Resting energy expenditure in diabetic and nondiabetic patients with liver cirrhosis: relation with insulin sensitivity and effect of liver transplantation and immunosuppressive therapy[1–3]

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ABSTRACT

Background: Hypermetabolism, insulin resistance, and diabetes are common in patients with liver cirrhosis.

Objective: We assessed whether diabetes and insulin resistance influence postabsorptive energy homeostasis in these patients and whether liver transplantation (LTx) and immunosuppressive drugs affect these relations.

Design: Twenty-six patients with liver cirrhosis (16 with and 10 without diabetes) were studied with an insulin clamp and indirect calorimetry. Eleven of these subjects were studied 9 mo after LTx to longitudinally assess its effects. To cross-sectionally explore a longer follow-up period, we studied 65 patients 6, 14, and 32 mo after LTx. Seven patients with chronic uveitis (receiving immunosuppressive therapy) and 20 healthy subjects served as control subjects.

Results: Diabetic and nondiabetic patients with cirrhosis had insulin resistance ($S_{I(clamp)}$; $P < 0.03$) and higher measured resting energy expenditure (REE) as a percentage of predicted REE than did healthy subjects (107.6 ± 1.8% compared with 97.4 ± 2.3%; $P < 0.03$), and these 2 alterations were associated ($R^2 = 0.119$, $P = 0.0002$). The longitudinal study showed an improvement in the 2 variables after LTx, but full restoration was not achieved. The cross-sectional analysis confirmed this observation in patients studied 6 mo ($n = 28$) after LTx. In patients studied 14 ($n = 21$) and 32 mo ($n = 16$) after LTx, $S_{I(clamp)}$ and measured REE as a percentage of predicted REE were not significantly different from those in control subjects.

Conclusions: In patients with liver cirrhosis, higher-than-normal postabsorptive REE was associated with insulin resistance regardless of diabetes. This abnormality persisted in patients studied 6–9 mo after LTx but improved simultaneously with the improvement in insulin sensitivity thereafter. *Am J Clin Nutr* 2002;76:541–8.

KEY WORDS Resting energy expenditure, liver cirrhosis, insulin resistance, liver transplantation, lipid oxidation, immunosuppressive therapy, diabetes

INTRODUCTION

Hypermetabolism may occur in patients with liver cirrhosis regardless of the clinical, laboratory, or histologic features of the disease or of its duration and severity (1), with the exception of primary biliary cirrhosis (2), suggesting that extrahepatic factors are the major determinants of hypermetabolism. Identification of these factors is therefore important because hypermetabolism, which induces malnutrition, may have a profound impact on the prognosis of patients with liver cirrhosis (1). Because patients with extrahepatic portal obstruction (3) or liver transplants (4) are hypermetabolic (despite having a normal liver function), portal hypertension and portosystemic shunting are suggested to be causative factors. In addition, the effects of the systemic inflammatory condition, which is mediated by increased concentrations of various cytokines (5), and the effects of an increased β-adrenergic activity were suggested to cause hypermetabolism in clinically stable patients with liver cirrhosis (1).

Insulin resistance is highly prevalent in patients with liver cirrhosis (6), is a primary event in the development of hepatogenous diabetes (7), and also influences the pathology and natural history of hepatogenous diabetes (8). The fact that the insulin-mediated oxidative disposal of carbohydrates (6, 9), lipids (9), and proteins (10) in liver cirrhosis is altered in association with increased β-adrenergic activity induced us to hypothesize that hypermetabolism in cirrhosis may be mediated by insulin resistance and overt diabetes.

This study was therefore undertaken to test whether postabsorptive energy homeostasis in patients with liver cirrhosis is related to insulin resistance and is made worse by diabetes and to test whether liver transplantation (LTx), which improves insulin sensitivity (11, 12), might also influence resting energy expenditure

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TABLE 1
Longitudinal study: anthropometric, laboratory, and clinical characteristics of cirrhotic patients before and 9 mo after liver transplantation (LTx) and of control subjects[1]

	Patients		Control subjects	
	Before LTx (n = 1 F, 10 M) [5][2]	After LTx (n = 1 F, 10 M) [3][2]	CU patients (n = 1 F, 6 M)	Healthy subjects (n = 2 F, 18 M)
Anthropometric variables				
Age (y)	54 ± 2[3]	55 ± 2	44 ± 5	43 ± 4
Body wt (kg)	74 ± 3	76 ± 4	76 ± 5	78 ± 3
Height (cm)	173 ± 1	173 ± 1	172 ± 4	173 ± 2
BMI (kg/m^2)	24.7 ± 1.0	25.2 ± 1.2	25.7 ± 1.8	26.0 ± 0.9
Age of transplant (d)	—	321 ± 25	330 ± 30[4]	—
Prednisone (mg/d)	—	—	6.5 ± 0.4	—
Cyclosporine A (mg·kg^{-1}·d^{-1})	—	3.6 ± 0.5	3.0 ± 0.5	—
Laboratory variables				
Fasting plasma glucose (mmol/L)	7.88 ± 0.75[5]	6.28 ± 0.50[5,6]	4.72 ± 0.16	4.94 ± 0.11
Fasting plasma free insulin (pmol/L)	135 ± 20[5]	45 ± 8[7]	54 ± 5[6]	42 ± 6
Insulin sensitivity				
Glucose infusion rate (mg·kg^{-1}·min^{-1})	2.51 ± 0.32[5]	3.85 ± 0.48[6]	5.07 ± 0.51	5.66 ± 0.31
$S_{I(clamp)}$ ([dL·(min·kg)$^{-1}$·(μU/mL)$^{-1}$] × 10^4)	3.84 ± 0.60[5]	6.33 ± 1.07[5,7]	7.93 ± 0.80[7]	10.53 ± 0.94
REE				
REE (MJ/d)	7.08 ± 0.21	7.12 ± 0.29	6.99 ± 0.38	7.05 ± 0.15
REE/kg body wt (kJ·kg^{-1}·d^{-1})	97.5 ± 2.5	95.0 ± 2.9	90.4 ± 1.1	91.6 ± 2.9
% of predicted REE (%)	108.5 ± 1.8[5]	106.9 ± 2.7[6]	100.5 ± 3.0	97.4 ± 2.3
Prevalence of hypermetabolism (n [%])	2 [18]	1 [9]	0 [0]	0 [0]
Respiratory quotient	0.78 ± 0.02	0.79 ± 0.01	0.82 ± 0.01	0.82 ± 0.00
Basal glucose oxidation (mg·kg^{-1}·min^{-1})	0.83 ± 0.30	1.09 ± 0.21	1.34 ± 0.17	1.34 ± 0.07
Basal lipid oxidation (mg·kg^{-1}·min^{-1})	1.10 ± 0.13	0.95 ± 0.07	0.79 ± 0.06	0.81 ± 0.04

[1] CU, chronic uveitis; $S_{I(clamp)}$, clamp-related index of insulin sensitivity; REE, resting energy expenditure.
[2] Number of diabetic patients in brackets.
[3] $\bar{x}$ ± SEM.
[4] Duration of immunosuppressive therapy.
[5,6] Significantly different from healthy subjects (ANOVA and Tukey's post hoc test): [5] $P < 0.03$, [6] $P < 0.05$.
[7] Significantly different from before LTx, $P < 0.01$ (Student's paired t test and Bonferroni correction).

(REE) and substrate oxidation rates despite the administration of immunosuppressive drugs. We pursued this aim with a longitudinal approach involving 11 cirrhotic patients who were studied before and 9 mo after LTx and with a cross-sectional approach involving 26 cirrhotic patients and 65 patients with liver transplants who were studied 6, 14, and 32 mo after LTx. The cross-sectional study was designed to explore a longer follow-up period after the surgical procedure and included a group of patients with a nonsystemic disease (chronic uveitis) who were treated with an immunosuppressive regimen similar to that of transplant patients.

SUBJECTS AND METHODS

Subjects

Longitudinal study

Eleven patients with postnecrotic cirrhosis (Child-Pugh class A or B) complicated by small unresectable hepatocellular carcinoma (for a single tumor, a tumor diameter < 5 cm; for multiple tumors, a tumor diameter < 3 cm) were recruited in the Hepato-Pancreatic Surgery and Liver Transplantation Unit of the National Cancer Institute. Five of these patients were also diagnosed as having diabetes (3 separate fasting plasma glucose values > 7 mmol/L), which was treated with diet or daily subcutaneous insulin injections. None had metastatic disease at the time of transplantation or when this study was performed. They were studied twice (before and 9 mo after LTx) by means of the euglycemic-hyperinsulinemic clamp and indirect calorimetry as described below. After transplantation, all patients were in stable clinical condition; liver function was normal, with the exception of slight increases in alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1). Two of the 5 patients with diabetes had normal fasting glucose concentrations after LTx; therefore, at the time the study was repeated, 3 of the 11 patients were still diabetic (2 were being treated with diet and 1 with insulin). The anthropometric, laboratory, and clinical characteristics of patients and control subjects [7 patients with chronic uveitis (CU) who were receiving immunosuppressive therapy similar to that of transplant patients and 20 healthy subjects] in this study are shown in **Table 1**.

Cross-sectional study

To explore a longer follow-up period, we performed a cross-sectional study that included *1*) 26 cirrhotic patients (16 with diabetes) who had features similar to those of the patients enrolled in the longitudinal study, *2*) 28 patients (7 with diabetes) who were studied 6 mo after LTx, 3) 21 patients (2 with diabetes) who were studied 14 mo after LTx, and *4*) 16 patients (1 with diabetes) who were studied 32 mo after LTx. The eleven patients who participated in the longitudinal study were also included in the cross-sectional analysis: all 11 were included in the group of cirrhotic patients before LTx, 9 of them were included in the subgroup of patients who were studied 6 mo after LTx, and 2 of them were included in the subgroup of patients who were studied 14 mo after

TABLE 2
Cross-sectional study: anthropometric, laboratory, and clinical characteristics of cirrhotic patients before liver transplantation (LTx) and of LTx patients who were studied 6, 14, and 32 mo after LTx [1]

	Before LTx (n = 2 F, 24 M) [16][2]	6 mo after LTx (n = 4 F, 24 M) [7][2]	14 mo after LTx (n = 3 F, 18 M) [2][2]	32 mo after LTx (n = 0 F, 16 M) [1][2]
Anthropometric variables				
Age (y)	53 ± 2[3]	50 ± 3	51 ± 2	55 ± 2
Body wt (kg)	76 ± 2	71 ± 2	74 ± 3	74 ± 3
Height (cm)	172 ± 1	170 ± 1	172 ± 2	173 ± 1
BMI (kg/m^2)	25.7 ± 0.7	24.6 ± 0.6	25.1 ± 0.9	24.8 ± 0.8
Age of transplant (d)	—	173 ± 12	404 ± 22	934 ± 75
Prednisone (mg/d)	—	5.0 ± 0.1[4]	—	—
Cyclosporine A (mg·kg^{-1}·d^{-1})	—	3.7 ± 0.5	3.6 ± 0.5	3.6 ± 0.5
Laboratory variables				
Fasting plasma glucose (mmol/L)	6.79 ± 0.43[5]	5.69 ± 0.33	5.10 ± 0.16[6]	5.28 ± 0.22[6]
Fasting plasma free insulin (pmol/L)	124 ± 12[5]	58 ± 4[6]	63 ± 8[6]	49 ± 5[6]
Insulin sensitivity				
Glucose infusion rate (mg·kg^{-1}·min^{-1})	2.33 ± 0.21[5]	3.62 ± 0.32[5]	5.42 ± 0.53[6]	5.41 ± 0.64[6]
$S_{I(clamp)}$ ([dL·(min·kg)$^{-1}$·(μU/mL)$^{-1}$] × 10^4)	3.45 ± 0.40[5]	6.03 ± 0.77[5]	9.54 ± 1.24[6]	8.93 ± 1.49[6]
REE				
REE (MJ/d)	7.19 ± 0.16	6.60 ± 0.16	6.67 ± 0.29	6.73 ± 0.21
REE/kg body wt (kJ·kg^{-1}·d^{-1})	95.4 ± 2.2	93.3 ± 2.1	91.6 ± 3.0	91.6 ± 3.0
% of predicted REE (%)	107.6 ± 1.8[5]	103.3 ± 1.8	103.0 ± 3.0	102.5 ± 2.4
Prevalence of hypermetabolism (n [%])	5 [19]	2 [7]	2 [9.5]	0 [0]
Respiratory quotient	0.79 ± 0.01	0.79 ± 0.01	0.81 ± 0.01	0.81 ± 0.01
Basal glucose oxidation (mg·kg^{-1}·min^{-1})	0.95 ± 0.16	1.02 ± 0.16	1.20 ± 0.18	1.21 ± 0.19
Basal lipid oxidation (mg·kg^{-1}·min^{-1})	1.02 ± 0.06	0.94 ± 0.06	0.84 ± 0.07	0.85 ± 0.07

[1] $S_{I(clamp)}$, clamp-related index of insulin sensitivity; REE, resting energy expenditure.
[2] Number of diabetic patients in brackets.
[3] $\bar{x}$ ± SEM.
[4] Seven patients were not taking prednisone.
[5] Significantly different from healthy subjects, $P < 0.03$ (*see* Table 1).
[6] Significantly different from before LTx, $P < 0.05$ (ANOVA and Tukey's post hoc test).

LTx. The anthropometric, laboratory, and clinical characteristics of these study groups are shown in **Table 2**.

The potential effect of diabetes on REE before LTx was also analyzed in the 26 patients with liver cirrhosis by comparing those with diabetes (n = 16) with those without (n = 10). All subjects who underwent the experimental procedures were fully informed of the possible risk of the study and gave their consent. The experimental protocol was approved by the Ethical Committee of the Istituto Scientifico H San Raffaele.

Experimental protocol

Subjects were instructed to consume an isoenergetic diet and to abstain from exercise activity for 3 wk before the studies. At the time of the studies, patients with diabetes were being treated with diet or insulin and were not receiving oral hypoglycemic agents. The use of drugs with potential metabolic effects was discontinued for a washout period of 3 d, and patients with diabetes were instructed to receive the last doses of intermediate- and short-acting insulin 24 and 12 h, respectively, before the study. Administration of immunosuppressive drugs was withheld on the morning of the study. At 0700 on the fourth day, subjects were admitted to the Metabolic Unit of the Division of Internal Medicine I of the Istituto Scientifico H San Raffaele after a 10-h overnight fast, and the following procedures were performed.

Indirect calorimetry

After subjects had lain quietly for 30 min, REE was measured for 45 min by continuous indirect calorimetry with a ventilated hood system (SensorMedics 2900 Metabolic Measurement Cart; SensorMedics, Milano, Italy) as previously described (7). The mean (±SE) CV within the session for both oxygen (1.6 ± 0.2%) and carbon dioxide (1.9 ± 0.3%) measurements were <2%, with ranges of 0.3–4.8% and 0.4–5.6% for oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), respectively. In our Metabolic Unit, the mean daily CV for REE in 15 healthy subjects, 10 patients with liver cirrhosis, and 10 patients with liver transplants assessed in 1999–2000 was 3.5 ± 0.3% for $\dot{V}O_2$ and 4.1 ± 0.5% for $\dot{V}CO_2$. Hypermetabolism was defined as previously described (1) as a measured REE ≥20% of the predicted value. Normometabolic patients were within the range of ±20% of the predicted value.

Euglycemic-hyperinsulinemic clamp

When indirect calorimetry was performed, a polytetrafluoroethylene catheter was inserted into an antecubital vein for infusions, and an additional catheter was retrogradely inserted into a wrist vein for blood sampling. The subject's hand was kept in a heated box (50 °C) throughout the experiment to allow sampling of arterialized venous blood. Blood samples for measurement of postabsorptive plasma glucose and free insulin were obtained in triplicate. Thereafter, a euglycemic-hyperinsulinemic clamp was performed as previously described (7, 13). Insulin was infused at 40 mU·m^{-2}·min^{-1}) to reach a plasma insulin concentration of ≈400 pmol/L, and the plasma glucose concentration was kept at 5 mmol/L for 150 min by variable infusion of a 20% (by vol) dextrose solution. Blood samples for plasma insulin and glucose were drawn every 15 min throughout the study.

Analytic procedures

Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA; 7), and the mean (±SE) CV was 1.7 ± 0.1% and 3.0 ± 0.4% in the fasting and clamp conditions, respectively. Plasma free insulin was measured as previously described (7), and the mean CV was 12.2 ± 1.7% and 5.7 ± 0.7% in the fasting and clamp conditions, respectively. The Kjeldahl method (14) was used to measure urine nitrogen in 24-h urine samples that the patients collected on the previous day.

Calculations

Indirect calorimetry

REE was calculated from the oxygen consumption and carbon dioxide production rates measured by means of indirect calorimetry (excluding the first 10 min of data acquisition) and from the urinary nitrogen excretion by using Weir's standard equation (15). Predicted REE was calculated by using the Harris-Benedict equations (16). Glucose, lipid, and protein oxidation were estimated as previously described (17).

Insulin sensitivity

The steady-state glucose infusion rate (GIR) was measured during the 120–150 min of the insulin clamp and was expressed as mg · kg body wt^{-1} · min^{-1}; the mean (±SE) CV was 4.9 ± 0.5%. The clamp-related index of insulin sensitivity ($S_{I(clamp)}$) was calculated as GIR/($\Delta I \times G$), where ΔI is the change in plasma insulin concentration during the last 30 min of the clamp from that of baseline and G is the plasma glucose concentration during the same interval (18).

Statistical analysis

All data are presented as means ± SEMs. With the use of standard linear regression, steady state was defined as a nonsignificant correlation of the variable with time ($P > 0.05$). Relations between variables were assessed by using linear regression analysis. In the longitudinal study of patients with liver cirrhosis, values before and after LTx were compared by using Student's paired t test, and the P values were Bonferroni corrected to adjust for the fact that the means were also used for comparison with the means for CU patients and healthy subjects. Values for the groups in the cross-sectional study were compared by using one-way analysis of variance and Tukey's post hoc test.

Measured REE as a percentage of predicted REE was stratified into 6 subgroups (I: ≥120%; II: ≥110% but <120%; III: ≥100% but <110%; IV: ≥90% but <100%; V: ≥80% but <90%; VI: <80%) within the 26 patients with liver cirrhosis, within the 65 transplant patients (regardless of the age of the transplant), and within the 27 control subjects (CU patients and healthy subjects). Differences in anthropometric variables, $S_{I(clamp)}$, and basal glucose and lipid oxidation between the members of each of the 3 groups (ie, the patients with liver cirrhosis, the LTx patients, and the control subjects) and between all the study subjects pooled together in subgroups I, II, III, and IV were assessed by using one-way analysis of variance and Tukey's test for post hoc comparisons. A trend effect of REE classes was also investigated by using the general linear models procedure. All analyses were performed with the use of SAS 6.12 (SAS Institute Inc, Cary, NC).

RESULTS

Prevalence of hypermetabolism in study groups

In the patients with liver cirrhosis, the prevalence of hypermetabolism did not differ between those with (3 of 16; 19%) and without (2 of 10; 20%) diabetes. In the LTx patients studied 6, 14, and 32 mo after LTx, the prevalence of hypermetabolism was 7% (2 of 28), 9.5% (2 of 21), and 0%, respectively. No hypermetabolic individuals were found among the CU patients and healthy subjects. Two LTx patients were hypometabolic with normal thyroid function.

Effect of liver transplantation: longitudinal study

Anthropometric and laboratory characteristics of the study subjects

In comparison with healthy subjects matched for anthropometric characteristics, the cirrhotic patients before LTx were characterized by postabsorptive hyperglycemia ($P < 0.01$) and hyperinsulinemia ($P < 0.01$). After LTx, these patients had significant improvements in plasma glucose ($P < 0.05$) and insulin concentrations ($P < 0.01$) (Table 1).

Insulin sensitivity

The patients with liver cirrhosis had significantly lower values for GIR and $S_{I(clamp)}$ than did the healthy subjects ($P < 0.03$; Table 1), which reflected a marked insulin resistance with respect to glucose metabolism in the patients; this insulin resistance significantly improved 9 mo after LTx ($P < 0.01$) but was still significantly higher than that of the healthy subjects ($P < 0.03$).

Resting energy expenditure and substrate oxidation rates

The groups did not differ significantly in REE; nevertheless, measured REE values as a percentage of predicted REE (predicted on the basis of the classical Harris-Benedict equations) were slightly but significantly higher in the cirrhotic patients than in the healthy subjects ($P < 0.03$; Table 1). After LTx, REE was not completely normalized in comparison with that of healthy subjects ($P = 0.05$). In addition, either before or after LTx, patients with liver cirrhosis showed a trend for lower glucose oxidation ($P = 0.08$) with a parallel trend for higher lipid oxidation ($P = 0.06$).

Effect of liver transplantation: cross-sectional study

Anthropometric and laboratory characteristics of the study subjects

The study groups did not differ significantly in anthropometric variables (Table 2). The laboratory features of the patients with liver cirrhosis were comparable to those observed in the longitudinal study: in comparison with the healthy subjects, the cirrhotic patients had postabsorptive hyperglycemia ($P < 0.03$) and hyperinsulinemia ($P < 0.03$). In the patients who were studied 6 mo after LTx, fasting plasma glucose concentrations were not significantly different from those of the control subjects ($P = 0.07$) even though 7 of the patients still had diabetes. The plasma free insulin concentrations of the patients who were studied 6 mo after LTx were significantly lower than those of the patients who were studied before LTx ($P < 0.05$) but were not significantly different from those of the healthy subjects. In patients studied 14 and 32 mo after LTx, plasma glucose and free insulin concentrations were not significantly different from those of the healthy subjects ($P = 0.15$ and 0.27, respectively).

Insulin sensitivity

The patients with liver cirrhosis had significantly lower values for GIR and $S_{I(clamp)}$ than did the healthy subjects ($P < 0.03$; Table 2), which reflected a marked insulin resistance in the

TABLE 3
Effect of diabetes in patients with liver cirrhosis: anthropometric, laboratory, and clinical characteristics[1]

	Patients with liver cirrhosis	
	Diabetic (n = 1 F, 15 M)	Nondiabetic (n = 1 F, 9 M)
Anthropometric variables		
Age (y)	54 ± 2[2]	50 ± 2
Body wt (kg)	77 ± 2	75 ± 4
Height (cm)	173 ± 2	172 ± 2
BMI (kg/m^2)	26.1 ± 0.9	25.1 ± 1.1
Laboratory variables		
Fasting plasma glucose (mmol/L)	7.90 ± 0.54[3]	5.00 ± 0.15
Fasting plasma free insulin (pmol/L)	130 ± 16	116 ± 20
Insulin sensitivity		
Glucose infusion rate (mg·kg^{-1}·min^{-1})	1.92 ± 0.41	2.97 ± 0.61
$S_{I(clamp)}$ ([dL·(min·kg)$^{-1}$·(μU/mL)$^{-1}$] × 10^4)	2.93 ± 0.88	4.25 ± 0.99
REE		
REE (MJ/d)	7.14 ± 0.22	7.25 ± 0.24
REE/kg body wt (kJ·kg^{-1}·d^{-1})	94.1 ± 2.5	97.5 ± 3.8
% of predicted REE (%)	107.6 ± 2.2	107.6 ± 3.1
Prevalence of hypermetabolism (n [%])	3 [19]	2 [20]
Respiratory quotient	0.78 ± 0.01	0.80 ± 0.01
Basal glucose oxidation (mg·kg^{-1}·min^{-1})	0.79 ± 0.22	1.20 ± 0.22
Basal lipid oxidation (mg·kg^{-1}·min^{-1})	1.07 ± 0.09	0.96 ± 0.08

[1] $S_{I(clamp)}$, clamp-related index of insulin sensitivity; REE, resting energy expenditure. The cirrhotic patients with or without diabetes described in this table are the same as those described in Table 2 under the heading "Before LTx."
[2] $\bar{x}$ ± SEM.
[3] Significantly different from nondiabetic, $P < 0.01$ (Student's unpaired t test and Bonferroni correction).

patients. The patients who were studied 6 mo after LTx had $S_{I(clamp)}$ values that were significantly lower than those of the healthy subjects ($P < 0.03$). In the patients who were studied 14 or 32 mo after LTx, $S_{I(clamp)}$ was not significantly different from that of healthy subjects ($P = 0.72$ and 0.61, respectively). GIR values showed a parallel behavior.

Resting energy expenditure and substrate oxidation rates

The groups did not differ significantly in REE; nevertheless, measured REE values as a percentage of predicted REE were slightly but significantly higher in the cirrhotic patients than in the healthy subjects ($P < 0.03$; Table 2). In the patients who were studied 6 mo after LTx, measured REE as a percentage of predicted REE was not significantly different from that of the healthy subjects ($P = 0.06$) or that of the patients who were studied 14 ($P = 0.287$) or 32 ($P = 0.08$) mo after LTx. In association with these results, oxidative substrate disposal was not significantly different between the groups, even though there were nonsignificant trends for higher lipid oxidation ($P = 0.07$) and lower glucose oxidation ($P = 0.08$) in the patients with liver cirrhosis.

Effect of immunosuppressive drugs

The CU patients were not significantly different from the other study groups in terms of anthropometric variables (Table 1). The CU patients were characterized by normal fasting concentrations of plasma glucose but were slightly hyperinsulinemic in comparison with the healthy subjects ($P = 0.05$). GIR and $S_{I(clamp)}$ values were significantly lower in the CU patients than in the healthy subjects ($P = 0.09$ and 0.05, respectively). In the CU patients, the values for REE ($P = 0.96$), measured REE as a percentage of predicted REE ($P = 0.47$), respiratory quotient ($P = 0.81$), and glucose ($P = 0.85$) and lipid oxidation ($P = 0.87$) were not significantly different from those of the healthy subjects. The CU patients were taking prednisone and cyclosporin A at doses similar to those taken by the LTx patients who were studied 6 mo after LTx. The LTx patients who were studied 14 and 32 mo after LTx were receiving similar doses of cyclosporin A but no prednisone.

Effect of liver cirrhosis and diabetes on resting energy expenditure

Among the patients with liver cirrhosis, those with diabetes did not differ significantly from those without diabetes in anthropometric variables (**Table 3**). The patients with diabetes had significantly higher fasting plasma glucose concentrations than did those without diabetes ($P < 0.01$); fasting plasma free insulin concentrations did not differ significantly between the 2 groups ($P = 0.58$). When either the GIR ($P = 0.09$) or, more properly, the $S_{I(clamp)}$ ($P = 0.11$) was used as a variable of a clamp-derived index of insulin sensitivity, there was a nonsignificant trend for insulin sensitivity to be lower in the diabetic patients than in the nondiabetic patients (Table 3). REE was not significantly different between the 2 groups when expressed as MJ/d ($P = 0.74$), as kJ · kg body wt^{-1} · d^{-1} ($P = 0.62$), or as a percentage of the predicted value ($P = 0.99$). Basal glucose oxidation and lipid oxidation were also not significantly different between the 2 groups ($P = 0.34$ and 0.36, respectively; Table 3).

Stratification

To further detect the metabolic features of the study groups with respect to different REE rates, the anthropometric variables, $S_{I(clamp)}$, and glucose and lipid oxidation in the postabsorptive state were summarized in **Table 4**, in which the subjects are classified on the basis of measured REE as a percentage of predicted REE as hypermetabolic (≥120%, subgroup I), normometabolic (<120% and ≥80%; subgroups II–V), and hypometabolic (<80%). The different subgroups did not differ significantly in anthropometric variables. When the results of all 118 subjects in the cross-sectional study were pooled together and analyzed, there were significant trends for $S_{I(clamp)}$ to be lower ($P < 0.003$) and for lipid oxidation to be higher ($P < 0.0001$) in subjects with higher values of measured REE as a percentage of predicted REE. When the analysis was performed within each of the 3 main groups shown in Table 4, measured REE as a percentage of predicted REE was significantly associated with lipid oxidation ($P < 0.05$) in the LTx patients.

Regression analysis

To further test the hypothesis that hypermetabolism in liver cirrhosis was associated with insulin resistance, we plotted the data from all of the subjects who participated in the cross-sectional study and performed a simple regression analysis that showed that measured REE as a percentage of predicted REE (**Figure 1**) was inversely associated with $S_{I(clamp)}$ ($R^2 = 0.119$, $P = 0.0002$).

DISCUSSION

The present study is the first study in which postabsorptive energy homeostasis, substrate oxidation, and insulin sensitivity were simultaneously monitored in a longitudinal fashion in patients with liver cirrhosis before and after LTx; the study also includes the longest cross-sectional follow-up study (32 mo) of these variables. The results indicate that elevated REE in patients

TABLE 4
Stratification of the study subjects on the basis of measured resting energy expenditure (REE) as a percentage of predicted REE[1]

Patient group and subgroup	REE		Frequency	Percentage of predicted REE	Age	BMI	Age of transplant	$S_{I(clamp)}$	Basal GOx	Basal LOx
	Description	Percentage of predicted								
		%	*n* (%)	%	*y*	kg/m^2	*d*	$[dL \cdot (min \cdot kg)^{-1} \cdot (\mu U/mL)^{-1}] \times 10^4$	$mg \cdot kg^{-1} \cdot min^{-1}$	$mg \cdot kg^{-1} \cdot min^{-1}$
Patients with liver cirrhosis ($n = 26$)[2]										
I	Hypermetabolic	≥120	5 (19.2)	124 ± 1[3]	51 ± 3	25.7 ± 2.8	—	3.2 ± 0.2	1.56 ± 0.38	1.07 ± 0.08
II	Normometabolic	≥110 but <120	5 (19.2)	114 ± 1	53 ± 3	26.1 ± 1.2	—	3.7 ± 0.7	1.36 ± 0.49	0.95 ± 0.21
III		≥100 but <110	13 (50.1)	105 ± 1	52 ± 1	25.6 ± 1.0	—	4.1 ± 0.6	0.71 ± 0.19	1.08 ± 0.08
IV		≥90 but <100	3 (11.5)	94 ± 2	58 ± 6	25.8 ± 0.3	—	3.9 ± 0.8	0.80 ± 0.40	0.84 ± 0.14
V		≥80 but <90	0 (0)	—	—	—	—	—	—	—
VI	Hypometabolic	<80	0 (0)	—	—	—	—	—	—	—
LTx patients ($n = 65$)[4]										
I	Hypermetabolic	≥120	4 (6.1)	125 ± 2	57 ± 3	24.3 ± 2.3	403 ± 68	5.8 ± 0.9	1.37 ± 0.26	1.13 ± 0.11[a]
II	Normometabolic	≥110 but <120	9 (13.8)	115 ± 1	55 ± 2	23.9 ± 0.9	410 ± 94	7.9 ± 2.7	1.26 ± 0.32	1.03 ± 0.12[a]
III		≥100 but <110	21 (32.3)	105 ± 1	52 ± 3	24.5 ± 1.1	376 ± 114	7.4 ± 1.1	1.09 ± 0.23	0.94 ± 0.08[b]
IV		≥90 but <100	20 (31.0)	96 ± 1	53 ± 3	25.7 ± 1.3	485 ± 128	8.4 ± 1.4	0.92 ± 0.30	0.85 ± 0.11[b]
V		≥80 but <90	9 (13.8)	87 ± 1	45 ± 4	24.5 ± 1.3	507 ± 148	6.9 ± 1.9	1.17 ± 0.31	0.66 ± 0.11[c]
VI	Hypometabolic	<80	2 (3.0)	76 ± 1	45 ± 2	25.8 ± 1.1	435 ± 184	7.0 ± 0.8	0.75 ± 0.00	0.56 ± 0.02[c]
CU patients and healthy subjects ($n = 27$)[5]										
I	Hypermetabolic	≥120	0 (0)	—	—	—	—	—	—	—
II	Normometabolic	≥110 but <120	3 (11.1)	115 ± 1	43 ± 5	23.4 ± 0.8	—	7.3 ± 2.1	1.42 ± 0.01	1.00 ± 0.03
III		≥100 but <110	7 (25.9)	104 ± 1	48 ± 5	28.3 ± 2.0	—	11.8 ± 1.6	1.36 ± 0.13	0.88 ± 0.07
IV		≥90 but <100	14 (51.8)	95 ± 1	44 ± 3	26.2 ± 1.0	—	9.8 ± 1.2	1.33 ± 0.13	0.77 ± 0.04
V		≥80 but <90	3 (11.1)	84 ± 1	35 ± 1	24.8 ± 1.2	—	9.9 ± 0.8	1.28 ± 0.08	0.70 ± 0.07
VI	Hypometabolic	<80	0 (0)	—	—	—	—	—	—	—

[1] $S_{I(clamp)}$, clamp-related index of insulin sensitivity; GOx, glucose oxidation; LOx, lipid oxidation; LTx, liver transplantation; CU, chronic uveitis. When all 118 subjects were pooled together and analysed cross-sectionally by using the general linear models procedure, there were significant trends for $S_{I(clamp)}$ ($P < 0.003$) and LOx ($P < 0.001$) across subgroups of measured REE as a percentage of predicted REE. Values in the same column with different superscript letters are significantly different, $P < 0.05$ (ANOVA and Tukey's test).

[2] Complete anthropometric and metabolic features of the patients with liver cirrhosis are shown in Table 2 (Before LTx) and in Table 3, in which the 16 patients with type 2 diabetes are compared with the 10 patients without diabetes.

[3] $\bar{x} \pm$ SEM.

[4] Complete anthropometric and metabolic features of LTx patients are shown in Table 2, in which the patients are shown with respect to the time of follow-up after LTx (6, 14, and 32 mo).

[5] Complete anthropometric and metabolic features of CU patients and healthy subjects are shown in Table 1.

with liver cirrhosis is associated with reduced insulin action on glucose metabolism, suggesting that insulin resistance may play a role in the pathogenesis of hypermetabolism in cirrhotic patients. To sustain this conclusion, we found that the higher REE characterizing the pretransplant condition progressively decreased after LTx and was associated with a progressive improvement in insulin sensitivity and that measured REE as a percentage of predicted REE was inversely associated with $S_{I(clamp)}$ (Figure 1). In addition, when we compared the patients with liver cirrhosis on the basis of the presence or absence of diabetes, we also showed that diabetes per se was not associated with measured REE as a percentage of predicted REE.

The data presented in the present study confirm that liver cirrhosis is also characterized by higher-than-normal REE (1) in patients with a mild form of the disease or with hepatocarcinoma because all the patients in this study were classified as Child-Pugh class A or B. The prevalence of true hypermetabolism in this group of cirrhotic patients was 19% (Tables 2 and 4), which is probably not significantly different from the prevalence (28–29%) in the Child A cirrhotic patients with hepatocarcinoma described by Müller et al (1). Note that 91% of the cirrhotic patients had an REE higher than predicted, whereas this was true in only 38% of the control subjects (CU patients and healthy subjects), in whom true hypermetabolism was not found. In the 65 LTx patients, the prevalence of true hypermetabolism was 6%; yet, 52% of the patients had a measured REE higher than predicted (Table 4).

Substrate oxidation rates in the postabsorptive state were also affected. In fact, we noticed that in the 26 patients with liver cirrhosis, the postabsorptive lipid oxidation rate, on average, contributed 62% of the REE (data not shown), and this contribution was higher than in the healthy subjects (50%); this finding was associated with a lower contribution of carbohydrate oxidation in

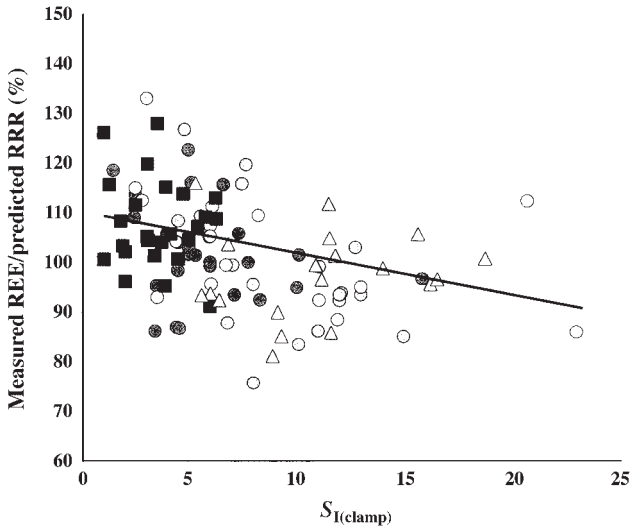

FIGURE 1. Linear regression analysis of data from the cross-sectional study in which measured resting energy expenditure (REE) as a percentage of predicted REE is plotted against the clamp-related index of insulin sensitivity [$S_{I(clamp)}$]. ■, patients with liver cirrhosis; ●, liver transplantation (LTx) patients who were studied 6 mo after LTx; ●, LTx patients who were studied 14 mo after LTx; ○, LTx patients who were studied 32 mo after LTx; △, healthy subjects. $R^2 = 0.119$, $P = 0.0002$.

the cirrhotic patients than in the healthy subjects (22% compared with 34%; data not shown), as previously described in patients with more advanced stages of liver disease (19, 20). In addition, higher postabsorptive lipid oxidation rates were significantly associated with hypermetabolism when data from all of the subjects who participated in the cross-sectional study were pooled together (Table 4). Therefore, in agreement with the results of previous studies, higher lipid oxidation seemed to play a role in the development of hypermetabolism; nevertheless, in the cirrhotic patients with measured REE > 110% of predicted REE, hypermetabolism was also paralleled by normal-to-higher carbohydrate oxidation rates (Table 4), suggesting that in these patients the oxidative pathways are deeply affected at the level of both glucose and fatty acid oxidative disposal.

The pathogenesis of hypermetabolism in liver cirrhosis is still unknown, but several factors have been suggested to be involved: portal hypertension, systemic inflammation, and increased sympathetic nervous system activity (1, 3–5). The results of the present study suggest that insulin resistance may also be causative. In this study, insulin sensitivity with respect to glucose metabolism was measured by using the gold standard technique, the euglycemic-hyperinsulinemic clamp: the patients with liver cirrhosis had dramatically lower $S_{I(clamp)}$ values than did the healthy subjects, and these values were significantly associated with the severity of the hypermetabolic state (Figure 1). The observation that in the other human model of marked insulin resistance without liver injury, ie, obesity, hypermetabolism was not prevalent and insulin sensitivity was not associated with resting thermogenesis (21) would suggest that the relation between the hypermetabolic state and insulin resistance is peculiar to liver cirrhosis.

Further support for this view comes from the data obtained after LTx, which were analyzed by using both the longitudinal and cross-sectional approaches. The longitudinal study showed that 9 mo after LTx, the cirrhotic patients were still characterized by higher-than-normal measured REE/predicted REE (22), and this was paralleled by the persistence of insulin resistance with respect to glucose metabolism. The cross-sectional study showed that in the patients studied 14 or 32 mo after LTx, the complete restoration of insulin sensitivity was associated with measured REE/predicted REE values that were not significantly different from those in the healthy subjects.

Other metabolic and nonmetabolic variables may have a profound impact on REE. Development of hepatogenous diabetes is common in patients with liver cirrhosis. In fact, 45% of the patients who participated in the longitudinal study and 35% of those who participated in the cross-sectional study were diagnosed as having diabetes. In addition, LTx patients, especially within the first 12 mo after the transplant, showed some prevalence of diabetes (27% in the longitudinal study and 25%, 14%, and 3% in the patients who were studied 6, 14, and 32 mo, respectively, after LTx in the cross-sectional study). Because diabetes per se usually induces an insulin-resistant state (23), the presence of diabetes may further worsen the alteration in REE in cirrhotic patients; however, in the present study REE was not significantly higher in the diabetic patients than in the nondiabetic patients (Table 3), suggesting that diabetes per se is not involved in the hypermetabolic state and that insulin resistance represents the major adverse metabolic event associated with elevated REE.

The patients with liver cirrhosis were also affected by hepatocellular carcinoma, and for this reason they were eligible for LTx, which was previously shown to provide a better prognosis than that provided by liver resection (24). An independent cancer-related role in elevated REE is unlikely because no patients had disseminated disease at the time of the study and because patients with other chronic hepatic diseases without the associated development of hepatocarcinoma were shown to develop hypermetabolism as well. Nevertheless, this possibility may not be excluded.

Immunosuppressive drugs used to avoid graft rejection might also be involved in the alterations in REE in LTx patients, and this effect might also be dose dependent. We therefore selected and studied a group of CU patients who were undergoing an immunosuppressive regimen similar to that of LTx patients but who were without a systemic disease: the CU patients were selected to be similar to the study subjects in terms of drug therapy, anthropometric features, and lifestyle. We found that these patients did not differ significantly from the healthy subjects in terms of REE and fuel oxidative partitioning (Table 1). The combined administration of prednisone and cyclosporine A were associated only with a mild degree of insulin resistance, and prednisone in particular may be responsible for this defect. The absence of a significant effect on REE suggests that these drugs may have only a minor role, if any, on the persistence of higher than predicted REE in cirrhotic patients after LTx.

In conclusion, the results of this study show that hypermetabolism in liver cirrhosis complicated by hepatocarcinoma may be partly explained by insulin resistance and that the presence of diabetes does not play a major additional role in elevated REE. Using both the longitudinal and cross-sectional approaches, we found that the metabolic profile of LTx patients who were studied 32 mo after LTx progressively improved in terms of insulin action and postabsorptive REE despite the use of immunosuppressive drugs. The data suggest that monitoring of both energy homeostasis and insulin action in patients with liver cirrhosis before LTx and during the first months after LTx is mandatory for designing a program

of nutritional and pharmacologic support to prevent malnutrition and metabolic complications in these patients.

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