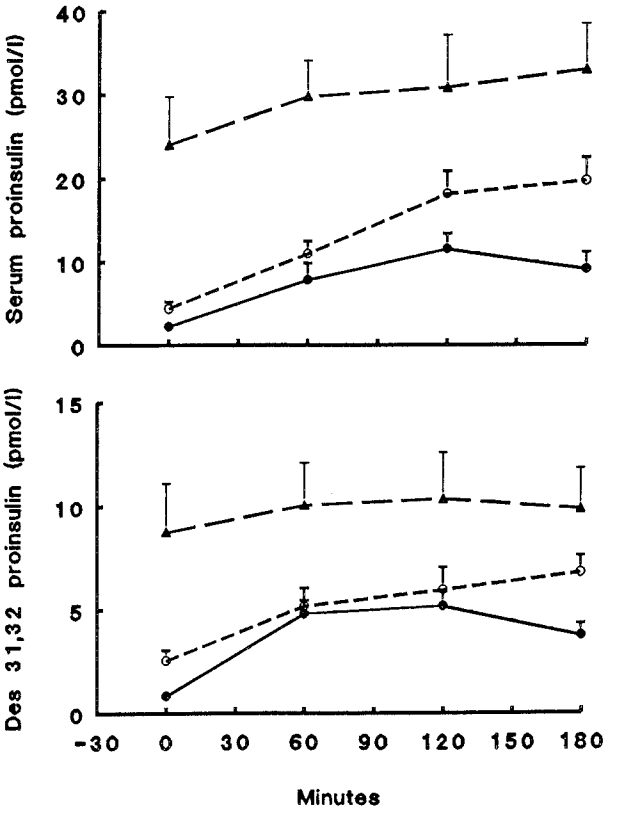

Fig 2. Serum intact proinsulin and des-31,32 proinsulin levels after an overnight fast and after ingestion of 75 g oral glucose at t = 0 minutes in seven nondiabetic cirrhotic patients (○), eight diabetic cirrhotic patients (▲), and eight normal control subjects (●). Mean ± SEM.

than in nondiabetic cirrhotic patients (2.6 ± 0.5, $P < .02$) or controls (0.8 ± 0.1, $P < .005$). Fasting proinsulin and des-31,32 proinsulin was 12.5% ± 1.4% of serum immunoreactive insulin in diabetic cirrhotic patients, higher than in nondiabetic cirrhotic patients (3.7% ± 0.5%, $P < .001$) or normal control subjects (7.8% ± 1.5%, $P = .035$). In nondiabetic cirrhotic patients, the proportion of immunoreactive insulin due to proinsulin and des-31,32 proinsulin was lower than in controls, but the difference did not reach statistical significance ($P = .074$). The fasting proinsulin to C-peptide molar ratio was significantly higher in diabetic cirrhotic patients (25.1 ± 8.6) than in controls (6.3 ± 1.4) or nondiabetic cirrhotic subjects (4.9 ± 1.4, $P < .05$ for both), but there were no differences between the control and nondiabetic cirrhotic groups.

After glucose ingestion, serum levels of intact proinsulin and des-31,32 proinsulin increased in nondiabetic cirrhotic and control subjects (Fig 2). In diabetic cirrhotics there was an increase in intact proinsulin ($P < .05$ for 180 minutes *v* basal), but the apparent increase in des-31,32 proinsulin was not statistically significant, and both the absolute and proportional increases in proinsulin and des-31,32 proinsulin were smaller than for insulin. Proinsulin plus des-31,32 proinsulin was no more than 16.7% of the immunoreactive insulin concentration in any of the three groups at any time point (Table 2). The highest concentrations of proinsulin and des-31,32 proinsulin were seen in diabetic patients with the highest fasting blood glucose (Fig 3). In diabetic cirrhotic patients, proinsulin showed a strong correlation with fasting blood glucose levels ($r = .95$, $P < .001$), as did des-31,32 proinsulin ($r = .87$, $P < .01$), proinsulin as a percentage of immunoreactive insulin ($r = .82$, $P < .02$), and the proinsulin to C-peptide molar ratio ($r = .87$, $P < .005$).

Table 2. Percentage of Immunoreactive Insulin as Intact Proinsulin and as Des-31,32 Proinsulin in the Basal State and After 75 g Oral Glucose in Eight Diabetic Cirrhotic Patients, Seven Nondiabetic Cirrhotic Patients, and Eight Normal Control Subjects

	Minutes After Glucose Ingestion			
	0	60	120	180
Proinsulin (%)				
Controls	5.6 ± 1.2	1.6 ± 0.3	4.9 ± 0.7	12.1 ± 3.5
Cirrhotics	2.3 ± 0.3	1.3 ± 0.4	1.4 ± 0.4	2.9 ± 1.2
Diabetic cirrhotics§‖	9.1 ± 1.0*‡	5.2 ± 0.9	5.6 ± 1.2	6.8 ± 0.9
Des-31,32 proinsulin (%)				
Controls	2.2 ± 0.4	1.0 ± 0.1	2.2 ± 0.3	4.6 ± 1.0
Cirrhotics	1.4 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	1.3 ± 0.7
Diabetic cirrhotics¶	3.4 ± 0.5†	1.8 ± 0.4	1.9 ± 0.5	2.1 ± 0.4

NOTE. Results are the mean ± SEM. Significance of differences between groups was sought by ANOVA followed by Tukey's multiple comparison testing.

*$P = .04$ compared with control subjects at $t = 0$ minutes.

†$P = .006$, compared with nondiabetic cirrhotic patients at $t = 0$ minutes.

‡$P < .001$ compared with nondiabetic cirrhotic patients at $t = 0$ minutes.

§$P < .02$ compared with nondiabetic cirrhotic patients and ‖$P < .001$ compared with controls for the mean of 60- to 180-minute percentages.

¶$P < .02$ compared with controls for the mean of 60- to 180-minute percentages.

DISCUSSION

In the present study, fasting serum levels of intact proinsulin and its major conversion intermediate des-31,32 proinsulin were increased threefold to 10-fold in our diabetic cirrhotic patients in comparison to nondiabetic cirrhotic and control subjects (Fig 2). Although fasting levels of proinsulin and des-31,32 proinsulin in our nondiabetic cirrhotic patients tended to be higher than in controls, these differences were not significant and a disproportionate elevation in proinsulin and des-31,32 proinsulin (relative to immunoreactive insulin and C-peptide) was seen only in diabetic patients. These findings are similar to those in subjects with impaired glucose tolerance due to obesity[13,24] and in type II diabetes without liver disease.[11-14,24-26]

There have been two previous studies of proinsulin levels in cirrhosis.[15,16] Kasperska-Czyzykowa et al[15] found a twofold elevation in proinsulin, insulin, and C-peptide levels in cirrhotics with normal fasting blood glucose concentrations, but the proportion of immunoreactive insulin accounted for by proinsulin was not increased in their patients. However, their proinsulin levels (absolute and as a proportion of total immunoreactive insulin) in both control and cirrhotic subjects were far higher than those found in the present or other studies using more specific proinsulin assays.[11,12,26-28] Ballmann et al[16] found no increase in proinsulin levels in diabetic or nondiabetic cirrhotic patients. Differences in assay methodology may account for the disparate findings. The proinsulin assay in the study by Ballmann et al[16] had a very low (0.1%) cross-reactivity with des-31,32 proinsulin; if this was the major circulating proinsulin-like molecule in cirrhosis, as found in some[8,12,27] but not all[11,26] studies of diabetic patients, it could explain their negative findings. Our finding that serum intact proinsulin levels exceeded those of des-31,32 proinsulin in all groups (Fig 2) does not support this explanation. The most likely explanation for the failure of Ballmann et al[16] to find an increase in proinsulin levels in their diabetic cirrhotic patients is that the mean fasting blood glucose level of the "diabetic" cirrhotics was only 5.8 ± 0.2 mmol/L, and none had a fasting blood glucose level above 6.6 mmol/L.

The relative concentrations of insulin, proinsulin, and split proinsulins in peripheral venous plasma depend on their relative rates of secretion and clearance. Horwitz et al,[29] by simultaneous portal and peripheral venous sampling for insulin, C-peptide, and proinsulin, showed that in normal subjects proinsulin secretion in response to an acute arginine or glucose stimulus is 2% to 5% that of insulin. This is similar to the relative molar proportions of insulin and proinsulin in normal pancreatic extracts.[30,31] The pancreatic content of intact proinsulin exceeds that of des-31,32 proinsulin,[32] whereas the metabolic clearance rate of des-31,32 proinsulin is similar to or greater than that of intact proinsulin.[10] If their relative secretion rates are determined by their islet content, one would expect serum intact proinsulin levels to be greater than those of des-31,32

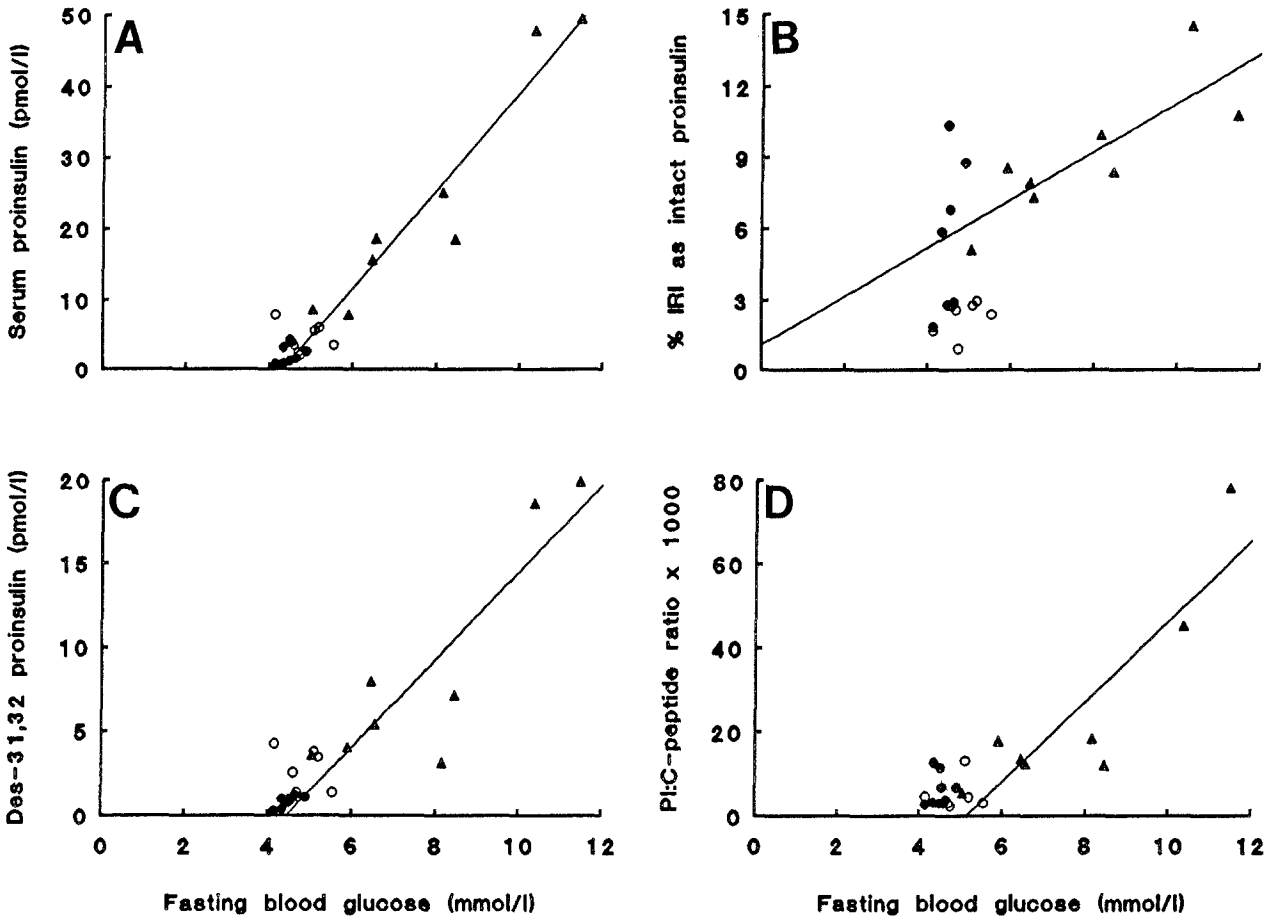

Fig 3. Relationships between fasting blood glucose levels and (A) serum intact proinsulin, (B) des-31,32 proinsulin, (C) percent of immunoreactive serum insulin (IRI) accounted for by proinsulin, and (D) the proinsulin (PI) to C-peptide molar ratio in the eight diabetic cirrhotic patients (▲), seven nondiabetic cirrhotic patients (○), and eight normal control subjects (●). Regression lines are for the diabetic cirrhotic group only. For serum proinsulin *v* blood glucose, $r = .95$ and $P < .001$. For des-31,32 proinsulin *v* blood glucose, $r = .87$ and $P < .01$. For %IRI as proinsulin *v* blood glucose, $r = .82$ and $P < .02$. For PI to C-peptide molar ratio × 1,000 *v* blood glucose, $r = .87$ and $P < .005$.

proinsulin, as was found in our control and cirrhotic subjects. Although proinsulin secretion is but a small proportion of that of insulin, it accounts for a higher proportion of steady-state plasma immunoreactive insulin because its metabolic clearance rate is only 20% to 25% that of insulin.[9,33] In normal subjects, 50% to 80% of secreted insulin is removed by the liver on first pass.[34] Even with peripheral insulin infusion, the liver accounts for approximately 70% of insulin clearance, although renal clearance increases linearly with plasma concentrations.[33,35] By contrast, the liver plays a much smaller role in the metabolism of proinsulin and des-31,32 proinsulin, with the kidneys accounting for about 70% of proinsulin clearance.[33] This is probably explained by the much lower affinity of insulin receptors for proinsulin and its intermediate conversion products than for insulin.[36,37] These considerations help to explain the relative plasma concentrations of insulin, proinsulin, and des-31,32 proinsulin basally and after glucose ingestion in our subjects.

The majority of patients in both cirrhotic groups (Table 1) had esophageal varices on endoscopy and would be expected to have a significant degree of portal-systemic shunting and reduced insulin clearance, as previously shown for both secreted and infused insulin.[3,5,7] C-peptide clearance is normal in nondiabetic cirrhotics with normal renal function.[4,7] Since proinsulin and C-peptide are handled similarly by the kidney,[33] one would expect proinsulin clearance also to be normal in our cirrhotics. Reduced insulin clearance but normal proinsulin clearance probably explains why in our nondiabetic cirrhotics proinsulin and des-31,32 proinsulin, although higher than in controls, accounted for a smaller proportion of total immunoreactive insulin both basally and after glucose ingestion (Table 2). The normal proinsulin to C-peptide molar ratio in nondiabetic cirrhotic patients is consistent with this explanation, and also suggests that in these markedly insulin-insensitive subjects[3,4] there was no disproportionate secretion of proinsulin. Reduced insulin clearance also explains why in diabetic cirrhotics, the changes in proinsulin in proportion to total immunoreactive insulin were relatively small as compared with findings in type II diabetics, despite their markedly increased proinsulin and des-31,32 proinsulin levels. In type II diabetes, proinsulin and des-31,32 proinsulin may account for as much as 50% of circulating immuno-

reactive insulin,[11-13] rather than the 12.5% in our diabetic cirrhotics. However, the disproportionate increase in proinsulin in our diabetic cirrhotics is clearly seen from the increased proinsulin to C-peptide molar ratio. Ward et al[14] showed that in type II diabetes, the disproportionate increase in proinsulin levels is due to increased secretion rather than impaired clearance.

The reason for the increased secretion of proinsulin and its intermediate conversion products relative to insulin and C-peptide is not known. Insulin insensitivity, whether due to obesity, growth hormone excess, or liver disease, is associated with increased insulin secretion rates,[3,7,13] and it has been suggested that the chronic increase in demand for insulin leads to depletion of mature islet β-cell insulin granules with subsequent release of immature proinsulin-rich granules.[14,38] Our findings do not support this hypothesis. Both our cirrhotic groups were markedly insulin-insensitive and to a similar degree.[4] However, a disproportionate increase in proinsulin secretion was seen only in diabetic patients who, although able to maintain similar fasting C-peptide levels (albeit at a higher blood glucose level), had a markedly impaired C-peptide response to oral glucose (Fig 1), suggesting that their 24-hour insulin secretion rates may be lower than in the nondiabetic cirrhotic group. Our previous demonstration[4] that cirrhotics with diabetes controlled by diet and oral hypoglycemic agents have a prompt release of insulin in response to tolbutamide, despite an absent first-phase insulin response to glucose, also suggests that depletion of insulin stores is not the explanation for the disproportionate increase in proinsulin release.

As in type II diabetes,[24,26] we found a strong correlation of serum proinsulin and des-31,32 proinsulin levels with fasting blood glucose concentrations in our diabetic cirrhotic patients (Fig 3). Several studies have suggested that the disproportionate increase in proinsulin levels in type II diabetes may be secondary to hyperglycemia.[24,38-40] Thus, reduction of plasma glucose levels in type II diabetic patients by diet or sulfonylureas has been shown to reduce the fasting proinsulin to insulin ratio.[38-40] However, Levy et al,[26] using the hyperglycemic clamp technique, showed that type II diabetic subjects have an increased proinsulin to insulin ratio even when comparison is made at the same plasma glucose concentration.

It is unlikely that hyperglycemia itself impairs the conversion of proinsulin to insulin; this is unaffected by an acute increase in glucose levels,[41] whereas chronic exposure of islets to hyperglycemia increases the rate of proinsulin to insulin conversion.[42] It is likely that hyperglycemia will only increase the proinsulin to insulin ratio in subjects with a primary islet β-cell defect; increasing the plasma glucose level to approximately 10 mmol/L for several hours in normal subjects by intravenous glucose infusion, although increasing plasma proinsulin and split proinsulin levels, does not increase the proinsulin to insulin ratio.[13,26] The present study and previous studies in type II diabetic patients and their normoglycemic first-degree relatives[13,24,26,38,39] suggest that a disproportionate increase in proinsulin levels is seen only when the islet β cells are failing and insulin secretion can no longer maintain normal fasting blood glucose levels. Fasting hyperglycemia may then be viewed as the mechanism by which insulin secretion rates are maintained, perhaps by an effect of glucose on the cytoskeleton[43] resulting in more efficient translocation of newly synthesized immature granules to the plasma membrane for exocytosis. This is consistent with the findings of Levy et al,[26] who showed that when comparisons are made at similar glucose levels, the disproportionate increase in proinsulin levels in diabetes is due to a subnormal insulin response rather than to increased proinsulin levels.

In conclusion, proinsulin levels are increased in both diabetic and nondiabetic cirrhotic patients, but a disproportionate elevation in proinsulin and des-31,32 proinsulin is seen only in diabetic cirrhotic patients. Nonetheless, because insulin clearance is reduced in cirrhosis, proinsulin plus des-31,32 proinsulin accounts for less than 15% of total plasma immunoreactive insulin. Hyperinsulinemia in cirrhosis is therefore not due to increased levels of proinsulin or its intermediate conversion products, and increased secretion of proinsulin does not explain the delayed onset of insulin action[17] or resistance of tissues to endogenously secreted insulin. This study further emphasizes the similarity of the islet β-cell defect in diabetic cirrhotic patients and those with type II diabetes. Indeed cirrhosis, by inducing tissue insulin resistance, may simply unmask the defect in insulin secretion in much the same way as pregnancy leads to the development of gestational diabetes in those at risk for type II diabetes.[44]

REFERENCES

1. Conn HO, Schreiber W, Elkington SG: Cirrhosis and diabetes. II. Association of impaired glucose tolerance with portal-systemic shunting in Laennec's cirrhosis. Dig Dis Sci 16:227-239, 1971

2. Kruszynska YT, McIntyre N: Carbohydrate metabolism, in McIntyre N, Benhamou P-J, Bircher J, et al (eds): Oxford Textbook of Clinical Hepatology. Oxford, UK, Oxford University Press, 1991, pp 129-143

3. Kruszynska YT, Home PD, McIntyre N: The relationship between insulin sensitivity, insulin secretion and glucose tolerance in cirrhosis. Hepatology 14:103-111, 1991

4. 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

5. Bosch J, Gomis R, Kravetz D, et al: Role of spontaneous portal-systemic shunting in hyperinsulinism of cirrhosis. Am J Physiol 247:G206-G212, 1984

6. Nygren A, Adner N, Sundblad L, et al: Insulin uptake by the human alcoholic cirrhotic liver. Metabolism 34:48-52, 1985

7. Kruszynska YT, Munro J, Home PD, et al: 24 Hour C-peptide and insulin secretion rates and diurnal profiles of glucose, lipids and intermediary metabolites in cirrhosis. Clin Sci 83:597-605, 1992

8. Temple RC, Clark PMS, Nagi DK, et al: Radioimmunoassay may overestimate insulin in non–insulin-dependent diabetics. Clin Endocrinol (Oxf) 32:689-693, 1990

9. Sonksen PH, Tompkins CV, Srivastava MC, et al: A comparative study on the metabolism of human insulin and porcine proinsulin in man. Clin Sci Mol Med 45:633-654, 1973

10. Tillil H, Frank BH, Pekar AH, et al: Hypoglycaemic potency and metabolic clearance rate of intravenously administered human proinsulin and metabolites. Endocrinology 127:2418-2422, 1990

11. Yudkin JS: Circulating proinsulin-like molecules. J Diabetic Complications 7:113-123, 1993

12. Temple RC, Carrington CA, Luzio SD, et al: Insulin deficiency in non–insulin-dependent diabetes. Lancet 1:293-295, 1989

13. Porte D: B-cells in type 2 diabetes mellitus. Diabetes 40:166-180, 1991

14. Ward WK, La Cava EC, Paquette TL, et al: Disproportionate elevation of immunoreactive proinsulin in type 2 (non–insulin-dependent) diabetes mellitus and in experimental insulin resistance. Diabetologia 30:698-702, 1987

15. Kasperska-Czyzykowa T, Heding LG, Czyzyk A: Serum levels of true insulin, C-peptide and proinsulin in peripheral blood of patients with cirrhosis. Diabetologia 25:506-509, 1983

16. Ballmann M, Hartmann H, Deacon CF, et al: Hypersecretion of proinsulin does not explain the hyperinsulinaemia of patients with liver cirrhosis. Clin Endocrinol (Oxf) 25:351-361, 1986

17. Kruszynska YT, Meyer-Alber A, Darakhshan F, et al: Metabolic handling of orally administered glucose in cirrhosis. J Clin Invest 91:1057-1066, 1993

18. Revers RR, Henry R, Schmeiser L, et al: The effects of biosynthetic human proinsulin on carbohydrate metabolism. Diabetes 33:762-770, 1984

19. Bergman RN, Ider YZ, Bowden CR, et al: Quantitative estimation of insulin sensitivity. Am J Physiol 236:E667-E677, 1979

20. Mohamed-Ali V, Yudkin JS: Simple and highly sensitive microplate immunoradiometric assays (IRMAs) for intact proinsulin and des-31,32 proinsulin. Diabetic Med 10:67, 1993 (suppl 1, abstr)

21. WHO Study Group: Diabetes mellitus. WHO Tech Rep Ser 727:80-82, 1985

22. Soeldner JS, Sloane D: Critical variables in the radioimmunoassay of serum insulin using the double antibody technic. Diabetes 14:771-779, 1965

23. Heding LG: Radioimmunological determination of human C-peptide in serum. Diabetologia 11:541-548, 1975

24. Saad MF, Kahn SE, Nelson RG, et al: Disproportionately elevated proinsulin in Pima Indians with non–insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 70:1247-1253, 1990

25. Bowsher RR, Wolny JD, Frank BH: A rapid and sensitive radioimmunoassay for the measurement of proinsulin in human serum. Diabetes 41:1084-1090, 1992

26. Levy JC, Clark PM, Hales CN, et al: Normal proinsulin responses to glucose in mild type 2 subjects with subnormal insulin response. Diabetes 42:162-169, 1993

27. Williams DRR, Byrne C, Clark PMS, et al: Raised proinsulin concentration as early indicator of B-cell dysfunction. Br Med J 303:95-96, 1991

28. Davies MJ, Rayman G, Gray JL, et al: Insulin deficiency and increased plasma concentration of intact and 32/33 split proinsulin in subjects with impaired glucose tolerance. Diabetic Med 10:313-320, 1993

29. Horwitz DL, Starr JI, Mako ME, et al: Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. J Clin Invest 55:1278-1283, 1975

30. Rastogi GK, Sinha MK, Dash RJ: Insulin and proinsulin content of pancreases from diabetic and non-diabetic subjects. Diabetes 22:804-807, 1973

31. Burr IM, Stauffacher W, Balant L, et al: Dynamic aspects of proinsulin release from perifused rat pancreas. Lancet 2:882-883, 1969

32. Given BD, Cohen RM, Shoelsen SE, et al: Biochemical and clinical implications of proinsulin conversion intermediates. J Clin Invest 76:1398-1405, 1985

33. Katz AI, Rubenstein AH: Metabolism of proinsulin, insulin and C-peptide in the rat. J Clin Invest 52:1113-1121, 1973

34. Blackard WG, Nelson NC: Portal and peripheral vein immunoreactive insulin concentrations before and after glucose ingestion. Diabetes 19:302-306, 1970

35. Ferrannini E, Wahren J, Faber OK, et al: Splanchnic and renal metabolism of insulin in human subjects. A dose response study. Am J Physiol 244:E517-E527, 1983

36. Peavy DE, Abram JD, Frank BH, et al: In vitro activity of biosynthetic human proinsulin: Receptor binding and biologic potency of proinsulin and insulin in isolated rat adipocytes. Diabetes 33:1062-1067, 1984

37. Yu SS, Kitabchi AE: Biological activity of proinsulin and related polypeptides in the fat tissue. J Biol Chem 248:3753-3761, 1973

38. Yoshioka N, Kuzuya T, Matgsuda A, et al: Effects of dietary treatment on serum insulin and proinsulin response in newly diagnosed NIDDM. Diabetes 38:262-266, 1989

39. Clark PM, Levy JC, Cox L, et al: Immunoradiometric assay of insulin, intact proinsulin and 32-33 split proinsulin and radioimmunoassay of insulin in diet-treated type 2 (non–insulin-dependent) diabetic subjects. Diabetologia 35:469-474, 1992

40. Duckworth WC, Solomon SS, Kitabchi AE: Effect of chronic sulphonylurea therapy on plasma insulin and proinsulin levels. J Clin Endocrinol Metab 35:585-591, 1972

41. Rhodes CJ, Halban PA: Newly synthesised proinsulin/insulin and stored insulin are released from pancreatic B cells predominantly via a regulated rather than a constitutive pathway. J Cell Biol 105:145-153, 1987

42. Nagamutsu S, Bolaffi JL, Grodsky GM: Direct effects of glucose on proinsulin synthesis and processing during desensitization. Endocrinology 120:1225-1231, 1987

43. Howell SL: The mechanism of insulin secretion. Diabetologia 26:319-327, 1984

44. Dornhorst A, Bailey PC, Anyakou V, et al: Abnormalities of glucose tolerance following gestational diabetes. Q J Med 77:1219-1228, 1990