Glucose Utilization and Production in Patients With Maturity-Onset Diabetes of the Young Caused by a Mutation of the Hepatocyte Nuclear Factor-1α Gene

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Mutations of the hepatocyte nuclear factor (HNF)-1α gene cause impaired insulin secretion and hyperglycemia in patients with maturity-onset diabetes of the young (MODY)3. Whether these mutations also affect glucose metabolism in tissues other than the β-cell has not yet been documented. We therefore assessed, in five MODY3 patients and a dozen healthy control subjects, insulin secretion, oxidative and nonoxidative glucose disposal, and glucose production during a two-step hyperglycemic clamp and a euglycemic hyperinsulinemic (0.4 mU · kg^{-1} · min^{-1}) clamp. Compared with healthy control subjects, MODY3 patients had higher fasting plasma glucose (+100%) but similar rates of fasting glucose production and oxidation. Both the early and late phases of insulin secretion were virtually abolished during the hyperglycemic clamp, and glucose production was suppressed by only 43% in MODY3 patients vs. 100% in healthy control subjects. The rate of glucose infusion required to produce a 5 mmol/l increase above basal glycemia was reduced by 30%, net nonoxidative glucose disposal (which is equal to net glycogen deposition) was inhibited by 39%, and net carbohydrate oxidation during hyperglycemia was 25% lower in MODY3 patients compared with control subjects. Insulin-stimulated glucose utilization and oxidation measured during the hyperinsulinemic clamp (at ~200 pmol/l insulin) were identical in MODY3 patients and in healthy control subjects, indicating that peripheral insulin sensitivity was not altered. Suppression of endogenous glucose production was, however, mildly impaired. It is concluded that MODY3 patients have severely depressed glucose-induced insulin secretion. The development of hyperglycemia in these patients appears to be caused by a decreased stimulation of glucose utilization, oxidation, and nonoxidative glucose disposal as well as by a blunted suppression of endogenous glucose output. These phenomena are essentially secondary to insulinopenia, whereas insulin sensitivity remains intact. *Diabetes* 47:1459–1463, 1998

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HNF, hepatocyte nuclear factor; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction.

Maturity-onset diabetes of the young (MODY) is an inherited form of NIDDM characterized by an autosomal dominant mode of transmission and an early age (usually <25 years old) at onset (1). It is a genetically heterogeneous group of disorders, with subtypes secondary to mutations of the genes coding for the hepatocyte nuclear factor (HNF)-4α (2) (MODY1), glucokinase (MODY2) (3), and HNF-1α (4,5) (MODY3). About 30% of patients with diabetes mellitus classified as MODY on clinical grounds do not display mutations of these genes, which indicate that additional genes will have to be identified (6).

Impaired insulin secretion, decreased insulin action, and increased endogenous (hepatic) glucose output may all contribute to the pathogenesis of hyperglycemia in NIDDM (7). In patients with MODY2 secondary to a mutation of the glucokinase gene, decreased glucose-induced insulin release, impaired postprandial suppression of hepatic glucose production, and blunted uptake of glucose by liver cells have been documented (8–10). These alterations are secondary to impaired glucose-sensing by pancreatic β-cells and hepatocytes that is due to low activity of the glucose-phosphorylating enzyme glucokinase.

HNF-1α is a nuclear transcription factor involved in the development and differentiation of hepatocytes (11,12). It is also expressed in other cell types, including endocrine pancreatic cells (13,14). Patients with MODY3 secondary to mutations of HNF-1α usually develop a severe form of diabetes in their late teens or early twenties (15). Such patients have been shown to have severe impairment of glucose-induced insulin secretion, which affects both the early and late phases of insulin release (16). In contrast, their insulin sensitivity appears essentially normal (15,17). No information, however, is available regarding glucose production and the pathways of glucose utilization in these patients. We therefore measured glucose production, glucose oxidation, and nonoxidative glucose disposal in a pedigree of patients with a mutation of HNF-1α and in healthy volunteers. These measurements were performed in the basal state, during a two-step hyperglycemic clamp in which plasma glucose was increased by 2.5 and 5.0 mmol/l and during a euglycemic-hyperinsulinemic clamp at a low (2.4 nmol · kg^{-1} · min^{-1}) rate of exogenous insulin infusion.

RESEARCH DESIGN AND METHODS

Patients. Five patients with MODY3 were included in this study. They all belonged to the same family (Fig. 1). Genetic analysis was performed after DNA extraction from peripheral blood samples. The proband was screened for muta-

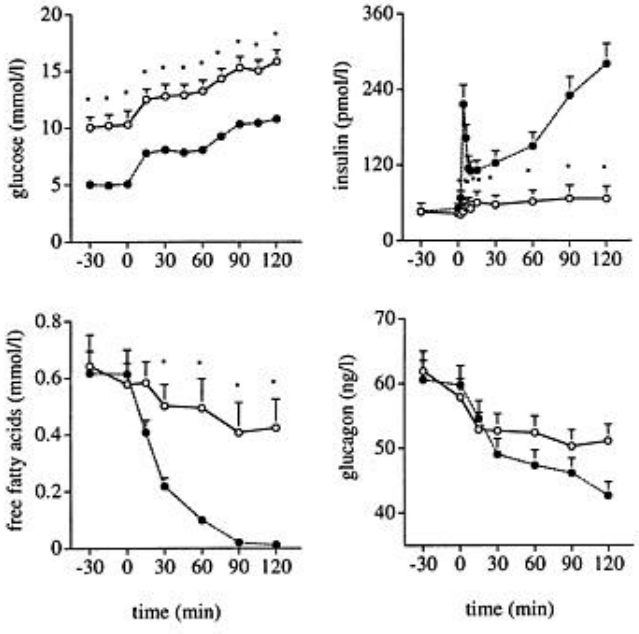

FIG. 1. Plasma glucose, insulin, free fatty acids, and glucagon concentrations during a two-step hyperglycemic clamp in MODY3 patients (○) and healthy subjects (●). *P < 0.05 vs. healthy subjects.

tions in the HNF-1α gene as previously described (2) by polymerase chain reaction (PCR) amplification of each exon and direct sequencing of the PCR product. The sequence of the mutation was confirmed by cloning the PCR product into the vector pGEM T (Promega, Madison, WI) and sequencing clones of both alleles. All patients identified had a frameshift mutation (C deletion in codon 291 of exon 4). A detailed genetic analysis of the pedigree of the MODY3 patients included in the present study has been published elsewhere (5). Patients' characteristics are shown in Table 1; they were not currently receiving any medication except for oral antidiabetic agents (n = 1) or insulin (n = 1), as indicated. These agents were discontinued 4 days before the study.

Two groups of six healthy volunteers were selected as a control group. All were in good physical condition and were not taking any medication. They had no first-degree relatives with obesity or diabetes mellitus. The experimental protocol was approved by the ethical board of Lausanne University School of Medicine, and all participants provided an informed written consent.

Experimental protocol

Study 1: Two-step (2.5 and 5 mmol/l) hyperglycemic clamp. The five MODY3 patients and group 1 of the healthy subjects took part in this study. The protocol started in the morning between 7:00 and 8:00 A.M. All participants had been fasting since 8:00 P.M. the day before. One venous cannula was inserted into an antecubital vein of the left arm. A primed (0.3 mg · kg^{-1} · mmol^{-1} fasting plasma glucose) continuous (1.5 µg · kg^{-1} min^{-1}) infusion of [$^{13}C_6$]glucose (Mass Trace, Woburn, MA) was administered for a period of 150 min in the basal state (time –150 to 0 min). Thereafter, a variable infusion of exogenous glucose, labeled with 0.05% $^{13}C_6$-glucose, was performed to acutely raise plasma glucose by 2.5 mmol/l for a period of 60 min (time 0–60) and by 5.0 mmol/l for an additional period of 60 min (time 60–120) (18). Another venous cannula was inserted into a vein of the right wrist. The right hand was placed in a thermostabilized box heated at 56°C to achieve partial arterialization of venous blood. Blood samples were collected at time –130, –30, –15, and 0 min basal, 30, 60 (hyperglycemia + 2.5 mmol/l), 90, and 120 min (hyperglycemia + 5 mmol/l) for the measurement of plasma hormone and substrate concentrations and of plasma ^{13}C-glucose enrichment. In addition, blood samples were collected every 5 min between 0 and 120 min for the bedside determination of plasma glucose concentrations. Respiratory gas exchanges were continuously monitored between –90 and 120 min using a ventilated canopy, as described elsewhere (19). A timed urine collection was performed to measure urinary nitrogen excretion. Net substrate oxidation rates were calculated using the equations of Livesey and Elia (20).

Study 2: Hyperinsulinemic-euglycemic clamp. The five MODY3 patients and group 2 of the healthy subjects took part in this study. General procedures were identical to those of study 1. At time 0 min, a bolus of 6,6-$^2H_2$-glucose (MassTrace, Woburn, MA) was administered (11.1 µmol/mmol fasting plasma glucose), and a primed continuous infusion of crystalline insulin (Actrapid HM; Novo Nordisk, Copenhagen, Denmark) was started. Plasma glucose was clamped at 5.0 mmol/l by a variable infusion of a 200-g/l solution of glucose (21) containing 1.25% 6,6-$^2H_2$-glucose to calculate whole body glucose turnover and endogenous glucose production (22). In MODY3 patients, 6,6-$^2H_2$-glucose was infused at a rate of 0.11 µmol · kg^{-1} · min^{-1} when the exogenous unlabeled glucose infusion was lower than 11 µmol · kg^{-1} · min^{-1}. Indirect calorimetry was performed as in study 1. Data were collected during 30 min after 150 min of hyperglycemia (150–180 min), except in two MODY3 patients, in whom euglycemia was attained only after 240 and 270 min.

Analytical procedures. For determination of ^{13}C-glucose or 6,6-$^2H_2$-glucose enrichments, plasma was deproteinized with 3% perchloric acid, neutralized with K_2CO_3, and partially purified over sequential cation-anion exchange resins (AG50 W-X8 and AG 1-X8; Bio-Rad, Richmond, CA). For ^{13}C-glucose determinations, the eluate containing glucose was evaporated to dryness, resuspended in 150 µl water, and the glucose was purified by high-performance liquid chromatography on an Aminex HPX-87/C column (Bio-Rad) eluted with H_2O at 0.6 ml/min at 80°C. The purified glucose was combusted to CO_2 in a Roboprep CN (Europa Scientific, Crewe, U.K.), and its ^{13}C:^{12}C ratio was analyzed by isotope ratio mass spectrometry on a Tracermass (Europa Scientific). Values were converted to atom percent excess. For 6,6-$^2H_2$-glucose determination, the eluate was evaporated to dryness, and pentacetyl glucose was prepared and analyzed with gas chromatography–mass spectrometry (Hewlett Packard, Palo Alto, CA) with selective monitoring of m/z 331 and 333 (10). Plasma insulin (kit from Biodata, Guidonia Martecelo, Italy) and glucagon (kit from Linco Research, St. Charles, MO) concentrations were measured by radioimmunoassay. Plasma and urine glucose concentrations were measured with a Beckman Glucose Analyzer II (Fullerton, CA). Plasma free fatty acid concentrations were measured colorimetrically using a kit from Wako (Neuss, Germany). Urine nitrogen concentrations were measured with the method of Kjehldahl (23).

Calculations. Glucose appearance and disappearance rates were calculated from plasma ^{13}C-glucose or 6,6-$^2H_2$-glucose dilution during labeled exogenous glu-

TABLE 1
Subject characteristics

	Sex (M/F)	Age (years)	Weight (kg)	Height (cm)	Fasting plasma glucose (mmol/l)	HbA_{1c} (%)	Treatment
Patients with MODY3							
1	M	26	67.8	176	12.9	10.2	Diet
2	F	31	76.0	161	12.7	7.2	Glimepiride/ metformin
3	M	28	76.0	171	7.0	5.6	Diet
4	F	50	53.5	154	7.8	7.8	Insulin
5	M	52	56.0	165	14.4	9.7	Diet
Healthy volunteers							
Group 1	3/3	32.3 ± 13.6	63.2 ± 13.9	168.7 ± 10.8	5.0 ± 0.6		
Group 2	3/3	38.5 ± 7.1	67.3 ± 7.9	171.7 ± 10.8	5.3 ± 0.4		

Data are means ± SE, unless otherwise indicated.

TABLE 2
Glucose kinetics during the two-step hyperglycemic clamp (study 1)

	Basal	Basal + 2.5 mmol	Basal + 5 mmol
Glucose appearance rate (μmol · kg^{-1} · min^{-1})			
MODY3 patients	14.8 ± 1.3	21.3 ± 3.4	31.3 ± 3.3
Control subjects	13.6 ± 0.5	17.4 ± 1.7	32.6 ± 2.5
Glucose infusion (μmol · kg^{-1} · min^{-1})			
MODY3 patients	—	14.2 ± 2.0	23.0 ± 2.3*
Control subjects	—	15.9 ± 1.4	32.8 ± 2.7
Endogenous glucose production (μmol · kg^{-1} · min^{-1})			
MODY3 patients	14.8 ± 1.3	7.0 ± 1.7*	8.3 ± 1.7*
Control subjects	13.6 ± 0.5	1.5 ± 0.3	–0.2 ± 0.4
Glucose clearance (ml · kg^{-1} · min^{-1})			
MODY3 patients	1.4 ± 0.1	1.7 ± 0.2	2.0 ± 0.2
Control subjects	2.8 ± 0.2	2.2 ± 0.3	3.2 ± 0.3

Data are means ± SE. *P < 0.05 vs. control subjects.

cose infusion (22). Metabolic clearance of glucose was calculated as (glucose appearance rate – urinary glucose excretion) / (plasma glucose concentration). Endogenous glucose production was calculated as (glucose appearance rate) – (glucose infusion rate).

Statistical analysis. All results in the text, tables, and figures are expressed as means ± SE, unless stated otherwise. Comparison of means was done with analysis of variance for repeated measurements and unpaired Student's t tests. All analyses were performed using the Statview 4.5. statistical package (Abacus Concepts, Berkeley, CA).

RESULTS

Study 1. Compared with healthy volunteers, patients with MODY3 had increased fasting glucose concentrations (10.0 ± 0.9 vs. 5.0 ± 0.2 mmol/l, P < 0.001). However, basal insulin (47 ± 5 vs. 46 ± 14 pmol/l), glucagon (60 ± 4 vs. 62 ± 4 ng/l), and free fatty acid (0.65 ± 0.10 vs. 0.62 ± 0.08 mmol/l) concentrations were comparable in both groups. Basal net carbohydrate, oxidation rate (8.1 ± 1.3 vs. 8.6 ± 0.7 μmol · kg^{-1} · min^{-1}), and endogenous glucose production (14.8 ± 1.4 vs. 13.7 ± 0.5 μmol · kg^{-1} · min^{-1}) were also similar. The metabolic clearance rate of glucose, however, was 47% lower in MODY3 patients than in healthy subjects (P < 0.02) (Table 2).

When plasma glucose was acutely increased by 2.5 mmol/l, healthy volunteers had a first-phase insulin secretion that peaked at 216 ± 31 pmol/l after 4 min. This first phase of insulin secretion was virtually abolished in MODY3 patients (Fig. 1). Insulin concentrations were increased by 199% compared with basal values when plasma glucose was raised by 2.5 mmol/l, and by 494% when glucose was raised by 5.0 mmol/l in healthy volunteers. In contrast, patients with MODY3 did not increase their insulin concentrations when plasma glucose was raised. Plasma insulin concentrations obtained at each step of glycemia (i.e., basal, basal + 2.5 mmol/l, and basal + 5.0 mmol/l) were correlated with both plasma glucose and glucose appearance rate in healthy volunteers, but not in patients with MODY3. Plasma free fatty acid concentrations were suppressed by 84% during the first step and by 97% during the second step of hyperglycemia in healthy control subjects but by only 23 and 35% in MODY3 patients. Plasma glucagon concentrations were suppressed by 22% during the first step and by 30% during the second step of hyperglycemia in healthy control subjects, whereas in MODY3 patients, suppression was slightly blunted at both steps, although not significantly (Fig. 1).

TABLE 3
Pathways of glucose disposal during the two-step hyperglycemic clamp (study 1)

	Basal	Basal + 2.5 mmol	Basal + 5 mmol
Net carbohydrate oxidation (μmol · kg^{-1} · min^{-1})			
MODY3 patients	8.1 ± 1.3	8.5 ± 1.2	9.7 ± 1.6
Control subjects	8.6 ± 0.7	10.8 ± 1.2†	13.1 ± 1.2†
Net nonoxidative carbohydrate disposal (μmol · kg^{-1} · min^{-1})			
MODY3 patients	—	4.3 ± 2.0	12.0 ± 2.6*†
Control subjects	—	5.1 ± 1.4†	19.8 ± 1.9†

Data are means ± SE. *P < 0.05 vs. control subjects; †P < 0.05 vs. basal.

The rate of glucose infusion required to increase plasma glucose concentrations was not statistically different in the two groups during the first step of hyperglycemia, but it was decreased by 30% in MODY3 patients during the second step. Glucose rates of appearance were similar in the two groups, at each step of hyperglycemia (Table 2). Urinary glucose excretion averaged over the 2-h period amounted to 1.4 ± 0.5 μmol · kg^{-1} · min^{-1} in MODY3 patients, whereas no glucose was found in the urine of healthy volunteers. Endogenous glucose production was virtually abolished in healthy volunteers already at the first step of hyperglycemia, but it was decreased by only 53 and 44% at each step in MODY3 patients (Table 2).

Glucose oxidation increased by 25% at the first step (P < 0.05) and by 52% at the second step of hyperglycemia (P < 0.01) in healthy subjects. In MODY3 patients, by contrast, glucose oxidation was increased by only 20% (NS) at the second step of hyperglycemia. Net nonoxidative carbohydrate disposal (i.e., the difference between glucose infusion rate and carbohydrate oxidation, which corresponds to net glycogen storage) was also 61% lower in MODY3 patients than in control subjects at the second step of hyperglycemia (Table 3).

Study 2. Insulin infusion increased plasma insulin concentrations to 200.1 ± 22.4 pmol/l in MODY3 patients and 192.6 ± 15.8 pmol/l in healthy control subjects (NS). In healthy volunteers, plasma glucose concentrations were clamped at 5.0 ± 0.1 mmol/l throughout the 3-h clamp period. In MODY3 patients, plasma glucose concentration decreased progressively during insulin infusion and averaged 5.3 ± 0.2 mmol/l during the 30 min during which data were collected. Plasma free fatty acid concentrations were decreased to 0.039 ± 0.014 mmol/l in MODY3 patients and 0.072 ± 0.032 mmol/l in healthy control subjects (NS). Parameters of glucose metabolism are shown in Table 4. Glucose disappearance rate, net carbohydrate oxidation, and nonoxidative glucose disposal were all increased to similar extents compared with basal values measured in protocol 1 and did not differ between

TABLE 4
Glucose metabolism during the hyperinsulinemic-euglycemic (2.4 nmol · kg^{-1} · min^{-1}) clamp (study 2)

	MODY patients	Control subjects
Glucose disappearance rate (μmol · kg^{-1} · min^{-1})	24.9 ± 4.1	22.6 ± 3.5
Net carbohydrate oxidation (μmol · kg^{-1} · min^{-1})	15.2 ± 1.9	15.1 ± 0.8
Nonoxidative glucose disposal (μmol · kg^{-1} · min^{-1})	9.7 ± 3.5	7.4 ± 2.5
Endogenous glucose production (μmol · kg^{-1} · min^{-1})	6.1 ± 0.7*	2.7 ± 0.8

Data are means ± SE. *P < 0.05 vs. control subjects.

MODY3 patients and healthy control subjects. Endogenous glucose production was suppressed to 41% of basal values in MODY3 patients and to 20% of normal basal values in healthy control subjects. It was significantly higher in MODY3 patients ($P < 0.05$).

DISCUSSION

Compared with healthy volunteers, patients with MODY3 secondary to mutation of HNF-1α display severe impairment of glucose-induced insulin secretion with nearly total abolition of both the early and late phases of glucose-induced insulin secretion. These results corroborate several previous reports. Byrne et al. (16) observed that glucose-induced insulin secretion, monitored by C-peptide deconvolution analysis, was severely impaired in patients with mutations of HNF-1α. Lehto et al. (15) observed a markedly impaired elevation of plasma insulin concentrations in MODY3 patients after intravenous glucose or intravenous arginine. Interestingly, nondiabetic relatives of MODY3 patients bearing the same genetic alterations also displayed impaired glucose-induced insulin secretion. These observations clearly point to a major secretory defect of pancreatic β-cells in this subset of MODY.

In MODY3 patients, net carbohydrate oxidation fails to increase in response to exogenous glucose infusion at both steps of hyperglycemia studied. Stimulation of nonoxidative glucose disposal is also blunted when compared with healthy subjects, while the suppression of endogenous glucose production is markedly impaired. MODY3 patients also show a lower inhibition of plasma free fatty acid concentrations, indicating impaired suppression of lipolysis. In contrast, infusion of exogenous insulin produces similar effects in MODY3 patients and in healthy volunteers. Even though the dose of insulin administered was chosen to obtain insulin concentrations that produce submaximal effects on most of the metabolic pathways studied, there is no evidence of an impaired ability of insulin to stimulate whole body glucose disposal, carbohydrate oxidation, nonoxidative carbohydrate disposal, or lipolysis in MODY3 patients. This leads to the conclusion that the impaired glucose metabolism observed during the hyperglycemic clamp study, i.e., when stimulation of glucose utilization is subordinated to endogenous insulin secretion, is essentially secondary to impaired endogenous insulin secretion and insulinopenia.

Even though four of the five MODY3 patients studied have overt hyperglycemia, they do not show increased basal endogenous glucose production. In this, they clearly differ from classical type 2 diabetic patients in whom an increased basal glucose output has been repeatedly documented (24–27). Their endogenous glucose production was significantly suppressed by more than 50% during mild hyperinsulinemia. This suggests that the blunted suppression of glucose production observed during the hyperglycemic clamp is essentially secondary to inadequate endogenous glucose production.

Suppression of endogenous glucose production by mild hyperinsulinemia was slightly impaired in MODY3 patients. However, this defect was markedly less than what is reported in classical type 2 diabetes (28,29). This suggests that alterations of hepatic glucose metabolism are minimal in MODY3 patients. Because HNF-1α regulates the expression of numerous hepatic genes, including those coding for key enzymes of glucose metabolism, we cannot exclude that mutation of its gene leads to mild dysregulation of glucose production. It is also possible that chronic insulinopenia decreases the hepatic expression of the glucokinase gene, leading to impaired hepatic glucose sensing (10,30). Finally, it is possible that minor alterations of glucose production are merely the result of chronic hyperglycemia, according to the concept of glucotoxicity (31).

In conclusion, the present data corroborate previous observations that MODY3 patients have severely decreased glucose-induced insulin secretion. As a consequence of the failure to increase plasma insulin concentrations, they display impaired suppression of endogenous glucose production and stimulation of net carbohydrate oxidation and nonoxidative carbohydrate disposal. The development of hyperglycemia appears essentially caused by a decreased stimulation of glucose utilization secondary to defects in insulin secretion.

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