

## CLINICAL STUDY

## Counterregulatory responses to hypoglycemia in patients with maturity-onset diabetes of the young caused by HNF-1 $\alpha$ gene mutations (MODY3)

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### Abstract

Mutations of HNF-1 $\alpha$  lead to severe  $\beta$  cell dysfunction, resulting in decreased glucose-induced insulin secretion. HNF-1 $\alpha$  is also expressed in liver, kidney and pancreatic  $\alpha$  cells, but the functional consequences of HNF-1 $\alpha$  mutations in these organs remain unknown. We therefore assessed the counterregulatory responses to hypoglycemia in six patients with HNF-1 $\alpha$  mutations (MODY3), five patients with non-insulin-dependent diabetes mellitus (NIDDM) and in nine healthy controls. Plasma glucagon concentrations and endogenous glucose production were measured every 15 min during a hyperinsulinemic clamp with progressive hypoglycemia. Plasma glucagon concentrations were similar at basal glycemia ( $73 \pm 6$ ,  $69 \pm 5$  and  $69 \pm 7$  ng/l) and reached peak values of  $88 \pm 9$ ,  $88 \pm 11$  and  $89 \pm 7$  ng/l at a glycemia of 3.6 mmol/l in MODY3 patients, patients with NIDDM and controls respectively (NS). Suppression of endogenous glucose production by insulin was blunted in MODY3 patients ( $3.3 \pm 1.2$   $\mu$ mol/kg per min) and in patients with NIDDM ( $4.4 \pm 0.6$   $\mu$ mol/kg per min) compared with controls ( $1.7 \pm 0.5$   $\mu$ mol/kg per min,  $P < 0.05$  compared with both MODY3 patients and patients with NIDDM). During hypoglycemia, endogenous glucose production increased to  $8.6 \pm 2.1$ ,  $8.8 \pm 0.7$  and  $7.0 \pm 1.0$   $\mu$ mol/kg per min in MODY3 patients, patients with NIDDM and controls respectively (all NS). These data indicate that mutations of HNF-1 $\alpha$  in MODY3 do not result in a decreased glucagon secretion or alterations of glucose production during hypoglycemia.

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### Introduction

Diabetes mellitus is a group of disorders of various etiological causes and pathophysiological mechanisms. Physiological and clinical studies indicate that hyperglycemia can develop as the result of decreased insulin secretion, insulin resistance, or defective neuroendocrine control of glucose homeostasis at the level of the central nervous system (1–4). One or several of these factors may therefore be involved in the development of impaired glucose tolerance or diabetes mellitus.

Maturity onset diabetes of the young 3 (MODY3) is a subtype of diabetes mellitus secondary to mutations of the hepatocyte nuclear factor 1 $\alpha$  (HNF-1 $\alpha$ ) gene (5). It is characterized by the development of a severe impairment of insulin secretion in response to various secretagogues in early adulthood (6, 7) as a result of HNF-1 $\alpha$  mutations in pancreatic  $\beta$  cells. Defective insulin secretion has also been observed in HNF-1 $\alpha$ -deficient mice, supporting the concept that insulin deficiency is responsible for the development of diabetes in patients with HNF-1 $\alpha$  mutations (8). In contrast,

insulin sensitivity has been reported to be essentially normal or mildly decreased (6, 9) in MODY3 patients. This minor impairment of insulin action is presumably secondary to the effects of chronic hyperglycemia (10), as the HNF-1 $\alpha$  gene is not expressed in skeletal muscle, the major tissue that determines insulin sensitivity.

HNF-1 $\alpha$  is also expressed in liver, kidney and pancreatic  $\alpha$  cells (11), but little information is available regarding the consequences of HNF-1 $\alpha$  mutations in these cells. In liver and kidney cells, mutations of HNF-1 $\alpha$  may be expected to affect glucose production. Arginine-induced glucagon secretion is impaired in MODY3 patients, suggesting that HNF-1 $\alpha$  leads to functional alterations of pancreatic  $\alpha$  cells (12). Alterations of glucose production and glucagon secretion secondary to HNF-1 $\alpha$  mutations may possibly play a part in the development of hyperglycemia or impair the counterregulatory responses to hypoglycemia in MODY3 patients.

To assess further the consequences of HNF-1 $\alpha$  mutations on liver and pancreatic  $\alpha$  cells, we measured endogenous glucose production and the concentrations

of counterregulatory hormones during moderate hyperinsulinemia with a progressive decrease in plasma glucose concentrations in a group of MODY3 patients and in a group of healthy volunteers. In order to evaluate the possible effects of chronic hyperglycemia, the same parameters were also measured in a small group of patients with non-insulin-dependent diabetes mellitus (NIDDM).

## Methods

### Patients

Six MODY3 patients (three men, three women), five patients with NIDDM (one man, four women) and 10 healthy individuals (five men, five women) were recruited to participate in this study. Their characteristics are shown in Table 1. A genetic analysis was performed after DNA extraction from peripheral blood samples. Screening for mutations in the HNF-1 $\alpha$  gene was performed by PCR, amplification of each exon and direct sequencing of the PCR product (5). Five patients from the same family (13) had a frameshift mutation (C deletion in codon 291 of exon 4). The sixth patient had a frameshift mutation (C insertion in codon 291 of exon 4). The experimental procedure in which the patients and healthy individuals agreed to participate had been approved by the Ethics committee of Lausanne University School of Medicine, and every participant provided an informed, written consent.

### Experimental protocol

The experiments began between 0700 h and 0800 h. The participants had been fasting since 2200 h the previous day. Oral antidiabetic medications had been discontinued for 72 hours, and insulin treatments for 24 hours.

On their arrival at the metabolic laboratory, each individual was weighed and measured. Thereafter they rested quietly in bed during the entire experiments, while watching video movies. One cannula was

inserted into an antecubital vein of the left arm for infusion of insulin, glucose, and 6,6- $^{2}\text{H}$ glucose. A second cannula was inserted into a wrist vein of the right arm for blood sampling. The right hand was placed in a thermostabilized box heated to 56 °C to achieve partial arterialization of venous blood. In MODY3 patients, a primed-continuous infusion of insulin (2.4 pmol/kg per min) was started at time 0 (14), and variable infusion of 20% dextrose was administered to maintain plasma glucose at basal values until time 120 min. Dextrose infusion was then reduced until plasma glucose could be maintained at 5 mmol/l during a period of 30 min. Thereafter, dextrose infusion was gradually reduced to decrease the plasma glucose concentrations by 0.5 mmol/l every 30 min until a plasma glucose concentration of 3.6 mmol/l was reached. In healthy controls, the experimental procedure was identical, except that basal glucose concentrations were maintained until 180 min to minimize the differences in duration of insulin infusions between MODY3 patients and patients with NIDDM and healthy controls at each glucose concentration step. During the entire procedure, the dextrose infused was mixed with 1.25% 6,6- $^{2}\text{H}$ glucose (Masstrace, Worcester, MA, USA) to measure glucose kinetics using the 'hot infusate' model (15). An infusion of 0.14  $\mu\text{mol/kg}$  per min 6,6- $^{2}\text{H}$ glucose was maintained when dextrose infusion was less than 11  $\mu\text{mol/kg}$  per min. Two blood samples were collected 15 min apart at each plateau of glycemia.

### Analytical procedures

Plasma glucose was measured using a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA, USA). Plasma insulin (kit from Biodata, Guidonia Montecello, Italy), glucagon (kit from Linco Research, St Charles, MO, USA), and cortisol (kit from Diagnostic Products Corporation, Los Angeles, CA, USA) were measured by radioimmunoassays. Plasma growth hormone concentrations were measured by a chemiluminescence immunometric assay (Nichols Institute,

**Table 1** Characteristics of the participants. Values are numbers or means  $\pm$  s.d.

Participant	Sex	Age (yr)	Weight (kg)	Height (m)	Treatment	HbA <sub>1c</sub> (%)	Diabetes duration (yr)	
MODY3 patients								
1	F	32	71	1.62	Glimepiride	8.8	8	
2	F	51	53	1.52	Metformin	9.7	26	
3	M	32	74	1.71	Insulin	7.2	7	
4	M	31	68	1.75	Diet	7.2	6	
5	M	27	92	1.72	Diet	10.8	4	
6	F	20	57	1.80	Glibenclamide	7.4	1	
		3 F/3 M	32 $\pm$ 10	69.1 $\pm$ 13.8	1.68 $\pm$ 0.10			
		1 F/4 M	53 $\pm$ 3	82.4 $\pm$ 8.4	1.69 $\pm$ 0.07	Insulin ( <i>n</i> = 5)	8.8 $\pm$ 0.3	8 $\pm$ 4
		5 F/5 M	42 $\pm$ 9	65.5 $\pm$ 12.6	1.70 $\pm$ 0.11	—	—	

HbA<sub>1c</sub>, glycated hemoglobin.

San Juan Capistrano, CA, USA). Plasma epinephrine and norepinephrine concentrations were measured by HPLC with electrochemical detection (16). Plasma 6,6- $^{2}\text{H}$ glucose was measured with gas chromatography-mass spectrometry as described elsewhere (17).

### Calculations

Glucose rates of appearance (GRa) and disappearance (GRd) were calculated from 6,6- $^{2}\text{H}$ glucose dilution analysis using the hot infusate model (15). Endogenous glucose production was calculated by subtracting dextrose infusion from GRa. Glycemic thresholds for secretion of counterregulatory hormone were determined as the first glucose concentration at which each hormone was statistically increased compared to values obtained at basal glycemia.

### Statistical analysis

All results in text, tables and figures are shown as mean  $\pm$  1 S.E.M. unless stated otherwise. Comparisons between MODY3 and patients with NIDDM and healthy individuals were made using MANOVA and unpaired *t*-tests with Bonferroni's correction. Determination of individual glycemic thresholds for counterregulatory hormones secretion and stimulations of glucose production were assessed by determining, by means of *t*-tests, the first level of glycemia at which hormone concentrations increased significantly compared to

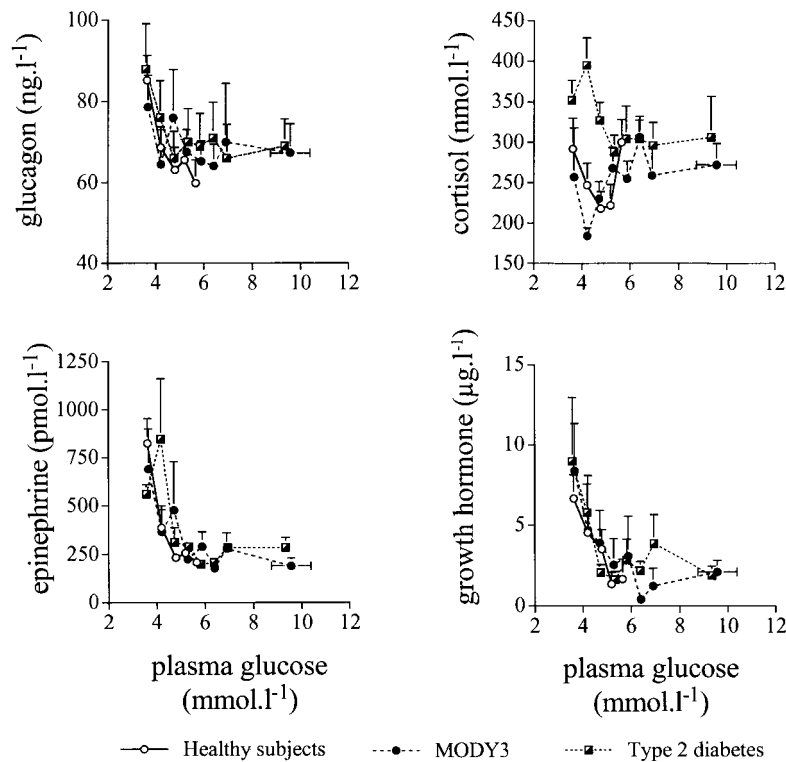
euglycemia. The statview 4.5 statistical package (Abacus Concepts, Berkeley, CA, USA) was used for these calculations.

### Results

Fasting plasma glucose concentration was increased in MODY3 patients ( $10.4 \pm 1.9$  mmol/l,  $P < 0.01$ ) and in patients with NIDDM ( $12.4 \pm 2.3$  mmol/l,  $P < 0.001$ ) compared with healthy controls ( $5.2 \pm 0.1$  mmol/l). Fasting plasma insulin concentrations were modestly increased in patients with NIDDM ( $102 \pm 32$  pmol/l) compared with MODY3 patients ( $58 \pm 13$  pmol/l) and healthy individuals ( $52 \pm 7$  pmol/l). Insulin infusion resulted in plasma insulin concentrations of  $205 \pm 20$ ,  $281 \pm 35$  and  $210 \pm 19$  pmol/l (NS) in MODY3 patients, patients with NIDDM and healthy individuals respectively.

During the initial period of insulin infusion, plasma glucose concentrations were maintained at each individual's basal values. Counterregulatory hormones concentrations during this period were not different in the three groups (Fig. 1). Endogenous glucose production was greater in both MODY3 and patients with NIDDM than in healthy individuals, and GRd was lower only in patients with NIDDM (Table 2).

During the second period of insulin infusion, plasma glucose concentrations was allowed to decrease progressively by reducing the rate of exogenous glucose infusion. The evolution of counterregulatory hormone



**Figure 1** Counterregulatory hormone concentrations as a function of glycemia in MODY3 patients, patients with NIDDM and healthy individuals.

**Table 2** Endogenous glucose production ( $\mu\text{mol/kg}$  per min) and glucose rate of disappearance ( $\mu\text{mol/kg}$  per min) during hyperinsulinemia at basal glycemia and at hypoglycemia.

	Basal glycemia	Hypoglycemia
<b>MODY3</b>		
Endogenous glucose production	3.3 $\pm$ 1.2*	8.6 $\pm$ 2.1
Glucose rate of disappearance	17.8 $\pm$ 2.7	21.3 $\pm$ 1.6
<b>NIDDM</b>		
Endogenous glucose production	4.4 $\pm$ 0.6*	8.8 $\pm$ 0.7
Glucose rate of disappearance	12.4 $\pm$ 1.3*	9.6 $\pm$ 1.0*
<b>Healthy individuals</b>		
Endogenous glucose production	1.7 $\pm$ 0.5	7.0 $\pm$ 1.0
Glucose rate of disappearance	24.4 $\pm$ 2.0	16.2 $\pm$ 1.2

\*  $P < 0.05$  compared with healthy individuals.

concentrations as a function of glycemia is shown in Fig. 1. Stimulation of counterregulatory hormone secretion by hypoglycemia and the glycemic threshold at which each hormone increased significantly were not different in MODY3 patients compared with patients with NIDDM and healthy controls (Fig. 1, Table 3). Endogenous glucose production at the final glycemia of 3.6 mmol/l was also similar in all groups of patients, but GRd was lower in patients with NIDDM (Table 2).

## Discussion

HNF-1 $\alpha$  is highly expressed in the liver and kidneys. As these two organs are responsible for endogenous glucose production (18), and as increased glucose production may participate in the pathogenesis of hyperglycemia, it was of interest to determine whether mutations of HNF-1 $\alpha$  led to dysregulation of glucose production. Our previous data indicated that MODY3 patients have normal basal glucose production rates (9). However, as hyperglycemia suppresses glucose production (17), the normal glucose production in MODY3 patients may in fact be considered to be abnormally increased. In contrast, the modest hyperinsulinemia elicited by infusion of 2.4 nmol/kg per min insulin efficiently suppressed glucose production, which confirms the results of a previous study (9). Compared with that in healthy individuals, the insulin-mediated suppression of glucose production in MODY3 patients

**Table 3** Glycemic thresholds (mmol/l) for counterregulatory hormone secretion and for increase in endogenous glucose production.

	Glucagon	Epinephrine	Growth hormone	Cortisol
MODY3	4.0 $\pm$ 0.2	3.7 $\pm$ 0.3	3.8 $\pm$ 0.3	3.2 $\pm$ 0.1
NIDDM patients	4.1 $\pm$ 0.1	3.5 $\pm$ 0.1	4.2 $\pm$ 0.2	4.1 $\pm$ 0.2
Controls	3.6 $\pm$ 0.1	3.8 $\pm$ 0.2	4.2 $\pm$ 0.2	3.8 $\pm$ 0.2

was, however, significantly impaired and was similar to that observed in patients with NIDDM. MODY3 patients had a slight, non-significant decrease in insulin-mediated glucose disposal. This corroborates our earlier observation that insulin sensitivity is not markedly decreased in this subtype of MODY (9). In contrast, patients with NIDDM had a significant decrease in insulin-mediated glucose disposal, indicating insulin resistance. This may be attributed, at least in part, to their greater body weight.

The glycemic threshold at which glucose production started to increase and the absolute values of glucose production at a glycemia of 3.6 mmol/l were similar in MODY3 patients, in patients with NIDDM and in healthy controls. Taken together, these data indicate that the control of glucose production was only modestly altered in MODY3 patients. Furthermore, as similar alterations were observed in patients with NIDDM, they suggest that these alterations may be essentially secondary to the effect of chronic hyperglycemia and glucose toxicity (10). Thus our data do not support the hypothesis of major alterations of hepatic or renal glucose production as a direct consequence of HNF-1 $\alpha$  mutations.

HNF-1 $\alpha$  is also expressed in pancreatic  $\alpha$  cells, and an impaired arginine-induced glucagon secretion has been observed in patients with HNF-1 $\alpha$  mutations (12) or with HNF-4  $\alpha$  (19). This raised the possibility that impaired glucagon release may place these patients at an increased risk of severe insulin-induced hypoglycemia. Our present data however indicate that glucagon secretion was not impaired in MODY3 patients during hypoglycemia. These results therefore do not support the hypothesis of an impaired counterregulatory response to hypoglycemia in MODY3. Our experimental procedure, however, produced only a moderate hypoglycemia, which does not permit us to undertake a quantitative evaluation of the amplitude of counterregulatory hormone responses in the different groups tested. The insulin infusion rate used in this study also produced modest hyperinsulinemia, and the ability of MODY3 patients to reverse the inhibition of glucose production during hypoglycemia at greater plasma insulin concentrations cannot be evaluated from our data.

In conclusion, our data indicate that mutations of HNF-1 $\alpha$  do not result in abnormal counterregulatory responses to hypoglycemia. Suppression of endogenous glucose production by insulin is mildly impaired in MODY3 patients. This may however be secondary to chronic hyperglycemia, as similar alterations are observed in patients with NIDDM.

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