MECHANISMS OF POSTPRANDIAL HYPERGLYCEMIA IN LIVER TRANSPLANT RECIPIENTS: COMPARISON OF LIVER TRANSPLANT PATIENTS WITH KIDNEY TRANSPLANT PATIENTS AND HEALTHY CONTROLS

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SUMMARY - Impaired glucose tolerance or diabetes mellitus are frequent complications after organ transplantation, and are usually attributed to glucocorticoid and immunosuppressive treatments. Liver transplantation results in total hepatic denervation which may also affect glucoregulation. We therefore evaluated postprandial glucose metabolism in a group of patients with liver cirrhosis before and after orthotopic liver transplantation. Seven patients with liver cirrhosis of various etiologies, 6 patients having received a kidney transplant, and 6 healthy subjects were studied. Their glucose metabolism was evaluated in the basal state and over 4 hours after ingestion of a glucose load with 6,6-2H glucose dilution analysis. The patients with liver cirrhosis were studied before, and again 4 weeks (range 2-6) and 38 weeks (range 20-76, n = 6) after orthotopic liver transplantation. Basal glucose metabolism was similar in liver and kidney transplant recipients. Impaired glucose tolerance was present in both groups, but postprandial hyperglycemia was exaggerated and lasted longer in liver transplant patients. Postprandial insulinemia was lower in liver transplant recipients, while C-peptide concentrations were comparable to those of kidney transplant recipients, indicating increased insulin clearance. Glucose turnover was not altered in both groups of patients during the initial 3 hours after glucose ingestion, but was higher in liver transplant early after transplantation during the fourth hour. Postprandial hyperglycemia remained unchanged in liver transplant recipients 38 weeks after liver transplantation, despite substantial reduction of immunosuppressive and glucocorticoid doses. We conclude that liver transplant recipients have severe postprandial hyperglycemia which can be attributed to insulinopenia (secondary, at least in part, to increased insulin clearance) and a late increased glucose turnover. These changes may be secondary to hepatic denervation.

Key-words: glucose metabolism, liver transplantation, kidney transplantation, insulinemia, c peptide.

RéSUMÉ - Mécanismes de l’hyperglycémie post-prandiale des receveurs de transplant hépatique : comparaison aux receveurs de transplant rénal et aux sujets témoins.

Une intolérance au glucose est une complication fréquente chez les patients ayant subi une transplantation d’organe, et est habituellement attribuée aux traitements par les immunosupresseurs et les glucocorticoïdes. La transplantation hépatique produit une dénervation hépatique totale, qui peut également affecter l’homéostasie glucidique. De manière à évaluer l’effet de cette dénervation hépatique, nous avons étudié un groupe de patients avec cirrhose hépatique, avant et après transplantation hépatique orthotopique. Sept patients avec cirrhose hépatique d’étiologies variées, 6 patients ayant eu une greffe rénale et 6 sujets sains ont été étudiés. Leur métabolisme glucidique a été mesuré par analyse de dilution isotopique du 6,6-2H glucose avant et après ingestion d’une charge orale de glucose. Le métabolisme glucidique basal était semblable chez les patients porteurs d’une transplantation hépatique ou rénale. Une intolérance au glucose était présente chez les deux groupes, mais l’hyperglycémie postprandiale était plus sévère et prolongée chez les transplantés hépatiques. L’hyperinsulinémie postprandiale était plus basse chez les transplantés hépatiques alors que les concentrations de C-peptide plasmatiques étaient identiques dans les deux groupes. Le turnover du glucose n’était pas modifié chez les deux groupes de patients transplantés au cours des trois premières heures suivant la charge orale de glucose, mais était significativement plus élevé au cours de la quatrième heure chez les transplantés hépatiques. L’hyperglycémie post-prandiale sévère présente 4 semaines après transplantation hépatique était inchangée 38 semaines après transplantation, malgré une importante réduction des doses d’immunosupresseurs et de glucocorticoides. Ces résultats indiquent que les patients porteurs d’une transplantation hépatique ont une hyperglycémie postprandiale sévère qui peut être principalement attribuée à une insulinopenie secondaire, en partie tout au moins, à une clairance accrue de l’insuline. Ces modifications pourraient être en relation avec la dénervation hépatique.

Mots-clés : métabolisme du glucose, transplantation hépatique, transplantation rénale, insulinémie, peptide c.
With the progress of immunosuppressive drug therapy over the last two decades, liver transplantation has become the treatment of choice for irreversible liver failure [1]. Impaired glucose tolerance or diabetes mellitus are frequent complications of organ transplantation [2, 3] which are usually attributed to glucocorticoid-induced insulin resistance [4] and inhibition of insulin secretion by cyclosporin and tacrolimus [5, 6].

In the case of liver transplantation, additional factors may also be involved. The liver plays a central role in the control of glycemia by releasing glucose during interprandial intervals and taking up glucose after meals [7]. These functions are regulated by the autonomic nervous system: hepatic glycogen synthesis is stimulated by the parasympathetic nervous system while glucose production is stimulated by the sympathetic nervous system [8]. In addition, studies performed in dogs indicate that portal glucose delivery triggers neural signals which increase both hepatic glucose uptake and insulin secretion and modulate insulin sensitivity [9, 10]. Activation of portal glucose sensors has also been shown to be involved in the counterregulatory response to hypoglycemia [11]. The liver is also responsible for the major portion of insulin degradation and clearance [12]. Alteration of any of these hepatic functions may possibly disturb glucose homeostasis.

Because of this prominent role of the liver in glucose regulation, and since liver transplantation results in a total hepatic denervation, we investigated basal and postprandial glucose metabolism in liver transplant recipients. We have recently reported that patients had severe postprandial hyperglycemia and relative hypoinsulinemia when studied 2-6 weeks after liver transplantation. Their postprandial hepatic glycogen synthesis was found similar to that measured in healthy controls and in kidney transplant recipients [13]. We have reexamined the same patients after an average period of 38 weeks after liver transplantation. Data obtained in these patients were compared to those obtained in kidney transplant recipients and in healthy controls.

### METHODS

**Subjects** – Seven patients with liver cirrhosis on a waiting list for a liver transplantation agreed to participate to this study. Liver failure was secondary to HCV hepatitis (n = 2), HBV hepatitis (n = 1), primary biliary cirrhosis (n = 1), autoimmune hepatitis (n = 1) and alcoholic cirrhosis (n = 2). Patients were studied before transplantation, and twice after liver transplantation, with a mean interval of 4 weeks (range 2-6) and of 38 weeks (range 20-76), post transplantation. Detailed description of the metabolic alterations present in these patients before liver transplantation has been reported elsewhere [14]. Two patients were on insulin therapy at the second occasion and their insulin treatment was discontinued the day before the study. Six kidney transplant recipients were studied also after a mean interval of 21 weeks (range 6-48) post transplantation. Six healthy volunteers were studied as a control group. Subjects were weighed on the morning of each metabolic study, and their body composition was assessed from skinfold thickness measurements [15]. Physical characteristics of the groups of subjects studied and glucocorticoid/immunosuppressive treatments are displayed in Table I.

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<td>Kidney transplant patients (6-48 wks post-transplant)</td>
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All data are expressed as mean ± 1 SD. * Figures in brackets indicate the number of patients treated.
Experimental protocol – All studies were performed in the morning after an overnight fast, and lasted 6.5 hours. Glucose rates of appearance and disappearance were measured by means of a primed (4 mg/kg)-continuous (40 µg/(kg.min) infusion of 6,6²H glucose (Masstrace, Worcester, MA) using Steele’s non steady state equations [16]. After allowing 2.5 hours for tracer equilibration, an oral glucose load (1.5 g glucose/kg lean body mass) was ingested over 5 min (time 0). Measurements were performed in the basal state (time -60 to time 0) and over 4 hours after glucose ingestion (time 0 to time 240). Glucose clearance was calculated as glucose rate of appearance/plasma glucose concentration. These data were expressed per kg lean body mass.

Analysis – Plasma glucose concentrations were measured with a Beckman glucose analyzer II (Beckman Instruments, Brea, CA). Plasma insulin (kit from Biodata Guidonia Montecello, Italy, with <14% cross-reactivity with proinsulin), glucagon (kit from Linco Research, St Charles, Missouri) and C-peptide (kit from Biodata) were measured by radioimmunoassay. Plasma 6,6²H glucose was measured by gas chromatography-mass spectrometry, as described [17].

Statistical analysis – Group comparisons were performed by multiple way analysis of variance and post hoc testing. All results are expressed as mean ± 1 SEM unless stated otherwise.

RESULTS

Before liver transplantation, liver cirrhosis patients had normal fasting glucose concentration (Fig. 1), glucose rates of appearance and disappearance (Fig. 2), but a marked hyperinsulinemia and hyperglucagonemia and slightly increased C-peptide concentrations (Fig. 3, 4). Following glucose ingestion, plasma glucose concentrations increased slightly more than in healthy controls but returned to basal values after 4 hours (Fig. 1). Glucose rate of appearance was comparable to that of healthy controls (Fig. 2), but glucose rate of disappearance was significantly lower during the second hour after oral glucose and postprandial stimulation of glucose clearance reduced on average by 59% (p < 0.05) (Fig. 5).

Four weeks after liver transplantation, fasting glucose concentration remained normal but postprandial glucose concentration was markedly increased compared to both healthy controls and kidney-transplant recipients (Fig. 1). Five patients had plasma glucose concentration of more than 220 mg/dl 2 hours after glucose ingestion. Basal insulin: glucose ratio was increased in liver transplant recipients (10.6 ± 0.6 µU/g) and in kidney transplant recipients (11.1 ± 1.7) compared to healthy volunteers (7.3 ± 0.8, p < 0.02). Postprandial insulin concentrations were significantly lower than observed in kidney transplant recipients between 60 and 150 min after glucose ingestion but were not different from those observed in healthy subjects (Fig. 3). C-peptide concentrations were identical in both groups of transplanted patients and were not significantly different from those observed in healthy subjects (Fig. 3). Glucagon concentrations...
were not different in liver and kidney transplant recipients (Fig. 4). Glucose rates of appearance were similar in both groups in the basal state and over the first 150 min after glucose ingestion. During the fourth hour after glucose ingestion, they were slightly higher in liver transplant recipients compared to both healthy controls and kidney transplant recipients (Fig. 2). In contrast, glucose disappearance was lower in liver transplant recipients compared to healthy controls or kidney transplant recipients over the first 2 hours after glucose ingestion (Fig. 5). Postprandial glucose clearance was significantly reduced in both groups of transplant patients compared to healthy controls. It was lower in liver than in kidney transplant recipients, but the difference did not reach statistical significance.

All alterations of glucose metabolism present 4 weeks after liver transplantation were only minimally improved in 6 patients re-studied after 38 weeks (Fig. 1-5).

**DISCUSSION**

The present data indicate that liver transplant recipients have a severely impaired glucose tolerance. After an ingestion of an average amount of 78 g glucose, 5 out of 7 patients had two hour plasma glucose concentration higher than 220 mg/dl 6 weeks after transplantation and 4 out of 6 patients 30 weeks after transplantation. It is therefore very likely that these patients would fulfill the diagnostic criteria of diabetes of the American Diabetes Association [18] after a standard 75 g oral glucose tolerance test. The development of postprandial hyperglycemia cannot be attributed to the sole glucocorticoid and immunosuppressive treatments because it was observed with a much lower incidence in kidney transplant recipients (2 out of 6 patients would have fulfilled the criteria for diabetes). Furthermore, glucose intolerance did not improve in liver transplant recipients over a 34-week period even though glucocorticoid doses were reduced on average by 38%.

Compared to other forms of diabetes mellitus, hyperglycemia associated with liver transplantation bears special features. Fasting glucose concentration was normal in all but one patient, as were fasting glucose production and clearance. Major alterations were observed only during the postprandial period when the hyperinsulinemia normally induced by glucocorticoid treatment [19-21] failed to occur. Postprandial plasma insulin concentrations were indeed markedly lower in liver transplant- than in kidney transplant recipients, even though the dose of glucocorticoid received was similar. This could be attributed to the lower dose of cyclosporin received by kidney transplant recipients. However this explanation appears unlikely because cyclosporin doses received by kidney transplant recipients and liver transplant recipients late after transplantation differ only slightly. In addition, a substantial reduction of the cyclosporin
dose late after liver transplantation only minimally improved postprandial insulin concentrations.

In contrast with significantly decreased insulin concentrations, plasma C-peptide concentrations and kinetics were identical in liver and kidney transplant recipients. This indicates that insulin secretion was unchanged, but insulin clearance was increased in liver transplant recipients, as already reported by Pershegin et al. [22]. Increased insulin clearance cannot be attributed to drug treatment because it was not observed after kidney transplantation nor to pre-existing alterations, as demonstrated by the results we obtained before transplantation showing a marked decrease in insulin clearance after oral glucose, as expected in patients with liver cirrhosis [23]. It cannot either be attributed to the persistance of some degree of porto-systemic shunting, since this would have decreased insulin clearance. Our observation could in part be secondary to reduced insulin clearance due to diminished kidney function in renal transplant recipients. It is however unlikely to be the major factor since liver transplant recipients have been shown to have lower insulin clearance than a group of patients treated with glucocorticoid for uveitis [22]. Several mechanisms may be postulated to account for this increased insulin clearance in liver transplant recipients. This process may possibly be regulated by hepatic innervation under normal conditions, and therefore be altered by hepatic denervation. Alternatively, loss of hepatic nerves may lead to dysregulation of hepatic blood flow.

The increase in plasma glucose concentrations during the first 60 minutes after glucose ingestion was comparable in liver and kidney transplant recipients, as where glucose rates of appearance and disappearance and glucose clearance. This suggests that early inhibition of endogenous glucose production, which has been shown to be a major determinant of post-prandial glycemia [24] was not affected by liver or kidney transplantation. Due to the low number of blood samples collected immediately after glucose ingestion the accuracy with which glucose kinetics was calculated may however be relatively low during this period. This observation nonetheless suggests that plasma insulin concentrations although decreased in liver transplant recipients, were sufficient to adequately suppress hepatic glucose output. Consistent with this explanation, the insulin-induced inhibition of glucose production has been observed to be unaffected by liver transplantation [22]. Plasma glucose concentrations however increased further after 60 min and remained elevated during the third and fourth hour after glucose ingestion in liver transplant recipients whereas it progressively decreased and returned to basal values in kidney transplant patients. This difference is best explained by a lower rate of glucose disappearance after liver transplantation during this period. This can be essentially attributed to the lower insulin concentrations stimulating less insulin-mediated glucose disposal. A lower insulin sensitivity appears unlikely to contribute to this lesser stimulation of glucose utilization since it has been observed that patients with liver transplant have the same degree of insulin resistance as patients receiving the same glucocorticoid doses for chronic uveitis [22, 25]. This hypothesis appears corroborated by our observation that the basal insulin: glucose ratio was increased in both liver and kidney transplant recipients. In addition, there was an increased glucose rate of appearance during the fourth hour post-glucose in patients studied 4 weeks after liver transplantation. This may be due to an early waning of the inhibition of glucose production secondary to low insulin concentrations. Alternatively, stimulation of glucose production during this period may be the reflection of subtle metabolic alterations induced by hepatic denervation. Another observation reported in the literature indeed indicates that hepatic denervation has functional consequences in liver transplant recipients: the increase in plasma epinephrine concentrations during hypoglycemia were reduced after liver transplantation [22] consistent with an ineffective stimulation of the sympathoadrenal system through portal glucose sensors secondary to hepatic denervation [26]. This late alteration of glucose appearance was however no longer statistically significant in the same patients 38 weeks after liver transplantation. Therefore it appears unlikely that increased glucose production secondary to hepatic denervation plays a major role in the development of postprandial hyperglycemia.

Our present observation of a marked post-prandial hyperglycemia in liver transplant compared to kidney transplant recipients nonetheless appears at odds with the observation by Pershegin et al. [22] that glucose metabolism was little affected by liver transplantation. Alterations of insulin clearance were also reported by these authors, which suggests that additional factors may be responsible for the discrepancy between their study [22] and the present study. An important point may be that metabolic responses to parenteral glucose (hyperglycemic and hyperinsulinemic clamps) were assessed in the study by Pershegin et al., while the response to oral glucose was monitored in our study. Since oral feeding normally elicits neural signals which affect hepatic metabolism, pancreatic islet secretions and insulin sensitivity [27], it is possible that failure to activate these signals were involved in the postprandial hyperglycemia of liver transplant recipients. Future studies targeted to monitor these postprandial signals will be required to assess this hypothesis.

In conclusion, this study indicates that liver transplant recipients have normal fasting plasma glucose but severe postprandial hyperglycemia. This metabolic alteration appears directly related to liver transplantation because it was not observed in kidney transplant
recipients receiving similar glucocorticoid and insulin treatments, and because it was minimally affected by the important reduction in glucocorticoid and immunosuppressive drug doses which took place between 4 and 38 weeks after transplantation. A relative hypoinsulinemia secondary to increased insulin clearance, and an increased glucose appearance reflecting presumably a modestly exaggerated glucose production three hours after glucose ingestion appear to be responsible for postprandial hyperglycemia. These alterations are likely to be directly or indirectly secondary to liver denervation. Due to their normal fasting glycemias, the prevalence of diabetes mellitus may be markedly underestimated in this group of patients. Systematic studies on larger groups of patients will be required to determine the prevalence of diabetes mellitus and its possible consequence after liver transplantation and to evaluate appropriate therapeutic strategies.

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REFERENCES
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