

Insulin, Glucagon, C-Peptide and Aminoacid Levels Following Liver Transplantation

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INTRODUCTION

Glucose intolerance (5,14), hyperinsulinemia, (3,9), insulin-resistance (2,4) and hyperglucagonemia (12,17,18) are common in patients with advanced liver disease. Several hormonal abnormalities are reported in cirrhotic patient and one thought to either contribute to or be responsible for the reduced plasma concentrations of the three principal branched chain acids (BCAA) valine, leucine and isoleucine that are characteristic of end-stage liver disease. As a result, these same hormonal abnormalities are thought also to contribute to the decreased molar ratio that exists in plasma between the BCAA and the aromatic amino acids (AAA) tyrosine, phenylalanine and tryptophan in cirrhotics (7,13,15,20). We report our observations concerning the plasma levels of insulin and glucagon and the major aminoacids before and after orthotopic liver transplantation (OLTx). The changes observed occur in cirrhotics postoperatively were compared to those observed to occur in a non-cirrhotic human recipient with normal liver function, who underwent liver transplantation for multiple hepatic adenomatosis and to those observed in five normal dogs which were submitted to liver replacement under controlled laboratory conditions.

MATERIAL AND METHODS

Case Material

The clinical characteristics of the eight cirrhotic patients and the single individual with multiple hepatic adenomatosis studied are shown in Table 1. As can be seen from the Table, the patient with multiple hepatic adenomatosis had normal liver function.

TABLE 1: PATIENTS TREATED WITH OLT*

| NAME | SEX | AGE (years) | DIAGNOSIS | BILIRUBIN (mg/100ml) | PROTHROMBIN Time (sec) | ALBUMIN (g/l) |
|------|-----|----------------|--|-------------------------|---------------------------|------------------|
| BR | M | 18 | Chronic active hepatitis | 38.8 | 18/13 | 2.7 |
| RY | F | 25 | Chronic active hepatitis | 9.9 | 15/13 | 2.6 |
| TH | F | 23 | Chronic active hepatitis | 2.2 | 16/13 | 2.2 |
| GH | M | 37 | Sclerosing Cholangitis | 19.7 | 15/13 | 2.6 |
| SJ | F | 41 | Sclerosing Cholangitis | 15.3 | 14.4/13 | 2.9 |
| KD | M | 34 | Sclerosing Cholangitis | 23.7 | 14/13 | 2.8 |
| SR | F | 29 | Wilson's Disease | 3.5 | 16.8/13 | 2.7 |
| SP | F | 43 | 1 Antitrypsin deficiency FOR NEOPLASM | 4.9 | 14/13 | 2.8 |
| GB | F | 29 | Adenomatosis | 0.5 | 13/13 | 3.0 |

Clinical characteristics, bilirubin, prothrombin time and albumin levels of the eight cirrhotic patients and the single patient with multiple adenomatosis.

In contrast, the patients with liver disease all had moderately advanced liver disease as documented by the abnormalities in their bilirubin, prothrombin time and albumin levels.

Cyclosporine and prednisone were used as the immunodepressive agents for all of the recipients studied (21,22).

The five mongrel dogs transplanted and studied weighed between 15-20 kg and like the human studied, were given cyclosporine and prednisone to prevent rejection using doses that, on a mg per kg basis, were similar to those used by the patients (10). The surgical procedures used for the patients and animals was identical (10,22).

Special Analysis

The plasma levels of immunoreactive insulin (IRI) were determined by radioimmunoassay using an insulin kit obtained from Serono Diagnostics (Braintree, MO). The detection limit of the assay was 5 U/ml. The plasma levels of C-peptide (CP) were determined by radioimmunoassay using a C-peptide kit obtained from Serono Diagnostics. The detection limit of the assay was 0.2 ng/ml.

The plasma level was determined with a glucagon kit. This kit was used for accuracy, precision and reliability (11). Samples were stored in chilled tubes containing aprotinin and used for assay.

The detection of insulin was determined by the double antibody technique (1), with a sensitivity of 0.1 ng/ml. Glucagon having a sensitivity of 1 ng/ml. Insulin/glucagon ratio was determined by the double antibody technique (16) using a ratio of 1:1. This formula was used: 6.000 D and a blank that glucagon has cyclosporine, to postoperatively, was found not to

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The unpaired Student's t-test was used for the data. A p

Glucagon, Insulin, and albumin levels were determined in patients studied. Normal preoperative levels were 1 ng/ml and remained normal for 24 hours following surgery.

The plasma levels of insulin (IRI) were determined (Fig. 1A). Following surgery there was a decrease in the IRI level.

The plasma levels of immunoreactive glucagon (IRG) were determined with a glucagon kit also obtained from Serono Diagnostics. This kit was chosen specifically because of its high degree of accuracy, precision and specificity as has been reported by Kenny et al (11). Samples for the glucagon assay were collected in chilled tubes containing 500 units of a trypsin inhibitor (aprotinin) and 1.2 mg of sodium EDTA/ml of whole blood collected for assay.

The detection limit for the assay was 15 pg/ml.

For representative blood samples obtained from each patient studied, both before and after transplantation, a gel filtration technique (1), was used to determine the amount of IRG-detectable glucagon having molecular weights of 3,500, 7,000 and 40,000 D.

Insulin/glucagon molar ratios were calculated as suggested by Muller (16) using the formula $(IRI \text{ (uU/ml)}/IRG \text{ (pg/ml)}) \times 23.33 = \text{Ratio}$. This formula assumes that insulin has a molecular weight of 6,000 D and a biologic activity of 25 units/mg. It also assumes that glucagon has a molecular weight of 3,500 D. The addition of cyclosporine, to achieve concentration of the drug found in blood postoperatively, to samples of blood assayed for IRG, CP, and IRI, was found not to effect the assay results.

Amino acid profiles were determined on deproteinized samples of plasma treated with 4% sulfosalicylic acid. The resultant supernatants were applied to an amino acid analyzer (Beckman Instruments, Somerset, NJ) and the levels of free BCAA and AAA including tryptophan were determined: The glucose determinations were made using a glucose-oxidase method (8).

Statistical Analysis

The unpaired Student's t-test was used for statistical analysis of the data. A p value <0.05 was considered to be significant.

RESULTS

End-Stage Liver Disease

Glucagone, Insulin and C-peptide levels (Fig. 1A). In the eight patients studied, the C-peptide plasma concentrations were all high normal preoperatively and rose 2 and 1/2-fold during the first 12 hours following liver transplantation to a maximum level of 10 ng/ml and remaining elevated for a least 30 days (Fig. 1A).

The plasma IRI levels followed a somewhat different pattern (Fig. 1A). Following transplantation, there was a two-fold increase in the IRI levels but this increase lasted only for a new

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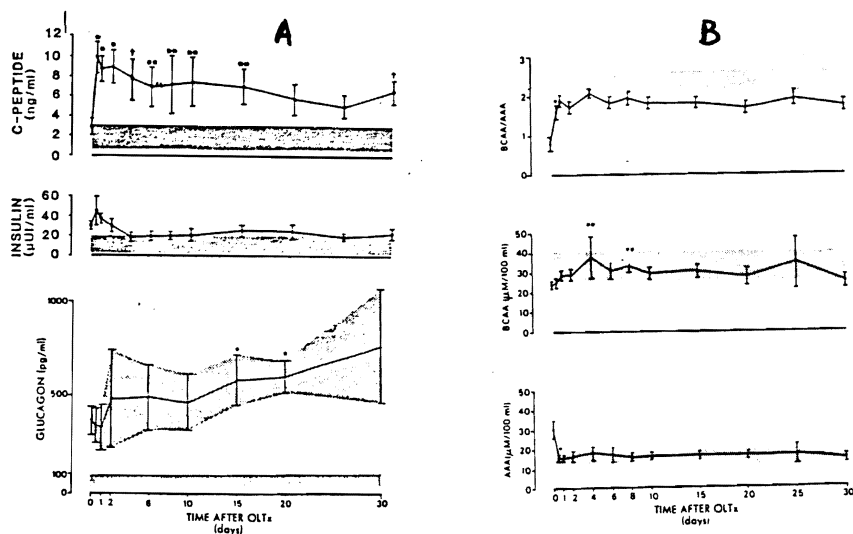
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hours and subsequently returned to near normal levels at four days post-transplantation.



When studied at six months, both the insulin and C-peptide plasma levels were slightly elevated (Table 2).

TABLE 2: LEVELS OF GLUCAGON, INSULIN, C-PEPTIDE AND IRI/IRG MOLAR RATIO

| | n | TOTAL GLUCAGON (pg/ml) | M.W. 3500 GLUCAGON (pg/ml) | INSULIN (uUI/ml) | C-PEPTIDE (ng/ml) | IRI/IRG |
|------------------------|----|------------------------------|----------------------------------|---------------------|----------------------|--------------------|
| NORMAL SUBJECTS | 10 | 90±15 | 75±16 | 20±5 | 3.2±0.5 | 3.3±0.3 |
| CIRRHOTIC PATIENTS | | | | | | |
| Before OLTx | 8 | 350±60 p 0.05 | 294±51 p 0.05 | 25±5 n.s. | 4 ±0.5 n.s. | 1.5±0.5 p 0.01 |
| 30 days after OLTx | 8 | 750±110 p 0.01 | 638±87 p 0.01 | 22±8 n.s. | 6 ±1 p 0.01 | 0.6±0.1 p 0.001 |
| 180 days after OLTx | 8 | 80±12 n.s. | 68±9 n.s. | 30±7.15 p 0.05 | 3.8±0.5 n.s. | 10.2±1 p 0.001 |

Levels of glucagon, C-peptide, insulin and IRI/IRG ratio in normal subjects, and in 8 cirrhotic patients who underwent liver transplantation. The determinations in the operated patients were made before transplantation, 30 days and 180 days after transplantation. The data for "normal subjects" refer to determinations obtained in 10 healthy people age 18-45 years. Each value is expressed as M ± S.D. Significance (p) in relation to the values of normal subjects.

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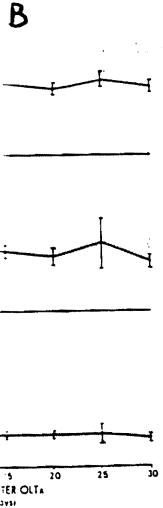
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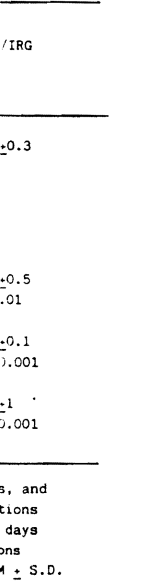
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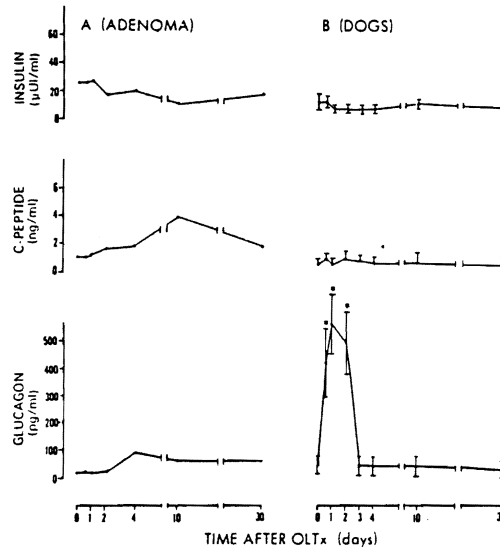
and C-peptide



Glucagon - Before operation, the plasma levels of IRG were four times the normal values (Table 2). After transplantation, the IRG level rose progressively. The highest mean plasma levels of IRG were found at 30 days following transplantation (Fig. 1A), but when restudied at 6 months had returned to the normal range (table 2). The resultant IRI/IRG molar ratio was decreased significantly as a consequence of the observed hyperglucagonemia with near normal insulin levels observed after the first 4 post-transplant days.

The gel filtration studies demonstrated that the hyperglucagonemia, both before and after transplantation, was predominantly due to the presence of IRG having a molecular weight of 3,500 D. This form of the hormone is thought to be the most biologically active fraction (6). A sharp increase of plasma glucose levels was observed probably related to the intense glucocorticoid therapy used during the first days following transplantation. Blood glucose levels returned to the normal range 6 days after operation and remained normal during all of the observation period (data not reported).

Amino Acids - The preoperative elevations of AAA (Fig. 1B) and the depressed levels of BCAA (Fig. 1B) returned to the normal range for each within 12 hours after transplantation. More importantly, the plasma amino acid levels and the BCAA/AAA ratio (Fig. 1B) subsequently achieved the normal range for the entire 30-day study period.



Control study - No changes were observed in the normal levels of IRI, IRG and the plasma amino acid levels following liver transplantation in the one patient with multiple hepatic adenomatosis studied (Fig. 2A). Similarly, no changes were observed for these same substances in the five normal dogs submitted to liver transplantation (Fig. 2B). However, this individual and these dogs had no abnormalities preoperatively that might otherwise have been expected to correct following successful transplantation. Importantly these data suggest that neither the doses of prednisone used nor the cyclosporine used to prevent rejection contributed to the hormonal and amino acid finding observed in the cirrhotic patients studied.

DISCUSSION

The purpose of this study was to determine if the hormonal abnormalities which are characteristic of end-stage chronic liver disease can be corrected by orthopic liver transplantation.

Both the hyperinsulinemia and the hyperglucagonemia evident preoperatively persisted for some time after successful transplantation suggesting that the hypersecretion of these two hormones which characterizes chronic liver disease was not promptly corrected with elimination of the liver disease per se. Although the plasma insulin level gradually returned to the normal range within days of the transplant procedure, the plasma glucagon level continued to be abnormally elevated and in fact became progressively more abnormal (more elevated) for several weeks before it also became normal sometime between 1 and 6 months postoperatively. These data coupled with the data obtained from a patient with multiple hepatic adenoma but normal liver function suggest that the abnormalities seen in the patients with advanced liver disease reflected alterations in hormone balance and energy metabolism which were due to the individual's advanced liver disease and not to the surgical procedure and the immunotherapy that follow the procedure. These observations also support the prior findings of Sherwin (17, 18) and others (6) that suggest that over production of the pancreatic hormones rather than defective hepatic uptake accounts for the hyperglucagonemia and hyperinsulinemia of advanced liver disease. Even more importantly, the failure to demonstrate any relationship between the changes in the plasma levels of glucagon and either the BCAA or AAA in these studies suggests that the abnormal plasma levels of both classes of amino acid present in patients with liver disease are not regulated or determined to a major degree by the hyperglucagonemia present in

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normal levels following liver transplantation. Multiple hepatic adenomas were observed in dogs submitted for individual and group studies and might otherwise be considered a successful transplant. Neither the increased C-peptide nor the increased insulin to prevent hyperglycemia. This finding ob-

In contrast to the observations of plasma glucagon, plasma insulin levels returned to near normal range within days of the transplant procedure despite apparent persistent hypersecretion of the hormone documented by the increased plasma level of C-peptide and a progressive rise in the C-peptide/insulin ratio. This apparent increase in insulin secretion was independent of the transient increase of glucose observed during the first 6 days after transplantation, and is well beyond the period of increased blood glucose seen postoperatively. These data suggest that the transplanted liver actively removes secreted insulin from the portal circulation, thereby maintaining a normal systemic plasma level of the hormone despite excess production and secretion of the hormone by the pancreas. Moreover, as the renal function of the patients and the animals studied did not become impaired (data not shown), it is most unlikely that these changes reflect an alteration in renal handling or clearance of either C-peptide or insulin. None the less, it is at least theoretically possible that the increased C-peptide levels reflect cyclosporine nephrotoxicity not apparent from the serial creatinine or BUN determinations made throughout the study.

The changes in the plasma levels of insulin and glucagon seen in the eight patients with advanced chronic liver disease who were studied were not seen in the one patient with normal hepatic function and multiple hepatic adenomatosis studied or in the five normal dogs that were transplanted experimentally. Thus the hormonal changes observed in the patients with advanced liver disease would appear to be specific for liver disease per se and not the transplant procedure or the immunodepressive regimen used.

These data are consistent with the recent suggestion that the reduced branched chain amino acid levels seen in cases of advanced cirrhosis are due to the increased plasma levels of insulin seen in such individuals. It has been proposed by the authors of these latter hypothesis that the systemic hyperinsulinemia of advanced chronic liver disease drives plasma BCAA into adipocytes, thereby producing an abnormal reduction in the plasma level of the branched chain amino acid which is characteristic of cirrhosis (19). The current observations that the plasma levels of the branched chain amino acids normalize concurrently with the plasma levels of insulin provides strong, albeit indirect, support for

the hypothesis.

Equally important, however, is the observation that the plasma level of branched chain amino acids normalize while the plasma glucagon level becomes progressively more abnormal. This suggests quite strongly that the plasma level of glucagon does not importantly regulate the plasma level of the branched chain amino acids.

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