Metabolic Effects of Cyclosporin A and FK 506 in Liver Transplant Recipients

ANDREW J. KRENTZ, BERTRAND DOUSSET, DAVID MAYER, PAUL MCMASTER, JOHN BUCKELS, ROBERT CRAMB, JANET M. SMITH, AND MALCOLM NATTRASS

Postoperative diabetes is a reported feature of the immunosuppressive agents cyclosporin A and FK 506. To date, however, no randomized comparative studies of the metabolic effects of these two drugs have been performed. In this study, extended (300 min) oral glucose tolerance tests (75 g) were performed a median of 8 mo (range 5–9 mo) postoperatively in 20 clinically stable liver transplant recipients randomly allocated to maintenance immunosuppression with either cyclosporin A (with or without azathioprine) or FK 506. None of the patients had clinically overt diabetes antedating transplantation. To avoid the confounding effects of corticosteroids, prednisolone was withdrawn at least 6 wk beforehand in each case. Ten healthy volunteers matched for age and body mass index served as control subjects. Overall blood glucose concentrations after the glucose challenge were significantly elevated in both groups of transplant recipients ($P < 0.005$ and $P < 0.001$ for cyclosporin A and FK 506 treatment groups, respectively) compared with the healthy control subjects. Venous whole-blood glucose concentration (mean ± SE) 120 min after the ingestion of oral glucose was significantly higher in both the cyclosporin A ($P < 0.05$) and FK 506 ($P < 0.01$) treatment groups compared with the control subjects (6.6 ± 0.5 vs. 8.8 ± 0.9 vs. 5.2 ± 0.2 mM, respectively). According to 1985 WHO criteria, 4 of 10 cyclosporin A–treated patients had impaired glucose tolerance, whereas 3 of 10 FK 506–treated patients had diabetes with 4 others having impaired glucose tolerance. Overall plasma immunoreactive insulin concentrations were significantly elevated in both the cyclosporin A ($P < 0.05$) and FK 506 ($P < 0.01$) groups, as were C-peptide concentrations ($P < 0.02$ and $P < 0.01$, respectively). By contrast, fasting lactate concentrations were significantly lower ($P < 0.05$) in the transplant patients compared with the control subjects. Total blood ketone body concentrations were slightly higher in both transplant groups with an increased ratio of 3-hydroxybutryate:acetoacetate in the cyclosporin A–treated ($P < 0.01$) and FK 506–treated ($P < 0.02$) patients. In conclusion, successful liver transplantation in humans is associated with significant postoperative glucose intolerance and hyperinsulinemia. These metabolic abnormalities are independent of corticosteroid therapy and are more pronounced in patients treated with FK 506 than in comparable patients receiving cyclosporin A with or without azathioprine. Alterations in the circulating ketone body ratio suggest a relatively more reduced hepatic intramitochondrial redox state in liver transplant recipients treated with these immunosuppressive agents. *Diabetes* 42:1753–59, 1993

CsA is the immunosuppressive drug of choice for whole-organ transplantation (1,2), despite reports of significant adverse metabolic effects including glucose intolerance and hyperinsulinemia (1,3,4). Although hyperinsulinemia suggests the possibility of tissue insulin resistance, a direct toxic effect of CsA on pancreatic β-cells has also been suggested (5). To reduce the incidence of adverse effects of CsA in organ transplantation, the drug is usually given in combination with other agents such as azathioprine and corticosteroids. However, not all clinical studies of CsA in humans have demonstrated alterations in glucose metabolism or β-cell function. Robertson et al. (6) found no significant alterations in glucose homeostasis or pancre-

From the Diabetic Clinic and the Department of Clinical Chemistry, The General Hospital, Birmingham; and the Liver Unit and the Department of Clinical Chemistry, The Queen Elizabeth Hospital, Birmingham, United Kingdom.

Address correspondence and reprint requests to Dr. Andrew J. Krentz, The General Hospital, Steelhouse Lane, Birmingham B4 6NH, United Kingdom.

Received for publication 13 May 1993 and accepted in revised form 22 July 1993.

CsA, cyclosporin A; BMI, body mass index; OGTT, oral glucose tolerance test; IRI, immunoreactive insulin; NEFA, nonesterified fatty acid; WHO, World Health Organization; RIA, radioimmunoassay; ANOVA, analysis of variance; IGT, impaired glucose tolerance; NIDDM, non-insulin-dependent diabetes mellitus.

TABLE 1
Clinical characteristics of study subjects

	Control subjects	CsA group	FK 506 group	*P* value
n	10	10	10	
Age (yr)	49 ± 8	46 ± 8	52 ± 8	>0.1
Sex (M/F)	5/5	3/7	3/7	>0.1
BMI (kg/m^2)	24 ± 4	23 ± 3	24 ± 4	>0.1

Data are means ± SE. The *P* value denotes one-way ANOVA including all three groups or Fisher's exact test, as appropriate.

atic islet β-cell function in patients with multiple sclerosis treated with CsA for 2 yr.

FK 506, a new macrolide immunosuppressive agent, has attracted considerable attention (7,8). However, after encouraging reports of efficacy, notably in reversing ongoing organ rejection combined with apparently low toxicity, FK 506 evidently also is characterized by adverse metabolic effects including the development of postoperative diabetes. Posttransplantation diabetes requiring long-term insulin treatment has been reported in ~10% of liver and kidney transplant recipients treated with FK 506 (9,10).

To date, no prospective comparative studies of the metabolic effects of these two agents have been performed. Furthermore, the coadministration of corticosteroids in many published studies confounds the interpretation of metabolic data as has the use of arbitrary and inconsistent diagnostic criteria. This study was prompted by our own observations that cases of postoperative diabetes appeared to be more frequent among patients randomized to FK 506 than patients receiving CsA.

RESEARCH DESIGN AND METHODS

Patients were participants in a phase III, randomized, prospective study comparing CsA with FK 506 as induction and maintenance immunosuppression in liver transplantation for nonmalignant conditions. Eligible patients were randomly allocated to receive either CsA and azathioprine in combination or FK 506 alone. Additionally, all patients received prednisolone postoperatively for 3 mo.

Thirty-five consecutive patients (18 treated with CsA and 17 treated with FK 506) receiving liver transplants were considered for inclusion in the metabolic study. Fifteen patients were either excluded or proved to be unsuitable. Reasons for exclusion included postoperative deaths ($n = 6$), withdrawals from the main study because of rejection or drug-related side effects ($n = 5$), glucose-induced vomiting ($n = 1$), failure to fast ($n = 1$), a personal history of pretransplant diabetes ($n = 1$), and unwillingness to participate ($n = 1$). Thus, a total of 20 patients participated in the metabolic study reported here (Table 1). Although the metabolic status of these patients before transplantation was not formally assessed, overt diabetes was not identified in any patient during pretransplant workup. The principal causes of liver disease included primary biliary cirrhosis ($n = 7$), sequelae of infectious hepatitis ($n = 6$), and sclerosing cholangitis ($n = 3$), with miscellaneous nonmalignant conditions accounting for the remainder.

Ten patients were receiving CsA in a maintenance dose of 6.33 ± 0.53 mg · kg^{-1} · day^{-1} (mean ± SE) (8 patients also were taking azathioprine [1.1 ± 0.1 mg · kg^{-1} · day^{-1}]; this drug was withdrawn in the other 2 patients because of adverse effects). All but 1 of the 10 CsA-treated patients were participants in the main randomized study of CsA versus FK 506. The remaining 10 patients had been randomized to FK 506 and were receiving a mean dose of 0.13 ± 0.01 mg · kg^{-1} · day^{-1} at the time of their metabolic studies. Results were compared with control data from 10 healthy volunteers matched for age and BMI (Table 1). None of the healthy volunteers was receiving medication that would influence the study and none had a history of diabetes in a first-degree relative.

Of the 20 liver transplant patients studied, transient hyperglycemia (defined as a venous plasma glucose concentration >11.1 mM) (11) had been documented within 14 days postoperatively in 5 of 10 CsA patients and 8 of 10 patients treated with FK 506. Although none had a history of diabetes antedating their liver transplantation, 3 patients (1 receiving CsA and 2 receiving FK 506) had a family history of diabetes in a first-degree relative. Although early postoperative diabetes did not require hypoglycemic therapy other than dietary measures in any of the CsA-treated patients, highly purifed monocomponent insulin was administered temporarily for a median of 4 wk (range 2 days to 3 mo) in 6 of the patients treated with FK 506, whereas 1 other patient received temporary treatment with a sulphonylurea. All hypoglycemic medication had been completely withdrawn in all patients at least 4 wk before metabolic studies. The presence or absence of early postoperative hyperglycemia was not a criterion for subsequent inclusion in this metabolic study; all suitable patients were included as detailed above.

The clinical significance of transient postoperative hyperglycemia after liver transplantation may be difficult to evaluate because of the routine use of dextrose-containing fluids and the hormonal stress response associated with a major surgical operation. For this reason, the metabolic studies were performed a median of 8 mo (range 5–9 mo) postoperatively when patients were clinically stable.

All patients received prednisolone in standard doses for 3 mo after transplantation. Our policy is to taper off and withdraw prednisolone completely by 3 mo postoperatively. No patient had received corticosteroids for at least 6 wk before the OGTT.

Three of the transplant recipients (1 receiving CsA and 2 receiving FK 506) were receiving medication known to be associated with potential effects on glucose homeostasis (12). In addition, 1 patient treated with FK 506 was receiving a replacement dose of thyroxine and another patient with a history of tuberculosis was receiving chemoprophylaxis with isoniazid. Details of all concomitant medication at the time of the metabolic study for each of the patients are presented in Table 2.

TABLE 2
Concomitant medication in the 20 liver transplant recipients randomized to CsA with or without azathioprine or FK 506

Recipient	CsA with or without azathioprine group	Recipient	FK 506 group
1	—	1	—
2	—	2	—
3	—	3	—
4	—	4	metoprolol and nifedipine
5	amitriptyline	5	thyroxine and ursodeoxcholic acid
6	ranitidine	6	—
7	—	7	—
8	erythromycin	8	—
9	—	9	phenytoin
10	—	10	isoniazid and pyridoxine

None of the patients had experienced episodes of rejection or serious infection during the 4 wk preceding metabolic studies; all had been consuming a weight-maintaining diet before the OGTT. All subjects were asked to refrain from alcohol the night preceding the study and to omit their usual medication on the morning of the test.

After a 10-h overnight fast, a flexible venous cannula was inserted into an antecubital vein and kept patent by flushing with 2 ml of saline (150 mM). Subjects then sat quietly for 30 min before withdrawal of basal blood samples (17 ml each sample) at −5 min and 0 min. A solution of 75 g anhydrous glucose in 250 ml of water was consumed over 5 min, and the usual conditions for an OGTT were observed. Free flowing venous blood (12-ml) samples were withdrawn at 15, 30, 60, 90, 120, 180, 240, and 300 min. At each sampling time point, the initial 2 ml of blood was discarded before collection of blood. Approximately 1.5–2.0 ml of blood was added to preweighed tubes containing 5 ml ice-cold perchloric acid (0.77 M), mixed, centrifuged promptly for 20 min at 4°C, and the supernatant decanted and frozen at −20°C pending assay of intermediary metabolites. Another 10 ml of blood was added to tubes containing lithium heparin, centrifuged and plasma separated and frozen at −20°C pending assay of IRI, C-peptide, and NEFAs.

Routine biochemical markers of hepatic function, including serum bilirubin, transaminases, and alkaline phosphatase, and serum creatinine were measured in each patient at the time of study as were trough blood CsA and plasma FK 506 concentrations. Oral glucose tolerance was categorized on the basis of the fasting and 120-min venous whole-blood glucose concentrations according to WHO criteria (11).

Ethical considerations. Informed written consent was obtained from all subjects. The study was approved by the Ethical Committee of South Birmingham Health Authority.

Assays. Glucose, lactate, pyruvate, alanine, 3-hydroxybutyrate, acetoacetate, and glycerol were assayed with the use of automated fluorimetric and spectrophotometric techniques (13). Blood acetoacetate was measured within 36 h of each study. The sum of 3-hydroxybutyrate and acetoacetate is referred to as total ketone bodies. Plasma IRI was measured by double-antibody RIA (14). Plasma C-peptide concentrations were measured by RIA (Guildhay, Guildford, UK). Plasma NEFA were measured with the use of an enzymatic method (Wako Chemicals, Neuss, Germany). Trough whole-blood CsA and plasma FK 506 concentrations were determined by monoclonal RIA (Incstar, Wokingham, UK) and an ELISA method (15), respectively.

Statistical analysis. Clinical characteristics, basal (fasting) metabolite and hormone concentrations and 120-min glucose concentrations were compared between the CsA, FK 506, and healthy control groups with the use of one-way ANOVA, including all three groups in the analysis, or Fisher's exact test, as appropriate. Where the variance (F) ratio indicated a significant ($P < 0.05$ or less) difference between the three groups, comparisons were then performed between paired groups of interest with the use of ANOVA.

Overall differences in metabolite and hormone concentrations between the three groups during the OGTT were examined by two-way ANOVA with the data classified by group and time (16). This method was also used to compare pairs of groups when the preliminary analysis indicated a significant overall difference between all three groups.

Data with a nongaussian distribution (17), including total ketone bodies and IRI, were logarithmically transformed before analysis, but are presented in their pretransformed state. Data are presented as means ± SE.

RESULTS

Clinical and biochemical characteristics of patients. The three groups were well matched for clinical characteristics with no significant differences ($P > 0.1$) in age, sex ratio, or BMI (Table 1). Although biochemical liver function tests were abnormal in 3 of 10 patients in each transplant group, serum concentations of bilirubin and hepatic enzymes were not elevated >50% above the upper limit of the laboratory reference range in any individual. Three of 10 CsA-treated patients and 5 of 10 FK 506–treated patients had serum creatinine concentrations above the upper limit of the normal reference range (>125 μM), although mean serum creatinine concentrations for the CsA and FK 506 groups were not significantly different (124 ± 10 and 133 ± 11 μM, re-

TABLE 3
Basal (fasting) metabolite, immunoreactive insulin, and C-peptide concentrations

	Control subjects	CsA group	FK 506 group	*P* value
n	10	10	10	
Glucose (mM)	4.6 ± 0.2	4.8 ± 0.2	5.1 ± 0.2	=0.10
Lactate (mM)	0.60 ± 0.08	0.36 ± 0.05*	0.37 ± 0.05*	<0.02
Pyruvate (mM)	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	>0.1
Alanine (mM)	0.28 ± 0.03	0.28 ± 0.01	0.26 ± 0.02	>0.1
Glycerol (mM)	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	>0.1
NEFA (mM)	0.60 ± 0.11	0.62 ± 0.07	0.58 ± 0.08	>0.1
Total ketone bodies (mM)	0.12 ± 0.02	0.32 ± 0.10	0.37 ± 0.18	>0.1
Insulin (pM)	28.2 ± 2.4	38.4 ± 2.4†	49.8 ± 8.4‡	<0.001
C-peptide (nM)	0.44 ± 0.43	0.60 ± 0.05‡	0.69 ± 0.11*	<0.02

Data are means ± SE. The *P* value denotes one-way ANOVA including all three groups.
*$P < 0.05$ vs. healthy control subjects.
†$P < 0.01$ vs. healthy control subjects.
‡$P < 0.02$ vs. healthy control subjects.

spectively, $P > 0.1$). Mean trough whole-blood CsA concentration at the time of the metabolic studies was 192 ± 23 ng/ml (therapeutic range 150–250 ng/ml); mean plasma FK 506 level was 0.43 ± 0.10 ng/ml (suggested therapeutic range 0.5–1.5 ng/ml).

Basal hormone and metabolite concentrations. Fasting plasma IRI concentrations (Table 3) were significantly different between the three groups ($P < 0.001$) with levels for both the CsA ($P < 0.01$) and FK 506 ($P < 0.02$) treatment groups being significantly higher than that for the healthy control subjects (Table 3). Fasting plasma C-peptide concentrations were also significantly elevated in each of the transplant groups compared with the healthy control subjects (Table 3). Fasting blood lactate concentrations were significantly lower ($P < 0.05$ for each) in both the CsA and FK 506 treatment groups. By contrast, blood pyruvate concentrations did not differ significantly (Table 3), and the difference in fasting blood lactate:pyruvate ratio between the groups (control subjects vs. CsA group vs. FK 506 group) was not statistically significant (9.5 ± 0.7 vs. 8.7 ± 1.0 vs. 8.6 ± 1.6, $P > 0.1$). Fasting concentrations of glucose, alanine, glycerol, and NEFAs were not significantly different ($P > 0.1$) between the three groups (Table 3). Fasting blood total ketone body concentrations were slightly higher in each transplant group relative to the healthy control subjects, although the difference between the three groups did not reach conventional statistical significance (Table 3).

Hormone and metabolite concentrations during OGTT. Overall blood glucose concentrations were significantly different ($P < 0.05$) between the three groups with glucose concentrations in both the CsA ($P < 0.005$) and FK 506 ($P < 0.001$) treatment groups being significantly higher than those for the control subjects by two-way ANOVA (Fig. 1). Mean blood glucose concentrations at 120 min were significantly elevated in both the CsA ($P < 0.05$) and FK 506 ($P < 0.01$) treatment groups relative to the group of healthy control subjects (6.6 ± 0.5 vs. 8.8 ± 0.9 vs. 5.2 ± 0.2 mM, respectively). Furthermore, mean blood glucose concentration at 120 min was significantly higher ($P < 0.05$) in the FK 506–treated patients compared with the CsA treatment group. In the FK 506–treated group, individual 120-min blood glucose concentrations were diagnostic of diabetes in 3 patients and of IGT in 4 others (11) (Table 4). Each of the FK 506–treated patients with diabetes and 3 of 4 patients with IGT had a history of diabetes in the immediate postoperative period. Each of these patients had required temporary exogenous insulin treatment to control their blood glucose concentrations. By contrast, none of the CsA-treatment group had diabetes 8 mo postoperatively, although 4 patients had IGT according to WHO criteria. Only 1 of the CsA-treated patients had a history of postoperative hyperglycemia after transplantation.

The difference in plasma IRI concentrations between the three groups was also statistically significant by two-way ANOVA ($P = 0.01$) (Fig. 2). Compared with the healthy control subjects, overall plasma insulin concentrations during the OGTT were significantly higher in both the CsA ($P < 0.05$) and FK 506 ($P < 0.01$) treatment groups. No significant difference was noted ($P > 0.1$) in overall plasma IRI concentrations between the two trans-

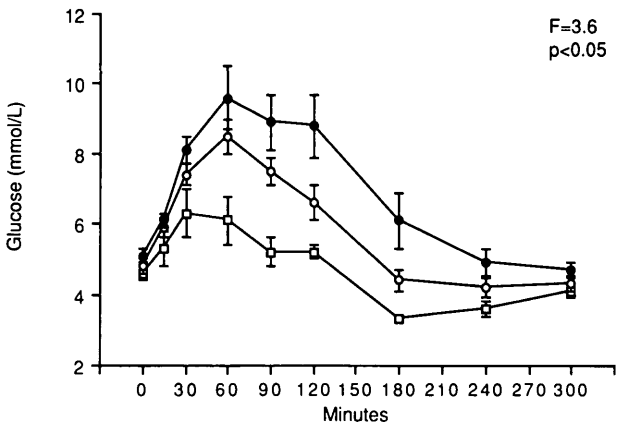

FIG. 1. Venous whole-blood glucose concentrations (means ± SE) in 10 healthy control subjects (□), 10 liver transplant recipients receiving maintenance treatment with CsA with or without azathioprine (○), and 10 liver transplant recipients receiving FK 506 (●). *F* (variance) ratio and *P* value refer to overall trend differences between the three groups by two-way ANOVA. Significant differences were apparent in overall glucose concentrations between the healthy control subjects and the CsA-treated patients ($P < 0.005$) and the healthy control subjects and the FK 506–treated patients ($P < 0.001$).

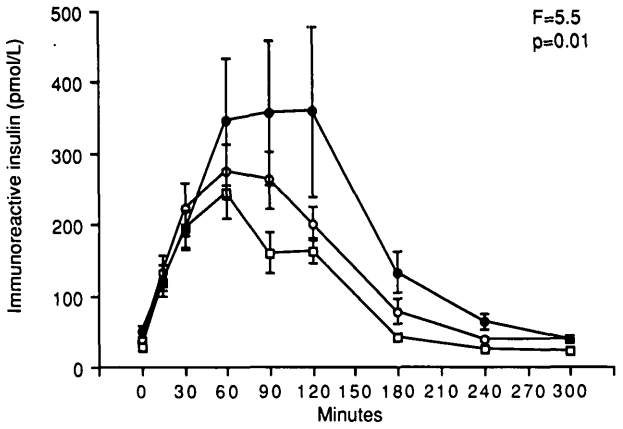

FIG. 2. Plasma immunoreactive insulin concentrations (means ± SE) in 10 healthy control subjects (□), 10 liver transplant recipients receiving maintenance treatment with CsA with or without azathioprine (○), and 10 liver transplant recipients receiving FK 506 (●). *F* (variance) ratio and *P* value refer to overall trend differences between the three groups by two-way ANOVA. Significant differences were evident in overall IRI concentrations between the healthy control subjects and the CsA-treated patients ($P < 0.05$) and the healthy control subjects and the FK 506–treated patients ($P < 0.01$).

plant groups. Plasma C-peptide responses were similar to those for IRI with significantly higher overall C-peptide concentrations in both the CsA-treated ($P < 0.02$) and FK 506–treated ($P < 0.01$) patients compared with the healthy control subjects.

No statistically significant ($P > 0.1$) differences were observed in the overall concentrations of lactate, pyruvate, alanine, glycerol, or NEFAs between the three groups by two-way ANOVA. Overall total blood ketone body concentrations were slightly, but not significantly ($P > 0.1$), higher than the control subjects in the two transplant groups after the oral glucose challenge. However, a statistically significant difference was noted between the three groups in the mean ratio of blood 3-hydroxybutrate:acetoacetate during the OGTT (Fig. 3).

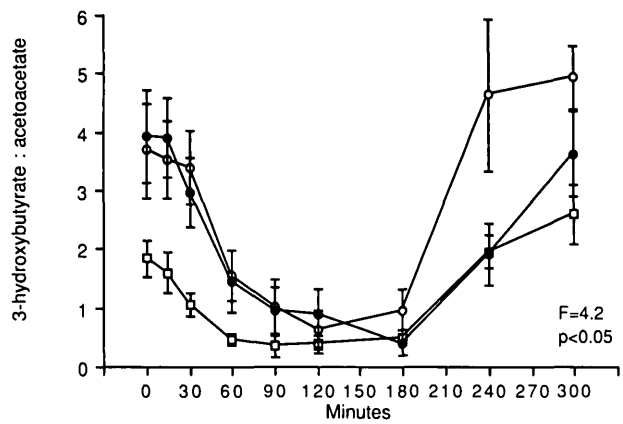

FIG. 3. Ratio (means ± SE) of blood 3-hydroxybutyrate:acetoacetate in 10 control subjects (□), 10 liver transplant recipients receiving CsA with or without azathioprine (○), and 10 liver transplant recipients receiving FK 506 (●). *F* (variance) ratio and *P* value refer to overall trend differences between the three groups by two-way ANOVA. Significant differences were also found between the CsA-treated patients ($P < 0.01$), the FK 506–treated patients ($P < 0.02$), and the control group.

The mean 3-hydroxybutrate:acetoacetate ratio was elevated in the CsA-treated ($P < 0.01$) and FK 506–treated groups ($P < 0.02$) compared with the healthy control subjects (1.26 ± 0.25 vs. 2.77 ± 0.51 vs. 2.48 ± 0.49 for the control, CsA, and FK 506 groups, respectively).

DISCUSSION

The results of this randomized study indicate that liver transplant recipients receiving maintenance immunosuppression with either CsA (with or without azathioprine) or FK 506 studied 8 mo postoperatively are characterized by a high prevalence of clinically significant glucose intolerance. Of 20 consecutive patients who were studied, 8 had IGT and 3 had diabetes, as defined by WHO diagnostic criteria. A number of possible explanations may exist for these findings. First, although patients with preexisting clinical diabetes were excluded from the study, it should be acknowledged that the pretransplantation metabolic status of the patients is not precisely known. Impairment of oral glucose tolerance is a well-recognized feature of patients with chronic liver disease (18), and although none of the patients we studied had clinically overt diabetes before transplantation, minor degrees of glucose intolerance may not have been detected during routine preoperative assessments. With the importance of the liver in disposal of a glucose load (19), abnormalities of glucose tolerance probably would have been present in most, if not all, of these patients with preterminal hepatic failure before transplantation. Our findings indicate that glucose tolerance is not normalized by liver transplantation in the majority of such patients. Second, hyperglycemia and hyperinsulinemia are features of patients with portal hypertension, a finding that has been ascribed to portal-systemic shunting (19). Possibly, vascular shunting after liver transplantation could produce similar metabolic abnormalities, although this would not explain the significantly elevated C-peptide concentrations in our patients that suggest increased islet β-cell secretion. Third, the finding may be a consequence of immunosuppressive therapy, although our study shows for the first time that it is not attributable to concurrent administration of corticosteroids. An important observation in this study is the difference in the degree of glucose intolerance between patients treated with CsA and those treated with FK 506. Statistically, it is unlikely that this was attributable to differences in pretransplantation status, because patients were randomly allocated to each immunosuppressive agent in a ratio of 1:1, nor is it likely that the observed discordance in glucose intolerance between the groups represents differences in the viability of the hepatic grafts. In support of this contention, only 3 of 10 patients in each group had minor abnormalities in clinical chemistry at the time of study, and no apparent association was noted between the abnormalities in biochemical liver function tests and the presence of glucose intolerance. The greater prevalence of disturbed glucose tolerance in the patients treated with FK 506 suggests a drug-related effect attributable to this immunosuppressive agent. Whatever the explanation, the finding is of clinical relevance because

TABLE 4
Individual fasting and 120-min venous whole-blood glucose concentrations in healthy control subjects and liver transplant recipients receiving CsA with or without azathioprine or FK 506

	Blood glucose concentration (mM)					
	Control subjects		CsA with or without azathioprine group		FK 506 group	
Recipient	0 min	120 min	0 min	120 min	0 min	120 min
1	4.8	4.4	4.7	4.5	6.2	7.2*
2	5.1	5.3	4.0	6.6	4.6	13.4†
3	4.6	4.8	5.0	7.7*	5.6	5.8
4	4.8	5.5	4.9	6.0	5.3	12.0†
5	4.6	5.2	4.9	5.7	4.6	9.4*
6	4.6	5.2	4.5	6.5	6.0	11.8†
7	3.7	6.3	5.0	9.0*	4.5	8.8*
8	4.5	5.9	5.2	4.2	4.5	5.6
9	5.4	5.9	4.2	7.6*	5.1	7.4*
10	4.0	3.9	5.6	7.8*	5.0	6.2
Means ± SEM	4.6 ± 0.2	5.2 ± 0.2	4.8 ± 0.2	6.6 ± 0.5‡	5.1 ± 0.2	8.8 ± 0.9§

*IGT (WHO, 1985).
†Diabetes (WHO, 1985).
‡$P < 0.05$ vs. healthy control subjects.
§$P < 0.01$ vs. healthy control subjects.

IGT is associated with an increased risk of atherosclerosis and progression to diabetes (21), whereas NIDDM is associated with long-term macrovascular (22) and microvascular complications (23). Note that accelerated atheromatous disease has been identified as a leading cause of premature death in long-term survivors of heart and renal transplantation (24,25). Although the mechanisms underlying this phenomenon remain unclear, current knowledge of metabolic risk factors for atherosclerosis (26) suggests that the combination of hyperglycemia and hyperinsulinemia could increase the risk of atheromatous disease in transplant patients. With improved survival after organ transplantation, these considerations could become increasingly important.

Others have suggested that adverse clinical effects associated with FK 506 may be dose related (9). It is therefore of interest that trough plasma FK 506 concentrations lay below the lower limit of the suggested therapeutic range at the time of study in 6 of 10 FK 506–treated patients as did the mean trough FK 506 concentration for the group as a whole. We are investigating whether combining FK 506 with azathioprine will allow a reduction in FK 506 dose thereby reducing the incidence of adverse effects including glucose intolerance in liver transplant recipients.

The finding of hyperglycemia despite elevated fasting and glucose-stimulated IRI and C-peptide concentrations implies tissue resistance to the actions of insulin. In vivo dose-response studies will be necessary to confirm this assumption, and these studies are in progress. Although our data do not exclude a coexisting defect in β-cell function, as has been reported for human β-cells in vivo (27,28), it is clear that the glucose intolerance observed in the transplant recipients is not attributable to absolute insulinopenia. Furthermore, differences in 2-h blood glucose and plasma IRI concentrations between the two transplant groups suggest the possibility of greater impairment of insulin sensitivity by FK 506.

Interestingly, 6 of 7 FK 506–treated patients found to have either IGT or diabetes 8 mo postoperatively had a history of temporary insulin-treated diabetes in the early posttransplantation period. Thus, defective glucose metabolism was manifest in these patients shortly after transplantation, and this defect was still evident, although to a lesser degree, 8 mo later. Concurrent administration of corticosteroids postoperatively during the first 3 mo may have aggravated the defect in glucose metabolism (29) necessitating temporary treatment with exogenous insulin in several patients. Other clinical investigators have reported an improvement in glucose tolerance in CsA-treated renal transplant recipients after withdrawal of corticosteroids sufficient to allow successful withdrawal of insulin therapy in some patients (30).

No statistically significant differences were observed in concentrations of other intermediary metabolites between the transplant recipients and the healthy control subjects in our investigation, although fasting total ketone body concentrations were slightly higher in both transplant groups. Statistically significant alterations in the blood ketone body ratio were, however, observed in the liver transplant recipients. The circulating concentration of acetoacetate is dependent on the intramitochondrial concentration ratio of NAD^+:NADH via the 3-hydroxybutyrate dehydrogenase reaction (31). The increased ratio of blood 3-hydroxybutyrate:acetoacetate observed in the liver transplant recipients implies a more reduced hepatic intramitochondrial redox state relative to the healthy control subjects. Whether the altered circulating ketone body ratio represents a subtle defect of mitochondrial function in the hepatic allografts is unclear.

Fasting blood lactate concentrations were significantly lower in both the CsA and FK 506 treatment groups. Given the combination of hyperglycemia and hyperinsulinemia during the OGTT, these low blood lactate concentrations are intriguing, being in marked contrast to the fasting and postprandial hyperlactatemia that characterizes chronic liver disease (32). This observation therefore

argues against dysfunction of the hepatic grafts as the cause of the metabolic disturbances in glucose and ketone body metabolism. However, the mechanisms underlying the low lactate concentrations remain unclear. Studies of lactate turnover may clarify the relative contributions of decreased lactate appearance and/or increased lactate disposal.

In conclusion, both CsA (with or without azathioprine) and FK 506 are associated with clinically significant glucose intolerance and hyperinsulinemia after successful liver transplantation in humans. These observations may have long-term implications for organ transplant recipients. In addition, alterations in the circulating ketone body ratio imply an altered hepatic intramitochondrial redox state after liver transplantation. Additional studies are underway to determine the specificity, pathogenesis, and clinical implications of these metabolic abnormalities.

ACKNOWLEDGMENTS

We thank Sue Ayres and Dr. Juan Umana for help with the organization of the study and with blood sampling during the OGTTs. We also wish to thank the staff of the Liver Unit, Queen Elizabeth Hospital, Birmingham, and the volunteers who acted as control subjects for the study. The expert technical assistance of Janet Sherwell, Sheila Nutter, and Peter Lewis of the Department of Clinical Chemistry, the General Hospital, Birmingham is gratefully acknowledged.

REFERENCES

1. Kahan BD: Cyclosporine. *N Engl J Med* 321:1725–38, 1989
2. Wonigeit K: Immunosuppressive treatment in liver transplantation. *Bailliere's Clin Gastroenterol* 4:813–24, 1989
3. Gunnarson R, Klintmalm G, Lundgren G, Tyden G, Wilczek H, Ostman J, Groth CG: Deterioration in glucose metabolism in pancreatic transplant patients after conversion from azathioprine to cyclosporine. *Transplant Proc* 16:709–12, 1984
4. Yamamoto H, Akazawa S, Yagamuchi Y, Yokota A, Yamasaki H, Nakanishi T, Tahara D, Matsuya F, Saito Y, Nagataki S: Effects of cyclosporin A and low dosages of steroid on post-transplantation diabetes in kidney tranplant recipients. *Diabetes Care* 14:867–70, 1991
5. Nielsen JH, Mandrup-Poulsen T, Nerup J: Direct effects of cyclosporin A on human pancreatic β-cells. *Diabetes* 35:1049–52, 1986
6. Robertson RP, Franklin G, Nelson L: Intravenous glucose tolerance and pancreatic islet β-cell function in patients with multiple sclerosis during 2-yr treatment with cyclosporin. *Diabetes* 38:58–64, 1989
7. Starzl TE, Todo S, Fung T, Demetris AJ, Venkataramanan R, Jain A: FK 506 for liver, kidney, and pancreatic transplantation. *Lancet* 2:1000–1004, 1989
8. Fung JJ, Todo S, Tzakis A, Alessiani M, Abu-Elmagd K, Jain A, Bronster O, Martin M, Gordon R, Starzl TE: Current status of FK 506 in liver tranplantation. *Transplant Proc* 23:1902–905, 1991
9. Fung JJ, Alessiani M, Abu-Elmagd K, Todo S, Shapiro R, Tzakis A, Van Thiel D, Armitage J, Jain A, McCauley J, Selby R, Starzl TE: Adverse effects associated with use of FK 506. *Transplant Proc* 23:3105–108, 1991
10. Scantlebury V, Shapiro R, Fung J, Tzakis A, McCauley J, Jordan M, Jensen C, Hakala T, Simmons R, Starzl TE: New onset of diabetes in FK 506 vs. cyclosporine-treated kidney transplant recipients. *Transplant Proc* 23:3169–70, 1991
11. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser. no. 727)
12. Ferner RE: Drug-induced diabetes. *Bailliere's Clin Endocrinol Metab* 6:849–66, 1992
13. Stappenbeck R, Hodson AW, Skillen AW, Agius L, Alberti KGMM: Optimized methods to measure acetoacetate, 3-hydroxybutyrate, glycerol, alanine, pyruvate, lactate, and glucose in human blood using a centrifugal analyzer with a fluorimetric attachment. *J Automat Chem* 12:213–20, 1990
14. Hales CN, Randle PJ: Immunoassay of insulin with insulin antibody precipitate. *Biochem J* 88:137–46, 1963
15. Tamura K, Kobayashi M, Hashimoto K, Kojima K, Nagase K, Iwasaki K, Kaizu T, Tanaka H, Niwa M: A highly sensitive method to assay FK 506 levels in plasma. *Transplant Proc* 19 (Suppl. 6):23–29, 1987
16. Armitage P, Berry G: *Statistical Methods in Medical Research*. 2nd ed. Oxford, Blackwell Scientific, 1987
17. Foster K, Alberti KGMM, Hinks L, Lloyd B, Posle A, Smythe P, Turnell DC, Walton R: Blood intermediary metabolite and insulin concentrations after an overnight fast: reference ranges for adults and interrelationships. *Clin Chem* 24:1568–72, 1978
18. Megyesi C, Samols E, Marks V: Glucose intolerance and diabetes in chronic liver disease. *Lancet* 2:1051–56, 1967
19. DeFronzo RA, Ferrannini E: Regulation of hepatic glucose metabolism in humans. *Diabetes Metab Rev* 3:415–59, 1987
20. Johnston DG, Alberti KGMM, Faber OK, Binder C, Wright R: Hyperinsulinism of hepatic cirrhosis: diminished degradation or hypersecretion? *Lancet* 1:10–13, 1977
21. Yudkin JS, Alberti KGMM, McLarty DG, Swai ABM: Impaired glucose tolerance. *Br Med J* 301:397–402, 1990
22. Pyorala K, Laasko M, Uusitupa M: Diabetes and atherosclerosis: an epidemiological view. *Diabetes Metab Rev* 3:463–524, 1987
23. Singh BM, Rutter JD, FitzGerald MG: The natural history of non-insulin-dependent diabetes mellitus. *Bailliere's Clin Endocrinol Metab* 2:343–58, 1988
24. Kriett JM, Kaye MP: The registry of the International Society for Heart and Lung Transplantation: eighth official report, 1991. *J Heart Lung Transplant* 10:491–98, 1991
25. Raine AEG, Ledingham JGG: Cardiovascular complications after renal transplantation. In *Kidney Transplantation, Principles and Practice*. Morris P, Ed. London, Grune & Stratton, 1984, p. 439–89
26. Reaven GM: Role of insulin resistance in human disease. *Diabetes* 37:1595–607, 1988
27. Rilo HLR, Zeng Y, Alejandro R, Carroll PB, Bereiter D, Venkataramanan R, Tzakis AG, Stazl TE, Ricordi C: Effect of FK 506 on function of human islets of Langerhans. *Tranplant Proc* 23:3164–65, 1991
28. Ricordi C, Zeng Y, Alejandro R, Tzakis A, Venkataramanan R, Fung J, Bereiter D, Mintz DH, Starzl TE: In vivo effect of FK 506 on human pancreatic islets. *Transplantation* 52:519–22, 1991
29. Rizza RA, Mandarino LJ, Gerich JE: Cortisol-indiced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action. *J Clin Endocrinol Metab* 54:131–38, 1982
30. Hricik DE, Bartucci MR, Moir EJ, Mayes JT, Schulak JA: Effects of steroid withdrawal on posttransplant diabetes mellitus in cyclosporine-treated transplant recipients. *Transplantation* 51:374–77, 1991
31. Williamson DH, Lund P, Krebs HA: The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondira of rat liver. *Biochem J* 103:514–27, 1967
32. Shnueli E, Record CO, Alberti KGMM: Liver disease, carbohydrate metabolism and diabetes. *Bailliere's Clin Endocrinol Metab* 6:719–43, 1992