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Impact of cyclosporine and low-dose steroid therapy on insulin sensitivity and beta-cell function in patients with long-term liver grafts

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Abstract To examine whether factors controlling glucose tolerance, i.e., insulin sensitivity (SI) and first- (Φ_1) and second-phase insulin secretion (Φ_2), are impaired in after orthotopic liver transplantation (OLT), they were assesssed in patients that had undergone OLT for cirrhosis ($n = 10$) with cyclosporin A and low-dose steroid therapy (5 mg prednisone per day) and were compared with those of healthy matched control subjects ($n = 10$). These factors were determined by means of computer-based analysis of frequently sampled intravenous glucose tolerance tests (FSIGTT). Glucose and insulin profiles (posthepatic insulin) did not differ between both groups, whereas C-peptide levels (prehepatic insulin) were elevated in the transplant group after the FSIGTT, indicating an increased hepatic insulin degradation. SI and Φ_1 did not differ between both groups. Φ_2, however, was significantly enhanced (23.94 ± 2.63 vs 13.88 ± 1.25 min^{-1}, $P < 0.05$). These results indicate that cyclosporine and low-dose steroid therapy do not impair SI and Φ_1. However, enhanced Φ_2 compensates the increased hepatic insulin clearance.

Keywords Liver transplantation · Glucose tolerance · Insulin sensitivity · Beta-cell secretion · Cyclosporin A · Corticosteroids

Abbreviations *CyA* Cyclosporin A · *FSIGTT* Frequently sampled intravenous glucose tolerance tests · *OLT* Orthotopic liver transplantation · Φ_1, Φ_2 First-phase, second-phase insulin secretion · *SI* Insulin sensitivity

Introduction

Insulin resistance and impairment of beta-cell secretion are commonly found in patients with liver transplants and are mostly caused by steroid therapy in combination with cyclosporin A (CyA) [8, 11, 31]. Especially the dose of steroids given to these patients plays a predominant role in the initiation of the impairment of glucose metabolism by decreasing insulin sensitivity (SI) [17, 31] and affecting beta-cell secretion [7]. On the other hand, long-term steroid therapy in combination with CyA is required to prevent graft rejection. To minimize both any disturbance of glucose metabolism and adrenal gland dysfunction, low-dose steroid treatment is considered to be a suitable medication over longer periods of time in patients with liver grafts [19].

The question arises whether the long-term application of CyA and low-dose steroid treatment causes any

impairment of glucose tolerance. To answer this question, it is necessary to determine the factors controlling glucose metabolism in human beings. Oral glucose tolerance tests are commonly used for assessment in accordance with the WHO criteria [25]. However, wide variations of plasma glucose and insulin levels are to be noted after oral glucose loading [30], mostly due to different capacities to suppress endogenous glucose production and different rises in splanchnic and peripheral glucose uptake [9, 18]. Furthermore, portosystemic shunting and abnormal systemic hemodynamics, which are also present after liver transplantation [14], may contribute to widely dispersed results after oral glucose loading in patients with liver grafts. Intravenous glucose loading, i.e., the frequently sampled intravenous glucose tolerance test (FSIGTT), is not influenced by different rises in splanchnic and peripheral glucose uptake and is regulated by glucose disposal in hepatic and predominantly peripheral tissues [2, 3]. Plasma C-peptide concentration is used to infer insulin secretion since it is secreted equimolarly with insulin and its extraction by the liver is negligible [6]. Glucose and insulin dynamics from the FSIGTT can be analyzed by means of the computer-assisted minimal modeling technique of glucose to calculate the net effect of insulin of promoting glucose dissolution and inhibiting endogenous glucose production, i.e., SI [3]. The minimal modeling technique of C-peptide is able to provide two indexes describing beta-cell sensitivity to glucose of first- (Φ_1) and second-phase insulin secretion (Φ_2) [33]. Thus, the combinaton of both techniques offers an integrated view of the regulation of glucose tolerance in patients with liver grafts. Glucose tolerance is maintained by the adjustment of SI, Φ_1, and Φ_2 from beta-cells after intravenous glucose loading [3]. In healthy subjects and in nondiabetic patients with liver cirrhosis, a compensatory relationship between these three factors exists, thus enabling normal glucose tolerance [3, 4, 20]. The purpose of this study was to measure SI, Φ_1, and Φ_2 in patients with long-term liver grafts undergoing low-dose steroid therapy and CyA treatment in order to assess the differences of glucose tolerance factors between these patients and healthy subjects.

Patients and methods

The clinical characteristics of the patients with liver grafts and the control subjects are shown in Table 1. Ten patients after liver transplantation, screened as having normal glucose tolerance (glucose concentrations of below 140 mg/dl after 75 g oral glucose load, in accordance with the WHO criteria), underwent the FSIGTT. All patients suffered from histologically proven liver cirrhosis before transplantation (Child C) and had the characteristic signs of severe portal hypertension at this stage. The mean time after orthotopic liver transplantation (OLT) was 44 ± 3 months (25–61 months). None of the patients with liver grafts had diabetes melletius before transplantation. Graft function and liver enzymes were in the normal range and stable over the long term. All patients had been receiving CyA and 5 mg/day prednisone for about 18 months. The immunosuppressive medication was taken 2 h before the tests started.

Table 1 Clinical characteristics and counter-regulatory hormones of controls and liver transplant patients. Values given as mean ± SEM (*STH* Somatotrophic hormone, HbA_{1c} hemoglobin A_{1c})

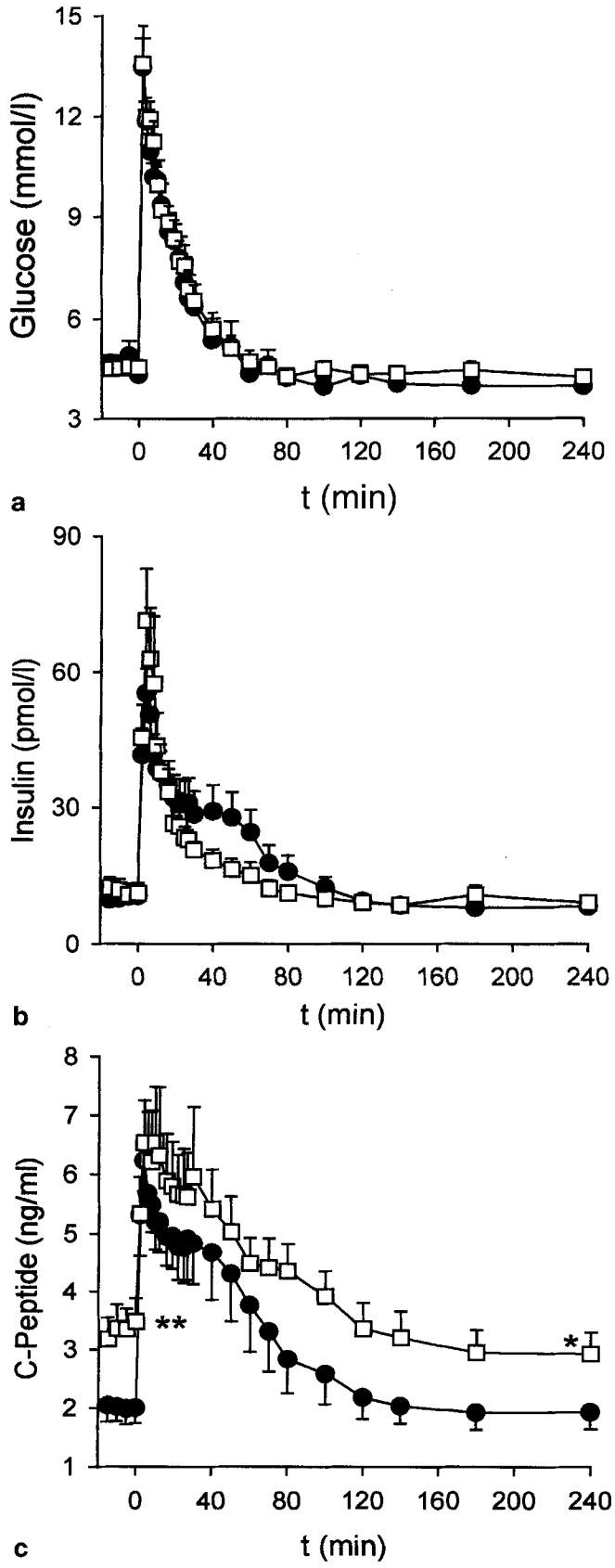
	Controls (n = 10)	CyA-group (n = 10)
Age	43 ± 3	46 ± 1
Gender (f = female, m = male)	f = 6, m = 7	f = 4, m = 6;
Fasting glucose (mmol/l)	4.52 ± 0.18	4.93 ± 0.65
Fasting insulin (pmol/l)	67.34 ± 11.6	81.68 ± 13.8
Fasting C-peptide (ng/ml)	1.73 ± 0.23	3.31 ± 0.42[a]
Fasting glucagon (ng/l)	99 ± 21	138 ± 29
Fasting STH (μg/l)	2.3 ± 0.1	3.8 ± 0.5
Fasting cortisol (ng/dl)	9.6 ± 1.8	11.2 ± 2.8
Total cholesterol (mg/dl)	213 ± 23	232 ± 19
Triglycerides (mg/dl)	196 ± 25	202 ± 16
HbA_{1c} (%Hb)	4.3 ± 0.2	4.6 ± 0.3

[a] $P < 0.01$ vs controls

These patients were compared with healthy control subjects matched according to body weight (body mass index, 24.8 ± 0.9 kg/m^2), age (43.8 ± 1.2 years), and sex (n = 10; 7 males, 3 females), on whom the FSIGTT was also performed. Exclusion criteria for controls and patients were present diabetes mellitus; cardiovascular, renal, and infectious diseases; alcoholism and drug abuse; estrogen therapy; and a positive family history of diabetes mellitus. Written consent was obtained from all subjects before the study. The study was approved by the ethic committee.

Plasma glucose concentration was measured in duplicate by the glucose oxidase method on a glucose analyzer (Beckmann Clinical System 700). Blood samples for plasma insulin were immediately centrifuged at 4 °C and stored at 20 °C until analysis. Insulin concentrations were measured by microparticle enzyme immunoassay (MEIA Insulin, IMx System, Abbott, Germany). Within the assay, the coefficient of variation was 5.3 %; the total assay variation was 6.2 %. C-peptide immunoradiometric assay was purchased from Diagnostic Products DPC, Bad Nauheim, Germany. Before the FSIGTT was started, glucagon (Glucagon RIA, Serono Diagnostics, Germany), cortisol (Enzymun-Test, Boehringer Mannheim), and serum growth hormone (STH, HGH MAIAclone, BioChem Immuno Systems) were measured. Cholesterol and triglycerides were determined enzymatically by means of commercially available kits (CHOL, SYS3; TG, SYS 3; Boehringer Mannheim, Germany). Hemoglobin A_{1c} was measured by high-performance liquid chromatography (normal range < 5.0 % Hb). Cyclosporine serum concentrations were measured by monoclonal antibody assay (EMIT 2000, Behring, Germany).

The factors controlling glucose tolerance were determined per single individual by means of the minimal modeling technique, i.e., by fitting the minimal models of glucose [2, 3] and C-peptide [33] kinetics to glucose and C-peptide data measured after a FSIGTT. SI measures the increase in fractional glucose clearance rate per unit change in plasma insulin [2, 3]. The minimal model for C-peptide [33] kinetics was used to assess beta-cell function from C-peptide from FSIGTT data. In keeping with the biphasic pattern of beta-cell insulin secretion, the model assesses the first phase, Φ_1, and the second phase, Φ_2, sensitivity to glucose. Φ_1 (dimensionless) is the amount of insulin secreted during the first



phase, X_0, normalized to the maximum increment of glucose concentration, ΔG, following the injection. Φ_2 (min^{-1}) measures the effect of glucose on the provision of new insulin [33]. The sampling protocol for measurement of glucose and insulin concentrations was used as described [3]. Glucose (300 mg/kg body weight, 50% solution) was administered intravenously within 2 min as described [2]. Glucose, insulin, and C-peptide profiles were analyzed using SAAM II software, version 1.0.2 (University of Washington, Seattle, Wash.). The precision of parameter estimates is expressed as fractional standard deviation (FSD, in percent).

Data are expressed as mean ± SE. To evaluate the differences between the control group and the patient group if normality failed, the Student's *t*-test or the rank sum test were assessed. Correlations were determined using linear regression, and a P value of less than 0.05 was considered significant.

Results

Table 1 presents the clinical characteristics of the control subjects and subjects with liver grafts. Fasting glucose and insulin did not differ between the patient and the control group (Table 1, Fig. 1). Fasting C-peptide, however, was significantly higher in the group with liver grafts ($P < 0.01$) than in the control group (Table 1, Fig. 1). The rise of insulin levels after glucose loading (Fig. 1) and the subsequent decrease were similar in the transplant group and in the controls. In contrast with the insulin profiles, the C-peptide course of the patients with grafts was different: a second increase followed the first peak immediately after glucose stimulus, the decrease of C-peptide was slower, and the levels remained higher (from 40 min on, to 240 min) in the transplant group in comparison with the control group (Fig. 1).

The analysis of glucose and insulin profiles revealed that SI among patients was not different from that among controls (Table 2). SI of both groups was negatively related to body mass index (patients: $r = -0.63$, $P < 0.05$, controls: $r = -0.80$, $P < 0.01$). Φ_1 (CV for X_0 9–21%) did not differ between patients and controls. Φ_2 responsiveness to glucose (CV 9–18%), however, was elevated in liver transplant patients (Table 2). Φ_2 was positively related to fasting insulin ($r = 0.72$, $P = 0.02$) and body mass index ($r = 0.86$, $P < 0.01$) in our control subjects, but not in our patients with liver grafts ($P > 0.05$). No relations were found between CyA serum concentrations and any glucose tolerance factor.

Fig. 1a–c Glucose, insulin, and C-peptide profiles after the frequently sampled intravenous glucose tolerance tests in controls (•) and in liver transplant patients (Cyclosporin A, □)

Table 2 Model-derived parameters of insulin sensitivity (*SI*) and first (Φ_1) and second beta-cell responsiveness (Φ_2) after glucose stimulus of controls and liver transplant patients. Values are given as mean ± SEM (*BMI* Body mass index, *CyA* cyclosporin A)

Patients (CyA)	BMI (kg/m^2)	CyA ng/dl	SI 10^{-4} min^{-1} per μU/ml	Φ_1 10^9	Φ_2 10^9 min^{-1}
1	21.9	150	6.85	202.1	22.16
2	22.5	145	6.36	149.9	29.37
3	21.8	90	3.26	286.3	33.51
4	24.5	73	7.66	77.7	16.57
5	22.9	60	1.56	168.6	20.54.
6	27.3	100	7.17	165.4	35.68
7	23.9	125	6.26	144.6	30.09
8	21.8	167	3.22	160.8	25.95
9	20.4	227	7.01	135.1	13.90.
10	21.3	210	4.36.	189.8	11.63
	22.8 ± 0.6	134 ± 17.7	5.38 ± 0.66	168.0 ± 16.9	23.94 ± 2.63[a]
Controls ($n = 10$)	24.8 ± 3.2	–	4.99 ± 1.20	189.9 ± 21.5	13.88 ± 1.25

[a] $P < 0.05$ vs controls

Discussion

This study yields evidence that long-term treatment with the combination of CyA and low-dose steroid treatment does not affect SI and Φ_1. This is important to note since both parameters are prognostic factors for the development of diabetes mellitus [13, 23]. The overall factor influencing SI in liver transplant patients, as generally recognized in healthy subjects, is body mass index [1]. Low-dose steroid therapy over a longer period of time does not affect the relationship between body mass and SI. The adverse effects of corticosteroids are dosage related [16]. Dosages below 7.5 mg/day are rarely associated with clinically significant effects on glucose metabolism [19]. Therefore, we can conclude that long-term treatment with steroids at a dosage of 5 mg/day has no detrimental effect on both predominant parameters of glucose metabolism, i.e., Φ_1 and SI. Diminished SI is commonly found in nearly all patients with liver cirrhosis [28] and is independent of liver function and clinical and nutritional state of the patient [24]. In this regard, we can assume that liver transplantation is capable of correcting this characteristic abnormality of glucose metabolism present in liver cirrhosis. The fact that the close relationship between SI and body weight in these patients is *restored* after liver transplantation may also reflect the normalization of insulin-mediated glucose disposal [22, 27] as well as fat and muscle recovery in these subjects [32].

Glucose and insulin levels in the fasting state and after the FSIGTT did not differ between controls and patients with liver grafts, whereas C-peptide concentrations were significantly elevated in the patient group. Since C-peptide is not removed by the liver, its kinetic behavior can be used as an indirect monitor of prehepatic insulin clearance [6, 33]. Therefore, the elevated fasting C-peptide in contrast with normal insulin levels and the rapid decay of insulin (*post*hepatic insulin) in contrast with the increased C-peptide concentrations (*pre*hepatic insulin) during the FSIGTT may point to an accelerated hepatic insulin clearance. Subsequently, the analysis of glucose and insulin dynamics during the FSIGTT, which evaluates the effect of posthepatic insulin, resulted in a similar SI in controls and the liver transplant group. The analysis of C-peptide profiles after the FSIGTT, however, assesses the prehepatic insulin kinetic and revealed normal Φ_1 response, but significantly enhanced Φ_2 in the group with liver grafts. Perseghin et al. were able to show that the suppression of beta-cell secretion under euglycemia and hyperinsulinemia was intact [27] in spite of higher fasting C-peptide levels in contrast with normal insulin levels in liver transplant patients. Francavilla et al. also measured elevated fasting C-peptide levels in contrast with lower insulin levels in liver transplant patients [10]. The authors of both studies suggested that this difference may have been due to increased hepatic insulin clearance. Portal hypertension and portal systemic shunting characterize the hemodynamic alterations in cirrhotics [14]. Although most of the hemodynamic abnormalites appear to be reversed after liver transplantation [26], hepatic blood flow is still increased for years [5, 12, 15] with subsequently accelerated higher first-pass-clearance of insulin. The discrepancy between insulin and C-peptide levels, however, only appears to be present in patients with advanced chronic liver diseases, and not in patients with normal liver function [10], indicating that pancreatic hypersecretion after OLT is specific to chronic liver disease per se and appears to be related to persistent hemodynamic abnormalites after OLT.

Φ_1 response after intravenous glucose loading contributes to the early loss of glucose by inhibiting hepatic glucose production [21]. Φ_2 normally adjusts the overall glucose tolerance to individual parameters such as body mass [3] and is strongly influenced by fasting insulin [29]. The close relationship of Φ_2 with fasting insulin in

our control group may reflect this interplay. This relation, however, was lacking in our transplant patients, probably due to the enhanced hepatic insulin clearance, which may produce higher variations of insulin concentrations in the fasting state. Steroid treatment with a dosage of 5 mg/day does not influence the fasting insulin levels since we found similar results in patients with single immunosuppressive CyA therapy (66.42 ± 4.27 pmol/l, paper under review).

Although we did not evaluate the hepatic and portal flow in these patients after OLT, this study yields some evidence that other factors than body mass index and immunosuppressive therapy are involved in the regulation of beta-cell function in patients with stable long-term liver grafts. The difference between lower fasting insulin and higher fasting C-peptide concentrations as well as the computer-assisted evaluation of pre- and posthepatic insulin and C-peptide kinetics by means of minimal modeling techniques point to an enhanced hepatic insulin clearance. The higher hepatic insulin degradation, probably caused by an increased hepatic blood flow, seems to play the predominant role in regulating insulin secretion in patients after OLT. The persistence of such hemodynamic alterations may have detrimental metabolic consequences in patients after liver transplantation, resulting in a prolonged insulin production and, finally, in an exhaustion of beta-cells and posttransplant diabetes mellitus.

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