Impaired Nonoxidative Glucose Metabolism in Patients With Liver Cirrhosis: Effects of Two Insulin Doses

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Glucose intolerance is encountered in the majority of cirrhotic patients. This alteration has been attributed to a defective insulin-mediated glucose uptake in peripheral tissue, where nonoxidative glucose disposal seems to be chiefly impaired. To further investigate insulin action under euglycemic conditions, we studied how physiological (100 μU/mL) and pharmacological (1,000 μU/mL) plasma insulin concentrations affect whole-body insulin-mediated glucose uptake, as well as oxidative and nonoxidative glucose disposal, in cirrhotic patients and controls. To this aim, a sequential two-step insulin euglycemic clamp combined with indirect calorimetry was performed in eight cirrhotic patients and six control subjects. During the first step of the clamp, total glucose uptake was reduced by 40% in cirrhotic patients versus controls (4.42 ± 1.39 *v* 7.63 ± 1.60 mg/kg/min, $P = .002$). By increasing insulin to pharmacological levels, glucose disposal increased in both groups. However, the maximum rate of glucose metabolism achieved in cirrhotic patients was lower than in controls at all times (10.29 ± 2.04 *v* 12.82 ± 0.51 mg/kg/min, $P = .012$). Glucose oxidation was lower in cirrhotics in the basal state, but similar in both groups during insulin/glucose infusion. On the other hand, the reduced nonoxidative glucose disposal observed in cirrhotic patients was not normalized even by increasing insulin to pharmacological levels. In conclusion, in liver cirrhosis a reduced insulin sensitivity is associated with a reduced insulin responsiveness that is mainly caused by defective nonoxidative glucose disposal.

AS MANY AS 70% of cirrhotic patients have impaired glucose tolerance, and of these, 10% to 20% eventually develop overt diabetes,[1] which has recently been recognized as a risk factor for long-term survival in cirrhosis.[2]

Although the underlying mechanisms of glucose intolerance in liver cirrhosis are not yet completely clarified, several studies have shown that the main alterations occurring in these patients concern glucose handling in peripheral tissues.[3] In fact, while insulin levels in cirrhotic patients are increased both in the basal state and after a glucose load,[4] insulin-mediated glucose uptake quantified using the hyperinsulinemic-euglycemic clamp technique has been repeatedly shown to be reduced.[5-7]

During hyperinsulinemic-euglycemic clamp studies, 85% of the glucose infused is taken up by muscle tissue.[8] In this tissue, 30% to 40% of glucose is oxidized and the remaining 60% to 70% is accounted for by nonoxidative pathways, primarily represented by glycogen formation.[9] By combining an insulin clamp with indirect calorimetry, the total glucose disposal and glucose oxidation rate can be measured and the nonoxidative glucose disposal calculated. Using these techniques, the glucose oxidation rate has been found to be normal in cirrhotic patients, while a defective nonoxidative glucose metabolism appears to be the main factor contributing to insulin resistance.[5-7] In normal subjects, when progressively higher levels of insulin are infused to increase glucose disposal, glucose oxidation increases until it reaches a plateau at a plasma insulin level of approximately 100 μU/mL. If glucose utilization is further augmented by increasing insulin to pharmacological levels, only nonoxidative glucose disposal continues to increase, becoming the most important route of glucose disposal.[9]

Information is lacking on the influence of pharmacological plasma insulin concentrations under euglycemic conditions on oxidative and nonoxidative glucose metabolism in cirrhotic patients. The purpose of the present study was to examine whether the reduced nonoxidative glucose metabolism in cirrhotic patients may be normalized at high insulin concentrations.

SUBJECTS AND METHODS

Patients

Eight patients with biopsy-proven liver cirrhosis (seven men and one woman, aged 54 ± 12 years) were studied. All patients were hospitalized and underwent metabolic study while in stable clinical condition. The severity of liver disease was assessed at admission according to the Child-Pugh score.[10] Characteristics of the patients are reported in Table 1.

None of the patients presented with overt diabetes requiring oral hypoglycemic agents or insulin therapy. Glucose tolerance evaluated by a 75-g oral glucose tolerance test was normal in three patients and impaired in five according to the World Health Organization classification criteria. In two patients, ascites that was present upon admission to hospital was treated with diuretic therapy before study. All medications that could interfere with glucose metabolism were suspended for at least the previous 3 days. Patients were following a balanced diet with no protein restriction. Six subjects hospitalized for minor problems with normal liver-function tests were studied as controls. None had significant weight change or were affected by any metabolic or endocrine diseases. Age and anthropometry were comparable in patients and controls at the time of study (Table 2). The purpose of the study was explained to all subjects before informed consent was obtained. The study was approved by the local ethics committee.

Protocol

All patients were studied after an overnight fast between 8 and 9 AM in a specially adapted room with controlled temperature (24°C). A polyethylene catheter was inserted into an antecubital vein for infusion of human insulin (Actrapid HM; Novo Nordisk, Copenhagen, Denmark) and 20% glucose solution. For blood sampling, a second catheter was inserted retrogradely into the wrist vein and kept patent with a saline infusion (~250 mL throughout the study). The patient's hand was kept in a heated box at a constant temperature of about 60°C to achieve venous blood arterialization. A hyperinsulinemic-euglycemic clamp was performed as previously described[11] according to the following

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Submitted October 11, 1996; accepted January 24, 1997.

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0026-0495/97/4607-0021$03.00/0

Table 1. Clinical Characteristics of the Cirrhotic Patients

Patient No.	Origin of Cirrhosis	Child-Pugh Score (class)	ALT (mU/mL)	Bilirubin (mg/dL)	Albumin (g/dL)
1	HBV-HCV	10 (B)	53	2.9	3
2	HCV	5 (A)	48	0.9	3.7
3	HCV	8 (B)	80	1.4	2.9
4	Wilson	9 (B)	64	0.9	2.6
5	HBV-HCV	6 (A)	60	0.4	3.2
6	HCV	9 (B)	45	2.3	3.4
7	HBV	11 (C)	95	1.3	3.1
8	HCV	5 (A)	35	1.1	3.4

NOTE. Normal values: ALT, <40 mU/mL; bilirubin, <1.1 mg/dL; albumin, >3.5 g/dL.

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

protocol. During the first 100 minutes, peripheral insulin concentration was increased to approximately 100 μU/mL by a primed continuous infusion of human insulin (1.0 mU/kg/min). During the second 100 minutes, insulin infusion was increased to a rate of 10 mU/kg/min to increase insulin concentration to approximately 1,000 μU/mL. A 10-minute insulin priming was performed at the beginning of each step to speed equilibration of insulin plasma levels. Plasma glucose concentration was clamped at the fasting level throughout the study by adjusting the glucose infusion rate according to plasma glucose determinations performed every 5 minutes. Arterialized venous blood was also collected in the basal state and at 20-minute intervals for determination of serum insulin. Plasma free fatty acid levels were measured in the basal state and at 80, 100, 180, and 200 minutes. Serum urea was assayed before and at the end of each step of the study. To determine total urinary nitrogen and glucose excretion, a timed urine collection were obtained before and during the study.

Indirect calorimetry measurements were performed in the basal state (30 minutes before insulin/glucose infusion) and during the steady state of each step of insulin infusion (80 to 100 minutes, first step, and 180 to 200 minutes, second step) using a metabolic measurement cart (Sensor Medics MMC Horizon, Anaheim, CA) with a canopy system as previously described.[12,13]

Methods

Plasma and urinary glucose were determined with a Beckman (Irvine, CA) glucose analyzer using a glucose oxidase method. Plasma insulin was determined by radioimmunoassay. Spectrophotometric methods were used to measure plasma free fatty acid and urea nitrogen levels. Total urinary nitrogen excretion was assessed in triplicate using an automatic nitrogen analyzer (Fisons NC 1500; Carlo Erba Instruments, Milan, Italy), based on the method of Duncan, on approximately 50 mg urine weighted on thin capsule. Atropine (4.84% nitrogen) was used as a standard. Mean oxygen consumption, carbon dioxide production, and urinary nitrogen excretion were used to calculate energy expenditure and the nonprotein respiratory quotient (npRQ). The urinary nitrogen excretion rate was corrected taking into account the modifications in serum urea nitrogen.[14] Total glucose metabolism was calculated at 60- to 100- and 160- to 200-minute intervals. Hepatic glucose production was not measured in the present study and was presumed to be completely suppressed by insulin infusion at each step both in cirrhotics and in controls, as previously reported.[8,15] Glucose and lipid oxidative disposal rates were calculated with the npRQ (with appropriate corrections when npRQ was >1) using the MMC built-in computer. Nonoxidative glucose disposal was calculated by subtracting the rate of glucose oxidation from the rate of total glucose metabolism. Student's *t* test for paired and unpaired data was used in statistical analysis of the results. All data are expressed as the mean ± SD.

Table 2. Age and Anthropometric Parameters of the Subjects

Parameter	Cirrhotic Patients	Controls
Sex (M/F)	7/1	4/2
Age (yr)	54 ± 12	49 ± 5
Weight (kg)	68 ± 9	66 ± 12
Height (cm)	169 ± 9	172 ± 9
Body mass index	24 ± 2	22 ± 2

NOTE. Results (except sex) are the mean ± SD.

RESULTS

Plasma glucose and insulin concentrations and total glucose disposal before and during the steady-state period of the two-step hyperinsulinemic glucose clamp are reported in Table 3. Basal glucose levels were similar in cirrhotics and controls (90 ± 3 mg/dL in cirrhotics *v* 93 ± 5 in controls, NS) and were maintained at the same level throughout the study (Table 3). During the whole study, the coefficient of variation for plasma glucose concentration was less than 5% in each subject. Basal insulin levels were significantly higher in cirrhotic patients (21 ± 11 μU/mL in cirrhotics *v* 8 ± 2 in controls, $P < .05$). Insulin levels increased both in cirrhotics and in controls during the two-step insulin infusion. Between 160 and 200 minutes, insulin levels tended to be higher in cirrhotic patients; however, the difference was not significant (1,332 ± 285 μU/mL in cirrhotics *v* 1,191 ± 102 in controls, NS). Total glucose disposal was significantly lower (−42%) in cirrhotics than in controls during the 1-mU/kg/min insulin infusion (Table 3). When insulin was infused at a rate of 10 mU/kg/min, glucose disposal increased significantly in both cirrhotics (+134%) and controls (+68%), but was still significantly lower (−20%) in the cirrhotic group ($P = .012$).

In the basal state, the glucose oxidation rate was lower in cirrhotics than in controls (0.92 ± 0.4 *v* 1.6 ± 0.6 mg/kg min, $P < .05$). During the study, it increased in both groups and was similar in cirrhotics and controls at both rates of insulin infusion. Individual rates of oxidative and nonoxidative glucose disposal plotted against the insulin concentration at each step of the clamp are reported in Fig 1. Nonoxidative glucose disposal was lower in cirrhotic patients than in controls at all insulin concentrations.

Resting energy expenditure and npRQ measurements are reported in Table 4. The npRQ was significantly lower in the basal state, indicating prevalent lipid utilization. During both steps of insulin infusion, npRQ tended to increase, and this increase was significantly higher in cirrhotics. When insulin was infused at 10 mU/kg/min, npRQ was greater than 1 in most cirrhotic patients, indicating net lipogenesis, a finding not observed in controls. The free fatty acid plasma level was significantly increased in cirrhotics compared with controls in the basal state (796 ± 298 mmol/L in cirrhotics *v* 442 ± 84 in controls, $P < .01$). However, these were suppressed normally during the first step (100 ± 48 *v* 63 ± 29 mmol/L, NS) and second step (75 ± 49 *v* 53 ± 25 mmol/L, NS) in both cirrhotics and controls.

DISCUSSION

We evaluated oxidative and nonoxidative glucose disposal in a group of patients with liver cirrhosis during a two-step insulin infusion euglycemic clamp. During the first 100 minutes,

Table 3. Blood Glucose and Insulin Levels and Total Glucose Disposal Before and During the Two-Step Hyperinsulinemic-Euglycemic Clamp

Group	Blood Glucose (mg/dL)			Insulin (μU/mL)			Total Glucose Disposal (mg/kg/min)	
	Basal	Step 1	Step 2	Basal	Step 1	Step 2	Step 1	Step 2
Cirrhotics	90 ± 3	91 ± 2	90 ± 1	21 ± 11*	104 ± 12	1,332 ± 285	4.42 ± 1.39†	10.29 ± 2.04‡
Controls	93 ± 5	92 ± 5	91 ± 4	8 ± 2	103 ± 11	1,191 ± 102	7.63 ± 1.60	12.82 ± 0.51

*$P = .04$.
†$P = .002$.
‡$P = .012$.

insulin was increased to 100 μU/mL, and from 100 to 200 minutes, to approximately 1,000 μU/mL. We found that total glucose utilization was lower in cirrhotic patients than in controls at both insulin levels. The glucose oxidation rate, which was reduced in cirrhotics in the basal state, increased to a similar level in both groups during the study. Nonoxidative glucose metabolism, which was lower in cirrhotics than in controls at 100 minutes, although augmented when insulin was further increased to 1,000 μU/mL, never reached the values observed in controls.

The insulin infusion rate used in the second step of the study (10 mU/kg/min) is among the highest doses in previous studies examining the relationship between insulin concentration and glucose disposal.[6,15-17] The insulin concentration achieved with this rate of insulin infusion is thought to produce the maximum rate of glucose metabolism in euglycemic conditions.[18] When insulin levels are increased to pharmacological levels in cirrhotic patients, a reduced responsiveness to insulin action has been reported,[15-17] ie, the maximum rate of glucose metabolism achieved at the highest rate of insulin infusion is lower in cirrhotic patients than in controls. The reduced insulin responsiveness in liver cirrhosis is confirmed by the present study, and evidence is provided that the alteration in nonoxidative glucose metabolism is not overcome even by inducing very high insulin levels (1,000 μU/mL). The exact mechanism causing a defective insulin action in cirrhotic patients is unknown. The dose-response curve observed in the present study and others can be explained both by impaired insulin binding of the hormone to its specific cell receptor, causing reduced glucose transport, and/or by an intracellular defect. Evaluation of insulin binding to peripheral blood cells or adipocytes of cirrhotic patients has yielded variable results, and even where observed, the defect was modest.[15,19,20] That the oxidative pathway of glucose metabolism is normal at high insulin concentration may suggest that in this condition glucose transport is maintained; however, some studies proposed that glucose transport is also affected in these patients.[21] Other abnormalities may also occur at postbinding intracellular sites. Glucose that is taken up but not oxidized in peripheral tissues is primarily used for synthesis of glycogen, but may also be converted to lipids or lactate.[22] Under physiological increments of insulin, glucose conversion to lipids is negligible and little of the glucose taken up by muscle is released as lactate.[18] However, in the present study when insulin was infused at the highest rate, we observed net lipogenesis in almost all cirrhotic patients, indicated by a npRQ greater than 1. In similar experimental conditions, net lipogenesis does not occur in normal subjects, as previously reported.[9] Measurement of lactate concentrations was not included in the present protocol. Muller et al,[5] after increasing insulin concentrations to 400 to 500 μU/mL in cirrhotic patients, observed a disproportionate increase in lactate concentration. Taken together, these observations may suggest that by elevating insulin to pharmacological levels and increasing intracellular glucose availability, only some lipid and/or lactate synthesis can be induced. With regard to glycogen storage, our data support the presence of a reduced capacity to store the administered glucose as glycogen in these patients. Some studies have examined this problem, and a reduced muscle glycogen synthase activity was actually found.[23] However, other studies failed to find a reduced muscle glycogen content even in cirrhotic patients of advanced stage.[21] Therefore, direct evidence that a reduced rate of glycogen synthesis is operative in cirrhotic patients is still lacking.

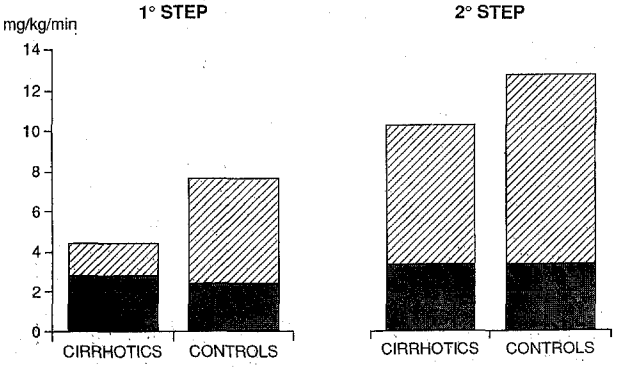

Fig 1. Oxidative (■) and nonoxidative (▨) glucose disposal in cirrhotic patients and controls. Data are the mean values obtained at steady state of the first (1.0 mU/kg/min, 1° step) and second (10 mU/kg/min, 2° step) insulin infusion during the euglycemic clamp.

A mechanism that may contribute to insulin resistance is a reduced glucose delivery to skeletal muscle owing to a defect in insulin-mediated vasodilatation.[24] A decreased effect of insulin to stimulate muscle blood flow has been found to occur in other insulin-resistant states such as obesity and type I diabetes mellitus.[25,26] This mechanism has not been specifically addressed in cirrhotic patients. However, the discrepancy observed in muscle glucose metabolism examined in vivo and in vitro in these patients[27] may suggest that a defect in microcirculation plays a role in the development of insulin resistance. On the other hand, it has been reported that leg blood flow, which is increased in cirrhotic patients in the basal state,[28,29] is similar in patients and controls during a euglycemic-hyperinsulinemic clamp study,[29] and a study in which muscle glucose uptake was measured by positron emission tomography[30] failed to support the hypothesis of "impaired capillary recruitment" as a cause of insulin resistance in patients with liver cirrhosis. Therefore, even though blood flow was not measured in this study, it seems unlikely that this mechanism could explain the reduction in insulin sensitivity and responsiveness observed in these patients.

Table 4. Resting Energy Expenditure and npRQ Before and During the Two-Step Hyperinsulinemic-Euglycemic Clamp

Group	Resting Energy Expenditure (kcal/min)			npRQ		
	Basal	Step 1	Step 2	Basal	Step 1	Step 2
Cirrhotics (n = 8)	0.96 ± 0.21	1.02 ± 0.22	1.13 ± 0.30	0.80 ± 0.05*	1.00 ± 0.05†	1.10 ± 0.07‡
Controls (n = 6)	1.09 ± 0.23	1.09 ± 0.20	1.15 ± 0.25	0.86 ± 0.05	0.90 ± 0.07	0.98 ± 0.05

*$P = .05$.
†$P = .01$.
‡$P = .004$.

The results of the present study suggest that in cirrhotic patients glucose oxidation is normally induced by hyperinsulinemia, but glucose storage is impaired. The impact of these observations on the fate of carbohydrates after a meal by cirrhotic patients should be evaluated with caution. In fact, after a meal, assuming complete absorption, the prolonged hyperglycemia associated with hyperinsulinemia may partially compensate for insulin resistance.[31] However, these compensatory mechanisms (ie, the mass effect of hyperglycemia and augmented insulin secretion) may become inadequate as time passes and cause cirrhotic patients to develop overt diabetes mellitus.[32,33]

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