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Ketosis Resistance in Fibrocalculous Pancreatic Diabetes: II. Hepatic Ketogenesis After Oral Medium-Chain Triglycerides

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A majority of patients with fibrocalculous pancreatic diabetes (FCPD) do not become ketotic even in adverse conditions. It is not clear whether this ketosis resistance is due to reduced fatty acid release from adipose tissue or to impaired hepatic ketogenesis. We tested hepatic ketogenesis in FCPD patients using a ketogenic challenge of oral medium-chain triglycerides (MCTs) and compared it with that in matched insulin-dependent diabetes mellitus (IDDM) patients and healthy controls. After oral MCTs, FCPD patients showed only a mild increase in blood 3-hydroxybutyrate (3-HB) concentrations (median: fasting, 0.13 mmol/L; peak, 0.52) compared with IDDM patients (fasting, 0.44; peak, 3.39) and controls (fasting, 0.04; peak, 0.75). Plasma nonesterified fatty acid (NEFA) concentrations were comparable in the two diabetic groups (FCPD: fasting, 0.50 mmol/L; peak, 0.79; IDDM: fasting, 0.91; peak, 1.04). Plasma C-peptide concentrations were low and comparable in the two diabetic groups. Plasma glucagon concentrations were higher in IDDM patients in the fasting state, but declined to levels comparable to those in FCPD patients after oral MCTs. Plasma carnitine concentrations were comparable in the two groups of patients. It is concluded that the failure to stimulate ketogenesis under these conditions could be partly due to inhibition of a step beyond fatty acid entry into the mitochondria.

FIBROCALCULOUS pancreatic diabetes (FCPD) is a peculiar type of diabetes seen in tropical developing countries.[1] It is secondary to tropical calcific pancreatitis and is classified as a subtype of malnutrition-related diabetes by the World Health Organization, but the role of nutritional factors in the pathogenesis of this disorder is debated.[2,3] A peculiar metabolic feature of FCPD patients is the resistance to development of ketosis even in adverse conditions. A number of hypotheses have been put forward to explain this phenomenon.[4-8] We have recently shown that ketosis resistance in these patients is multifactorial and that it is not fully explained by any one of the previous hypotheses.[9] There appears to be a contribution of both a diminished supply of nonesterified fatty acids (NEFAs) from adipose tissue and a diminished hepatic ketogenic response.

We tested hepatic ketogenesis in FCPD patients using the ketogenic stimulus of oral medium-chain triglycerides (MCTs), and compared it with that in insulin-dependent diabetes mellitus (IDDM) patients and control subjects.

SUBJECTS AND METHODS

Eight FCPD patients, seven IDDM patients, and seven healthy control subjects were studied. Clinical characteristics of these subjects are shown in Table 1. FCPD patients were diagnosed by Mohan's criteria,[10] and all of them reported a history of abdominal pain suggestive of pancreatitis and showed pancreatic calculi on plain x-ray of the abdomen and on sonography. None of these patients showed significant ketonuria (> 40 mg/dL; Keto-Diastix, Miles India, Ames Division, Baroda, India) either at diagnosis or during subsequent follow-up when insulin treatment was stopped for weeks to months (usually for socioeconomic reasons). The IDDM patients were ketosis-prone young diabetics attending our clinic. A plain x-ray of the abdomen and sonography of the pancreas were normal in these patients. The control subjects (five men and two women) were healthy volunteers without a family history of diabetes; they were the same age as the study patients but heavier (mean weight, 55.4 kg; body mass index [BMI], 21.2 kg/m^2).

FCPD and IDDM patients were selected so that the two groups were comparable in age, BMI, daily insulin dose, and duration of diabetes since diagnosis. All were on regular twice-daily split-mix insulin treatment and were studied when control of the diabetes was thought to be satisfactory and stable. None of the FCPD patients were on oral pancreatic enzyme treatment. Routine tests of liver and kidney function were normal in all.

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Table 1. Clinical Characteristics of the Patients

Characteristics	FCPD (n = 8)	IDDM (n = 7)	Controls (n = 7)
Sex (M/F)	7/1	5/2	5/2
Age (yr)	28.8 ± 4.0	21.0 ± 1.5	26 ± 1.0
Height (m)	1.61 ± 0.02	1.65 ± 0.05	1.62 ± 0.04
Weight (kg)	51.4 ± 3.4	51.3 ± 4.1	55.4 ± 3.4
BMI (kg · m^{-2})	19.8 ± 1.03	18.6 ± 0.8	21.2 ± 1.1
Waist to hip ratio	0.91 ± 0.02	0.86 ± 0.03	0.82 ± 0.02
Triceps skinfold (mm)	11.6 ± 1.5	11.0 ± 1.4	10.5 ± 1.8
Subscapular skinfold (mm)	16.7 ± 1.7	12.5 ± 1.7	16.3 ± 2.8
Insulin dose			
U/d	40 ± 6	38 ± 4	
U/kg/d	0.9 ± 0.2	0.8 ± 0.1	
HbA_1 (%)	10.3 ± 0.9	8.7 ± 0.8	
Diabetes duration (yr)	4.3 (1.5-8.0)*	4.0 (3.0-6.0)*	

NOTE. Results are the mean ± SEM except where indicated.
*Median (range).

On the day before the test, the total daily dose of insulin was divided into three equal doses of short-acting (regular) insulin. The last dose was administered at least 10 hours before commencement of the test. After an overnight fast (8 to 10 hours), venous blood was drawn via an indwelling cannula in an antecubital vein. The mean of two baseline samples drawn 15 minutes apart was taken as the fasting value. Each subject was then given a 50-mL drink of MCT solution (Mead Johnson Nutritionals, Bristol-Myers Pharmaceuticals, Uxbridge, UK), which contains 3% caproic (C6), 68% octanoic (C8), 24% decanoic (C10), and 5% lauric (C12) fatty acids. Blood samples were drawn hourly for 5 hours after the MCT drink.

Blood samples for C-peptide and glucagon assays were collected in Trasylol (final concentration, 500 IU/mL), and the plasma was stored at −70°C until transported on dry ice to the United Kingdom for C-peptide assay and to Denmark for glucagon assay. Plasma C-peptide level was measured by a kit (Novo, Bagswaerd, Denmark)[11] with a detection limit of 0.02 nmol/L and intrabatch and interbatch coefficients of variation of 5% and 8%, respectively, and pancreatic glucagon (detection limit, 5 ng/L; intrabatch and interbatch coefficients of variation, 6% and 9.5%, respectively) was assayed by wick chromatography radioimmunoassay[12] using a specific antibody supplied by Dr L. Heding, Novo, Copenhagen, Denmark. Plasma glucose (glucose oxidase), triglycerides, and liver and kidney biochemistry were determined on an Abbott VP Super Auto Analyzer (Irving, TX) using standard kits. Hemoglobin A_1 (HbA_1) level was measured by a colorimetric method using fructose standards.[13] 3-Hydroxybutyrate (3-HB) and glycerol levels were measured on perchloric acid extract of whole blood by an enzymatic assay.[14] Plasma NEFA were assayed with an enzymatic kit (Wako Chemicals, Neuss, Germany) that is equally sensitive to fatty acids of different lengths (including C8 and C10). Plasma carnitine level was measured by an enzymatic method.[15]

The study protocol was approved by the hospital ethics committee, and all participants provided consent to participate. A physician attended the patients throughout the study.

Statistical Analysis

The significance of differences between groups was assessed by the Mann-Whitney test, and serial changes during the test by the Wilcoxon test. Correlations were tested by Spearman's method.

RESULTS

There was no significant difference between FCPD and IDDM patients with respect to age, weight, BMI, daily insulin dose, and HbA_1, although IDDM patients had slightly less body fat than FCPD patients as seen from subcutaneous fat measurements (triceps and subscapular skinfolds) and the waist to hip ratio (Table 1). Control subjects were heavier than both diabetic groups.

Fasting plasma glucose and glucagon and blood 3-HB concentrations were higher in IDDM patients than in FCPD patients ($P < .05$ for all); plasma NEFA, C-peptide, and blood glycerol concentrations were similar in IDDM and FCPD patients.

After oral MCTs (Fig 1), plasma glucose concentrations did not change in FCPD patients. They increased minimally in IDDM patients and were higher in IDDM than in FCPD patients throughout the test. Plasma NEFA concentrations did not show a significant change from the fasting value in IDDM patients, but the levels showed a significant increase at 3, 4, and 5 hours in FCPD patients ($P < .01$ for all). Blood 3-HB concentrations increased slowly in FCPD subjects, achieving significance only 5 hours after the drink ($P < .05$). On the contrary, IDDM subjects showed a brisk and progressive increase in blood 3-HB concentrations ($P < .01$ for all). For any given plasma NEFA concentration, blood 3-HB was higher in IDDM subjects than in FCPD subjects, such that the 3-HB/NEFA ratio was higher in IDDM than in FCPD patients (peak 3-HB/NEFA ratio, median [range]: IDDM, 3.9 [2.5-7.7]; FCPD, 1.0 [0.4-4.4]; $P < .01$). The blood glycerol concentration was similar in the two diabetic groups and did not show any significant change after oral MCTs. Fasting plasma triglyceride concentration was higher in FCPD patients (median, 1.11 mmol; range, 0.89-3.91) than in IDDM patients (0.78 mmol; 0.55-1.21; $P < .05$). There was no significant change after oral MCTs in either group.

Plasma C-peptide concentration remained unchanged throughout the test in FCPD and IDDM patients, and there was no significant difference between the two groups at any time. Plasma glucagon concentration was higher in IDDM than in FCPD patients at fasting and 1 hour; it decreased within 2 hours of the MCT drink and was no different from that in FCPD patients from 2 hours onward. The higher plasma glucagon concentration in IDDM patients was accounted for by a very high concentration in two patients. There was no correlation between plasma C-peptide or glucagon concentrations and 3-HB concentration in IDDM and FCPD patients.

In control subjects, plasma glucose and 3-HB concentrations were lower than in both diabetic groups; NEFA and glycerol concentrations were similar. In these subjects, plasma glucose, NEFA, and glycerol concentrations remained unchanged throughout the test, and blood 3-HB increased after the MCT drink ($P < .001$ for all) and was higher than in FCPD patients at 1 and 2 hours but lower than in IDDM patients throughout the test. Fasting plasma triglyceride in control subjects (0.81 mmol; 0.67-1.07) was lower than in FCPD patients ($P < .05$), but similar to that in IDDM patients. It increased after the MCT drink (peak, 1.67 mmol; 1.42-1.85; $P < .05$) and became similar to the level in FCPD subjects.

Plasma carnitine concentrations were similar in FCPD (median, 48.4 μmol/L; 30.4-78.1), IDDM (39.7 μmol/L; 35.3-67.0), and control (44.0 μmol/L; 29.8-62.0) subjects. Similarly, there was no difference in the acylcarnitine concentration in the three groups (median, 72.5, 65.7, and 73.2 μmol/L, respectively).

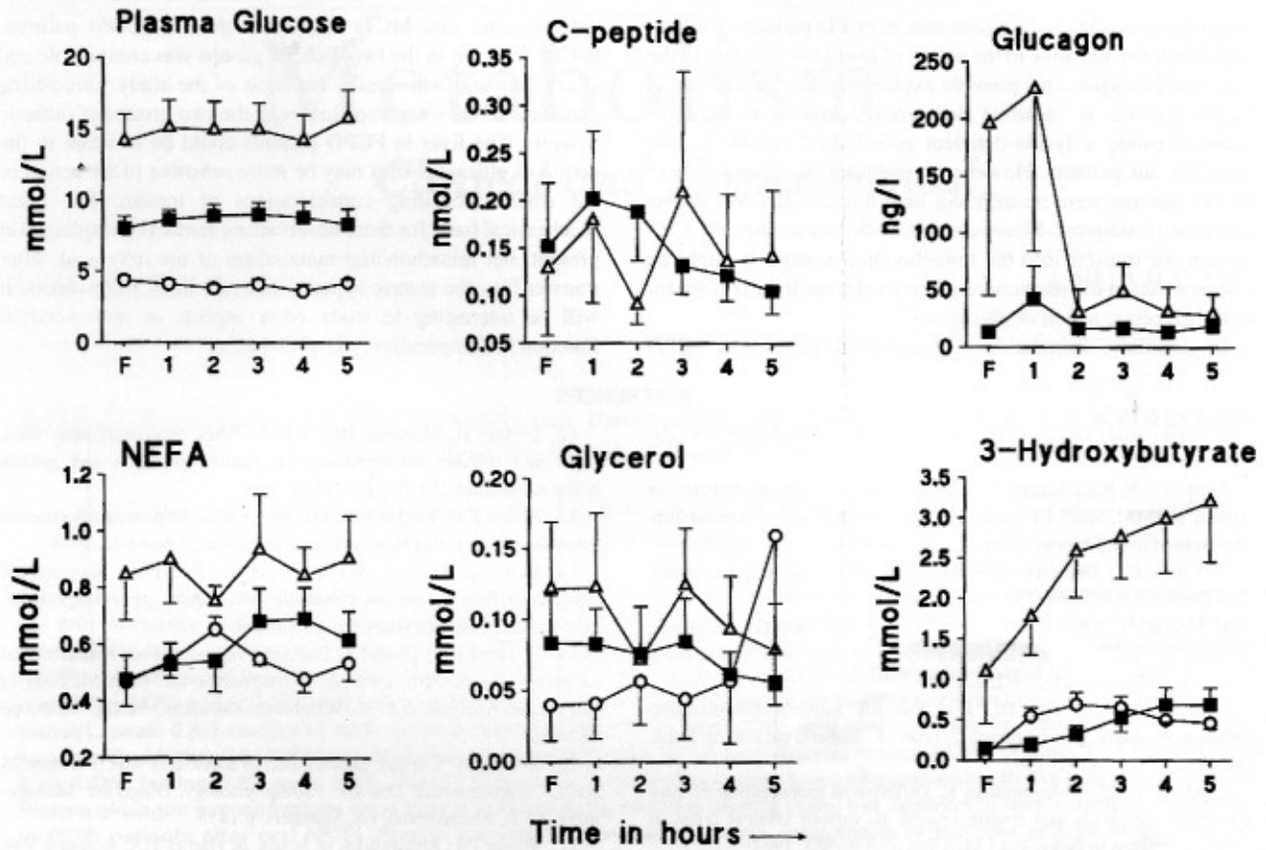

Fig 1. Circulating concentrations of glucose, C-peptide, glucagon, NEFA, glycerol, and 3-HB in FCPD (■) and IDDM (△) patients after oral MCTs. Glucose, NEFA, glycerol, and 3-HB in nondiabetic controls (○) are shown for reference (mean ± SEM).

DISCUSSION

We have previously reported lower blood 3-HB concentrations in our untreated FCPD patients than in IDDM patients despite comparable plasma C-peptide, glucagon, and NEFA concentrations.[9] We suggested that the ketosis resistance of FCPD patients is probably multifactorial, and that both steps in ketogenesis (adipose tissue lipolysis and hepatic ketogenesis) could be involved.

We have tested regulation of hepatic ketogenesis by feeding MCTs that liberate fatty acids, the precursors for ketogenesis. MCTs are absorbed in the intestine without prior pancreatic digestion,[16] an important consideration in FCPD patients who have severe exocrine pancreatic deficiency. Moreover, medium-chain fatty acids (C8 and C10) cross mitochondrial membranes and enter the matrix without the help of the carnitine palmitoyl transferase system (CPT),[17] thus stimulating mitochondrial β-oxidation even in carnitine-deficient states.

After oral MCTs, blood 3-HB concentrations increased slowly and to a lesser extent in FCPD than in IDDM patients. This suggests that hepatic ketogenesis was stimulated less in FCPD patients, despite the strong ketogenic stimulus.

The oral dose of MCTs did not cause a significant increase in peripheral venous concentrations of NEFAs in control and IDDM subjects, probably because of avid hepatic uptake and metabolism. On the contrary, the peripheral venous concentration of NEFAs increased significantly in FCPD patients, probably because of incomplete uptake and metabolism by the liver. Similar findings are reported in patients with liver cirrhosis, who show an increase in peripheral venous concentration of NEFAs because of the failure of hepatic uptake and metabolism.[18]

In addition to the circulating concentration of NEFAs, hepatic ketogenesis is influenced by the relative concentrations of insulin and glucagon.[19,20] FCPD and IDDM subjects showed small and comparable endogenous insulin secretion as reflected by plasma C-peptide concentration. The long interval (10 hours) since the last dose of injected soluble insulin makes it unlikely that any significant amount was present in the circulation. However, it does appear that FCPD patients have a higher ambient hepatic insulin concentration, but there is no simple way of proving or disproving this. Plasma glucagon concentrations were elevated in IDDM patients at the start of the test, but quickly decreased to levels comparable to those in FCPD patients, with the concentrations being similar in the two groups during most of the study. Thus, despite a similar metabolic and hormonal milieu, hepatic ketogenesis was less active in FCPD subjects. Our results for circulating triglycerides suggest that FCPD patients use NEFAs in lipogenic pathways in the liver, although this seems to be a slow process, because there was no increase in plasma triglycerides during the study period.

The failure to stimulate ketogenesis to a substantial extent

under these conditions suggests that in FCPD patients, the liver could be more sensitive to the action of insulin or resistant to the action of glucagon. One possible explanation for our findings in FCPD patients is carnitine deficiency,[21] known to occur in subjects eating a lysine-deficient cereal diet[8] similar to that eaten by our patients. However, circulating carnitine levels in FCPD patients were no different than those in IDDM patients and control subjects. Moreover, MCTs do not require the CPT system for transfer into the mitochondrial matrix. It might be relevant that in the malnourished rat model the liver is resistant to the glycemic action of glucagon.[22]

In summary, stimulation of hepatic ketogenesis in FCPD patients after oral MCTs was lower than in IDDM patients. β-Cell function in the two diabetic groups was comparable and α-cell function was similar for most of the study. Circulating carnitine levels were also similar in the two groups of diabetic patients. The liver in FCPD patients could be resistant to the action of glucagon, or it may be more sensitive to the action of the small circulating concentrations of insulin. The exact biochemical basis for these observations remains unexplained at present, but mitochondrial metabolism of the fatty acids after transfer into the matrix appears different in FCPD patients. It will be interesting to study other aspects of mitochondrial function in this peculiar type of diabetes.

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