CLINICAL STUDY

Insulin secretion and insulin sensitivity in diabetic and non-diabetic subjects with hepatic nuclear factor-1 α (maturity-onset diabetes of the young-3) mutations

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Abstract

Objective: To evaluate insulin secretion and sensitivity in affected (diabetes mellitus or impaired glucose tolerance; $n = 7$) and in unaffected (normal glucose tolerance; $n = 3$) carriers of hepatocyte nuclear factor-1 α (maturity-onset diabetes of the young-3 (MODY3)) gene mutations.

Methods: Insulin secretion was assessed by an i.v. glucose tolerance test (IVGTT), hyperglycemic clamp and arginine test, and insulin sensitivity by an euglycemic hyperinsulinemic clamp. Results were compared with those of diabetic MODY2 (glucokinase-deficient) and control subjects.

Results: The amount of insulin secreted during an IVGTT was decreased in affected MODY3 subjects (46 ± 24 (s.d.) pmol/kg body weight (BW)) as compared with values in MODY2 (120 ± 49 pmol/kg BW) and control (173 ± 37 pmol/kg BW; $P = 0.0004$) subjects. The amount of insulin secreted during a 10 mmol/l glucose clamp was decreased in affected MODY3 subjects (171 ± 78 pmol/kg BW) and MODY2 subjects (302 ± 104 pmol/kg BW) as compared with control subjects (770 ± 199 pmol/kg BW; $P = 0.0001$). Insulin secretion in response to arginine was decreased in affected MODY3 subjects. Milder and heterogeneous defects were observed in the unaffected MODY3 subjects; the amount of insulin secreted during the hyperglycemic clamp was 40–79% of that of controls. The response to arginine was abnormally delayed. Insulin sensitivity was decreased in diabetic but not in non-diabetic MODY3 subjects.

Conclusions: Beta-cell dysfunction in response to glucose and arginine is observed in affected and unaffected MODY3 subjects. The MODY3 and MODY2 subtypes present different insulin secretion profiles. Secondary insulin resistance might contribute to the chronic hyperglycemia of MODY3 patients and modulate their glucose tolerance.

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Introduction

Maturity-onset diabetes of the young (MODY) is an autosomal dominant subtype of non-insulin-dependent diabetes mellitus (NIDDM), characterized by early age of onset (1). MODY is genetically heterogeneous and so far mutations in five genes on chromosomes 20q, 7p, 12q, 13q and 17 have been shown to cause MODY. These genes encode the transcription factor hepatocyte nuclear factor 4- α (HNF-4 α /MODY1) (2, 3), the enzyme glucokinase (GCK/MODY2) (4, 5), and the transcription factors HNF-1 α (HNF-1 α /MODY3) (6, 7), insulin promoter factor-1 (IPF1) (8) and HNF-1 β /TCF2

(9) respectively. About 15–25% of MODY families show no mutations in these loci suggesting that at least one additional locus remains to be identified (10). The genetic heterogeneity of MODY is associated with clinical and metabolic heterogeneity (1). Mutations in GCK/MODY2 result in mild chronic hyperglycemia due to reduced pancreatic beta-cell responsiveness to glucose, and decreased net accumulation of hepatic glycogen and increased hepatic gluconeogenesis following meals (5, 11–13). In contrast, MODY1 and MODY3 are characterized by major hyperglycemia associated with microvascular complications (14, 15). Data on IPF1- and HNF-1 β -related diabetes are scarce.

HNF-1 α is a transcription factor involved in tissue-specific expression of many genes in the liver, kidney, the endocrine and exocrine pancreas, and a variety of other tissues (16, 17). However, the target genes responsible for the MODY3 phenotype and the mechanisms implicated in the hyperglycemia remain unclear. We and others have shown that MODY3 is associated with a beta-cell defect (15, 18, 19). The present report describes detailed evaluations of insulin secretion and insulin sensitivity in individuals carrying *HNF-1 α* mutations who have a range of glucose tolerance from normal to impaired to NIDDM.

Methods

Subjects

The MODY kindreds participating in the present study belong to a cohort of Caucasian families with multiple cases of diabetes collected for the study of the genetic determinants of the different types of diabetes mellitus (DM) (20). All kindreds are of French ancestry, except FS4, which is of Swiss ancestry. A 75-g oral glucose tolerance test (OGTT) was performed in 18 heterozygous carriers of *HNF-1 α* mutations from nine different MODY3 kindreds, and in 27 unaffected first-degree relatives, homozygous for the wild-type allele, who were used as a control group (7). Subjects with *HNF-1 α* mutations had normal glucose tolerance (NGT; $n=4$), impaired glucose tolerance (IGT; $n=4$) or DM ($n=10$) according to criteria of the World Health Organization (21). Basic demographic and clinical data of family members are shown in Table 1. Evaluations of insulin secretion and insulin sensitivity by an i.v. glucose

tolerance test (IVGTT), arginine test and glucose clamps were then performed in ten carriers of *HNF-1 α* mutations from six kindreds, who accepted these evaluations. They had NGT ($n=3$), IGT ($n=2$) or DM ($n=5$). Patients were divided into two groups according to the glucose tolerance status: one group consisted of the seven hyperglycemic individuals with IGT or DM (affected MODY3 subjects), and the other group consisted of the three subjects with NGT (unaffected MODY3 subjects). *HNF-1 α* mutations in these subjects were G31D (subject M3#10), R55G56fsdelGAGGG (M3#3 and M3#4), Y122C (M3#2 and M3#6), S142F (M3#1, M3#7 and M3#8), R171X (M3#5) and P291fsdelC (M3#9). Eleven healthy individuals, unrelated to the patients and with no diabetic relatives, were used as controls for these evaluations of insulin secretion and insulin sensitivity. Affected MODY3 subjects were also compared with a group composed of six diabetic carriers of *GCK/MODY2* mutations. Data from the diabetic MODY2 subjects have been partially reported previously (12, 22). Profiles of the basic demographic and clinical data of patients and controls who underwent these metabolic investigations are shown in Table 2. All subjects were in good general health; those with diabetes had no evidence of diabetic complications. All diabetic subjects were treated by sulfonylurea and subjects M3#1 and M3#5 also received biguanides. As not all of the 18 carriers of *HNF-1 α* mutations tested by OGTT accepted the additional metabolic evaluations, we have also estimated the beta-cell function from the OGTT data (see below). Subjects were compared with normoglycemic first-degree relatives, carriers of wild-type alleles. All subjects participating in the study gave written informed consent.

Table 1 Demographic and clinical profile of family members: OGTT studies. Data expressed as mean $\pm$ s.d. Statistics are Kruskal–Wallis test (P values) followed by Tukey–Kramer HSD test when appropriate. Sex was compared with a contingency table chi-square test.

	MODY3 subjects			First-degree relatives ^a	P
	DM	IGT	NGT		
Subjects (n)	10	4	4	27	–
Sex (M/F)	5/5	1/3	4/0	15/12	0.15
Age (years)	42 $\pm$ 10	22 $\pm$ 7	32 $\pm$ 11	34 $\pm$ 10	0.04
Body mass index (kg/m ²)	22.9 $\pm$ 1.1	21.9 $\pm$ 1.6	25.4 $\pm$ 6.9	22.7 $\pm$ 3.0	0.74
Fasting glucose (mmol/l)	8.7 $\pm$ 3.2*	5.0 $\pm$ 0.4	5.6 $\pm$ 0.4	5.1 $\pm$ 0.5	<0.003
Fasting insulin (pmol/l)	40 $\pm$ 28	45 $\pm$ 38	48 $\pm$ 19	42 $\pm$ 37	0.77
2-h glucose (mmol/l)	16.1 $\pm$ 4.8*	9.2 $\pm$ 1.3*	5.4 $\pm$ 1.1	5.7 $\pm$ 1.2	<0.0001
2-h insulin (pmol/l)	80 $\pm$ 67	123 $\pm$ 116	140 $\pm$ 118	172 $\pm$ 115	0.11
Relative insulin response (l/mmol) ^b	0.23 $\pm$ 0.21*	1.03 $\pm$ 1.01*	1.85 $\pm$ 1.18	2.86 $\pm$ 2.46	<0.0001
Triglycerides (mmol/l)	0.74 $\pm$ 0.21	0.79 $\pm$ 0.17	1.04 $\pm$ 0.49	0.90 $\pm$ 0.39	0.48
Total cholesterol (mmol/l)	5.20 $\pm$ 1.05	5.12 $\pm$ 0.30	4.53 $\pm$ 0.10	5.09 $\pm$ 0.94	0.74
High density lipoprotein cholesterol (mmol/l)	1.41 $\pm$ 0.23	1.26 $\pm$ 0.28	1.20 $\pm$ 0.23	1.49 $\pm$ 0.45	0.50
Low density lipoprotein cholesterol (mmol/l)	3.45 $\pm$ 0.98	3.50 $\pm$ 0.60	2.86 $\pm$ 0.45	3.12 $\pm$ 0.79	0.56

* Statistically significant differences ($P < 0.05$) as compared with the unaffected relatives.

^a Normoglycemic homozygous carriers of wild-type alleles.

^b ($\Delta 30-0$ min insulin/ $\Delta 30-0$ min glucose)/basal insulin.

Table 2 Demographic and clinical profile of subjects and controls from insulin secretion and insulin sensitivity studies. Data expressed as mean $\pm$ s.d. Statistics are Kruskal–Wallis test (*P* values) followed by Tukey–Kramer HSD test when appropriate (see Methods). Sex was compared with a contingency table chi-square test. Data from the three unaffected MODY3 subjects are not included in the statistics.

	Sex	Age (years)	BMI (kg/m ²)	Age of diagnosis (years)	Hb A1C ^a (%)	FPG ^b (mmol/l)	2hPG ^b (mmol/l)
Affected MODY3 subjects	2M/5F	30 $\pm$ 11	22.6 $\pm$ 2.6	15 $\pm$ 3	6.4 $\pm$ 1.3	7.7 $\pm$ 2.0*	13.2 $\pm$ 4.2*
Unaffected MODY3 subjects	3M/0F	31 $\pm$ 7	23.8 $\pm$ 4.0	–	5.2 $\pm$ 0.1	5.1 $\pm$ 0.3	4.7 $\pm$ 1.2
Control group	6M/5F	28 $\pm$ 4	21.5 $\pm$ 1.6	–	–	4.8 $\pm$ 0.5	4.8 $\pm$ 1.0
MODY2 subjects	5M/1F	34 $\pm$ 10	21.5 $\pm$ 2.7	16 $\pm$ 3	6.2 $\pm$ 0.2	7.9 $\pm$ 0.3*	10.7 $\pm$ 0.9*
Statistical significance (<i>P</i>)	0.14	0.46	0.50	0.67	0.72	0.0008	0.0008

* Statistically significant differences (*P* < 0.05) as compared with the control group.

^a Hb A1C: glycated hemoglobin A1C; standard values in our laboratory for non-diabetic subjects: 4.9 $\pm$ 0.7%.

^b FPG and 2hPG stand for fasting and 2-h plasma glucose, respectively, during an OGTT.

Study design

Insulin secretion and insulin sensitivity were evaluated during two experimental protocols performed less than 1 week apart. The first protocol involved the measurements of insulin secretory responses during an IVGTT, hyperglycemic clamp and i.v. injection of arginine. The second protocol consisted of an euglycemic hyperinsulinemic clamp to assess insulin action. These evaluations were performed at the Clinical Immunology Department, Hôpital Necker-Enfants Malades, Paris, France, except for subject M3#9 who was studied at the Division of Clinical Physiology, University Medical Center, Lausanne, Switzerland. Subject preparation and blood sampling details were described previously (23).

Insulin secretory responses during IVGTT, hyperglycemic clamp and arginine administration The IVGTT was performed in all subjects with MODY-3 mutations and the hyperglycemic clamp and the arginine test were performed in nine out of the ten subjects. Following a 20-min baseline sampling period, all subjects received a bolus of 30% dextrose (0.5 g/kg body weight (BW)) over exactly 3 min. Completion of glucose administration was marked as time 0. From time 15 to 120 min, a hyperglycemic clamp was performed during which plasma glucose was clamped at 10 mmol/l by means of a variable rate i.v. infusion of 30% dextrose (Infusomat, Secura Braun, Melsungen, Germany). Plasma glucose concentration was determined at 5-min intervals and the glucose infusion rate was automatically adjusted by a microcomputer using algorithms previously described (24). In order to standardize the prevailing level of glycemia for the arginine test (25), the arginine bolus was administered at the end of the glucose clamp: at time 120 min, 5 g of 10% arginine chlorhydrate was injected i.v. in 30 s and sampling was continued for an additional 15 min.

Euglycemic hyperinsulinemic clamp This test was performed in seven out of the ten MODY-3 subjects. After a 30-min baseline sampling period, insulin

(Actrapid, Novo-Nordisk Industry, Copenhagen, Denmark) was administered as a primed continuous infusion for 2 h with a priming dose of 10 mU/kg BW and an infusion rate of 1 mU/kg BW per min. Plasma glucose concentration was determined at 5-min intervals and the rate of an i.v. 30% dextrose infusion (Infusomat) was automatically adjusted by a microcomputer to maintain plasma glucose at a pre-set level of 4.7 mmol/l (24).

Assays Throughout the experiments, blood was regularly sampled for glucose, insulin and C-peptide measurements. Plasma glucose was measured by the glucose oxidase technique (Beckman II analyzer, Beckman, Fullerton, CA, USA). Plasma insulin and plasma C-peptide were measured by RIAs. Cross-reactivity with intact pro-insulin is at least 30% for insulin and 15% for C-peptide assays.

Data analysis

OGTT Beta-cell function in response to the oral ingestion of 75 g glucose was quantified as the ratio of the incremental plasma insulin response above basal at 30 min to that of plasma glucose, divided by basal plasma insulin levels ((Δ 30–0 min insulin/ Δ 30–0 min glucose)/basal insulin), as previously described (26, 27). This measure of insulin secretion was termed 'relative insulin response'.

IVGTT, hyperglycemic clamp and arginine test

First-phase insulin secretion following an IVGTT was expressed as the respective sum of insulin and C-peptide values of 1 and 3 min. Hyperglycemic clamp results are expressed as the average insulin and C-peptide values from 60 to 120 min. Responses to arginine were expressed as the incremental area above the value at time 120 min of insulin and C-peptide, calculated by the trapezoidal rule using values at 122, 123, 124, 125 and 130 min. During the study period, insulin secretion rates (ISRs; pmol/kg BW per min) were computed

for each 1-min interval from time 0 to 20 min, and for each 2-min interval thereafter (see below). The amount of insulin (pmol/kg BW) secreted during the IVGTT (0–10 min), hyperglycemic clamp (60–120 min) and arginine test (120–130 min) was computed from the estimated ISR during these periods.

Estimations of ISR ISRs were derived by deconvolution of the plasma C-peptide concentrations (28) using version 3.4a of the ISEC software (29). Individual kinetics parameters of C-peptide clearance are computed by ISEC from standard kinetic parameters (30), taking into account the age, sex, body surface area and glucose tolerance status of the subject.

Euglycemic hyperinsulinemic clamp Average values of plasma glucose and insulin, and of glucose infusion rates during the last 30 min of the clamp (90–120 min) were used in the calculations. Glucose clearance was computed by dividing the average glucose infusion rate (mmol/kg BW per min) by the average plasma glucose concentration (mmol/l) (31). Insulin sensitivity was expressed as an insulin sensitivity index (ISI) computed as the ratio glucose clearance/plasma insulin concentration, multiplied by 10^6 ($\mu\text{l/kg BW per min per pmol/l insulin}$). Two unaffected MODY3 subjects were not tested with an euglycemic hyperinsulinemic clamp, and ISI was then computed with data from the hyperglycemic clamp (90–120 min), as this represents an adequate alternative for individuals with preserved insulin secretion (32). The use of glucose clearance, instead of glucose infusion rates, in the computation of ISI minimizes the bias related to different mass action effects of plasma glucose at different glucose levels (euglycemia vs hyperglycemia) on rates of glucose disposal (31).

Statistical analysis Data are expressed as mean $\pm$ standard deviation (S.D.) unless stated otherwise. A Kruskal–Wallis test (rank sums) for non-parametric data was used for comparisons between groups. When this test was significant, comparisons between pairs were made using the Tukey–Kramer HSD test with log-transformed data (33). Qualitative traits were analyzed by contingency table chi-square tests. Individual and/or average data are given in the text, tables and figures for the group of unaffected MODY3 subjects, but the data were not included in the statistics due to the small size of the group. For summarizing the responses of each subject to the different tests of insulin secretion and action described above, individual tests were considered normal when values were higher than 1 S.D. below the mean of controls for the respective test, moderately decreased ($\downarrow$) when values were between 1 and 2 S.D. below the mean of controls, and severely decreased ($\Downarrow$) when lower than 2 S.D. below the mean of controls. These computations were performed with

data normalized by log-transformation. A conservative approach was used and both the insulin and C-peptide results, as well as the amount of secreted insulin computed from the ISR, when appropriate, had to be abnormal to deem the test result abnormal. Statistics were performed with JMP software (SAS Institute Inc., Cary, NC, USA).

Results

OGTT

The relative insulin response following the oral ingestion of glucose was significantly lower in MODY3 subjects with DM (0.23 ± 0.21 l/mmol) or IGT (1.03 ± 1.01 l/mmol) than in their normoglycemic first-degree relatives, carriers of wild-type alleles (2.86 ± 2.46 l/mmol; Kruskal–Wallis $P < 0.0001$; Table 1). The response in unaffected MODY3 subjects was decreased but not significantly different from that of the normoglycemic wild-type relatives (1.85 ± 1.18 l/mmol).

IVGTT and hyperglycemic clamp

Fasting plasma glucose was significantly higher in affected MODY3 and in MODY2 subjects than in controls, while fasting insulin and C-peptide concentrations were not significantly different in any of the groups (Table 3). Plasma glucose levels peaked 1 min after the injection of an i.v. glucose bolus, showing a trend towards higher levels in affected MODY3 subjects and in MODY2 subjects than in controls ($P = 0.06$). The first-phase insulin secretion was lower in diabetic MODY3 subjects than in subjects from the other groups. Insulin and C-peptide values at 1 and 3 min, as well as the amount of insulin secreted from 0 to 10 min computed from the ISR, were significantly decreased as compared with values in control subjects (Table 3 and Fig. 1). C-peptide values at 1 and 3 min and the amount of insulin secreted from 0 to 10 min in affected MODY3 subjects were also significantly lower than values in MODY2 subjects, while insulin values at 1 and 3 min, although decreased, failed to reach significance. The first-phase insulin secretion was well preserved in unaffected MODY3 subjects. When considering insulin and C-peptide values at 1 and 3 min and the amount of insulin secreted from 0 to 10 min, at least one of these parameters was normal (higher than 1 S.D. below the mean of the controls) in all unaffected MODY3 subjects (Table 3 and Fig. 1).

From time 60 to 120 min of the hyperglycemic clamp study, plasma glucose concentrations were 12.4 ± 1.6 , 10.5 ± 0.8 and 10.1 ± 0.5 mmol/l in affected MODY3, MODY2 and control subjects respectively ($P = 0.04$), and were 10.7 ± 0.7 mmol/l in unaffected MODY3 subjects. Concomitant mean plasma insulin and C-peptide values, as well as the amount of insulin

Table 3 IVGTT, hyperglycemic clamp and arginine test. Data are mean $\pm$ s.d. Insulin and C-peptide (90–120 min) during the hyperglycemic clamp represent the mean $\pm$ s.d values of samples obtained at 60, 80, 90, 100, 110 and 120 min. Statistics are Kruskal–Wallis test (*P* values) followed by Tukey–Kramer HSD test when appropriate (see Methods). Data from the three unaffected MODY3 subjects are not included in the statistics.

Subjects (glucose tolerance status)	IVGTT						Hyperglycemic clamp		Arginine test	
	Glucose 0 min (mmol/l)	Insulin 0 min (pmol/l)	C-peptide 0 min (nmol/l)	Glucose peak (mmol/l)	Insulin 1 + 3 min (pmol/l)	C-peptide 1 + 3 min (nmol/l)	Insulin 60–120 min (pmol/l)	C-peptide 60–120 min (nmol/l)	Insulin area (pmol/l × min)	C-peptide area (nmol/l × min)
M3#1 (DM)	12.1	12	0.35	28.4	18	0.42	23 ± 6	0.58 ± 0.08	768	5.15
M3#2 (DM)	7.2	42	0.51	21.9	240	1.79	–	–	–	–
M3#3 (DM)	8.6	12	0.32	26.7	60	0.75	20 ± 4	0.34 ± 0.07	450	2.89
M3#4 (DM)	9.9	12	0.58	25.5	42	1.38	30 ± 7	0.66 ± 0.22	912	3.86
M3#5 (DM)	9.3	28	0.54	21.4	60	1.22	28 ± 3	0.73 ± 0.10	510	5.25
M3#6 (IGT)	5.2	30	0.44	15.2	114	1.06	70 ± 5	1.01 ± 0.07	1236	4.15
M3#7 (IGT)	5.7	54	0.75	19.6	246	1.75	58 ± 11	0.88 ± 0.09	3780	9.10
M3#8 (NGT)	4.1	18	0.41	14.9	756	3.53	95 ± 10	1.53 ± 0.07	4476	9.77
M3#9 (NGT)	5.1	44	0.57	21.2	264	3.34	194 ± 77	2.72 ± 0.44	–	–
M3#10 (NGT)	5.2	38	0.83	22.9	444	3.59	170 ± 22	2.53 ± 0.22	1950	5.05
Affected MODY3 subjects	8.3 ± 2.4*	27 ± 17	0.50 ± 0.15	22.7 ± 4.6	111 ± 94*	1.20 ± 0.50*#	39 ± 22*	0.70 ± 0.24*	1276 ± 1259*	5.07 ± 2.16*
Unaffected MODY3 subjects	4.8 ± 0.6	33 ± 14	0.60 ± 0.21	19.7 ± 4.2	488 ± 249	3.49 ± 0.13	153 ± 50	2.26 ± 0.64	–	–
Control group	5.0 ± 0.3	44 ± 19	0.72 ± 0.31	18.5 ± 3.0	799 ± 353	3.97 ± 0.81	286 ± 92	2.74 ± 0.45	4471 ± 2411	10.69 ± 2.61
MODY2 subjects	7.1 ± 0.5*	30 ± 12	0.50 ± 0.22	21.2 ± 2.8	429 ± 246*	3.08 ± 1.26	74 ± 54*	1.23 ± 0.28*	1416 ± 414*	7.56 ± 1.75
Statistical significance (<i>P</i>)	0.0003	0.14	0.13	0.06	0.0004	0.0007	0.0002	0.0001	0.0015	0.006

Symbols represent statistically significant differences (*P* < 0.05) as compared with the control group (*), and MODY2 subjects (#).

Figure 1 (A) ISRs during the IVGTT (0–20 min) and hyperglycemic clamp (20–120 min) in affected MODY3 (●), unaffected MODY3 (○), MODY2 (■) and control (□) subjects. Results expressed as mean (IVGTT) or mean $\pm$ S.E.M. (hyperglycemic clamp) for the sake of clarity. (B) Amount of insulin secreted during the IVGTT (0–10 min) and hyperglycemic clamp (90–120 min). Results expressed as mean $\pm$ S.D. or individual data (hatched bars) for unaffected MODY3 subjects. Statistics are the Kruskal–Wallis test (P values) followed by a Tukey–Kramer HSD test for comparison between pairs when appropriate; the symbols represent statistically significant differences ($P < 0.05$) as compared with the control group (*) and MODY2 subjects (#).

secreted during this time-interval computed from ISRs, were greatly decreased in affected MODY3 subjects, and moderately but significantly decreased in MODY2 subjects as compared with controls (Table 3 and Fig. 1). The three unaffected MODY3 subjects showed heterogeneous responses. When considering mean plasma insulin and C-peptide values, and the amount of insulin secreted during the hyperglycemic clamp, one subject presented decreased responses (lower than 2 S.D. below the average of controls), while the remaining two subjects presented at least one of these parameters within the normal range (higher than 1 S.D. below the mean of the controls).

Arginine test

At the end of the hyperglycemic clamp (time 120 min), plasma glucose was 10.8 ± 1.2 , 9.8 ± 0.2 and 10.0 ± 0.3 mmol/l in affected MODY3, MODY2 and control subjects respectively ($P < 0.04$), and was 10.2 ± 0.8 mmol/l in unaffected MODY3 subjects. Concomitant plasma insulin values were 41 ± 26 , 78 ± 64 , 339 ± 118

($P < 0.0002$) and 139 ± 48 pmol/l; C-peptide values were 0.69 ± 0.28 , 1.14 ± 0.30 , 3.08 ± 0.62 ($P < 0.0001$) and 2.28 ± 0.73 nmol/l respectively in affected MODY3, MODY2, control subjects and unaffected MODY3 subjects. To account for the observed heterogeneity of plasma insulin and C-peptide values, the responses to the arginine test were expressed as the increment over levels at time 120 min. The area under the insulin curve, computed from time 120 to 130 min, was significantly reduced in affected MODY3 and MODY2 subjects as compared with controls, while the area under the C-peptide curve was significantly reduced only in affected MODY3 subjects (Table 3). The amount of insulin secreted in response to arginine was computed from time 120–130 min from the increment in ISR over levels at time 120 min (Fig. 2). The levels in affected MODY3 subjects were significantly reduced as compared with those in controls, while MODY2 subjects had well-preserved responses. Here again, when excluding the two affected MODY3 subjects with IGT, the responses of the remaining five affected MODY3 subjects with DM were significantly lower than those of

Figure 2 (A) Increase in ISR over the 120 min value during the i.v. arginine test in affected MODY3 (●), unaffected MODY3 (○), MODY2 (■) and control (□) subjects. Results expressed as mean $\pm$ S.E.M. for the sake of clarity. (B) Amount of insulin secreted during the arginine test (120–130 min). Results expressed as mean $\pm$ S.D. or individual data (hatched bars) for unaffected MODY3 subjects. Statistics are the Kruskal–Wallis test (P value) followed by a Tukey–Kramer HSD test for comparison between pairs when appropriate; the symbols represent statistically significant differences ($P < 0.05$) as compared with the control group (*).

the MODY2 subjects who all had DM (data not shown). The two unaffected MODY3 subjects who were tested had quantitatively normal responses (Table 3, Fig. 2). However, insulin secretion in response to arginine was delayed in both individuals as compared with subjects in the other groups (Fig. 2).

Hyperinsulinemic euglycemic clamp

Average plasma glucose values observed from 90 to 120 min were 4.7 ± 0.1 mmol/l for affected MODY3

Figure 3 ISI in affected MODY3, unaffected MODY3 and control subjects. Results computed from data of the euglycemic hyperinsulinemic clamp, except for two unaffected MODY3 subjects, for whom it was computed from data of the hyperglycemic clamp (see Methods). Results expressed as individual values (●) and group mean $\pm$ S.D. (○).

subjects and 4.8 ± 0.3 mmol/l ($P = 0.84$) for control subjects. Insulin values were 334 ± 39 and 369 ± 44 pmol/l ($P < 0.06$) respectively. Concomitant glucose infusion rates were significantly lower in affected MODY3 subjects (5.73 ± 2.19 mg/kg BW per min) than in controls (9.54 ± 3.04 mg/kg BW per min; $P < 0.02$). Individual ISIs computed from hyperinsulinemic euglycemic clamps or from hyperglycemic clamps are shown in Fig. 3. Average values were 21.8 ± 8.5 and 30.2 ± 9.8 μ l/kg BW per min per pmol/l insulin ($P < 0.07$) for affected MODY3 and control subjects respectively, and 25.8 ± 7.3 μ l/kg BW per min per pmol/l insulin for unaffected MODY3 subjects.

Discussion

The present study shows that affected MODY3 subjects with DM or IGT present a severe defect of insulin secretion in response to glucose and arginine. Both the first-phase insulin secretion in response to glucose, assessed by IVGTT, and the second-phase insulin secretion, assessed by the hyperglycemic clamp, were substantially decreased (Tables 3 and 4). The amount of insulin secreted during the IVGTT and the hyperglycemic clamp by affected MODY3 subjects was less than 27 and 23% respectively of the amount secreted by the control subjects during these two tests. Furthermore, the increase in the amount of insulin secreted in response to arginine in affected MODY3 subjects was less than 32% of that observed in control subjects. Defects of insulin secretion were also detected in unaffected MODY3 subjects with NGT. The first-phase insulin secretion in response to glucose was well preserved, but the second phase, assessed by the hyperglycemic clamp was variably decreased in these subjects. The amount of insulin secreted during the

Table 4 Summary of observed defects in MODY3 subjects. Computations performed with log-transformed data: N: value higher than 1 s.d. below the mean of controls; ↓: value between 1 and 2 s.d. below the mean of controls; ↓↓: value lower than 2 s.d. below the mean of controls; D: delayed response.

Subject	Glucose tolerance status	OGTT (insulin secretion)	IGTT	Hyperglycemic clamp	Arginine test	Insulin sensitivity ^a
M3#1	DM	↓	↓↓	↓↓	↓↓	↓↓
M3#2	DM	↓	↓↓	—	—	↓
M3#3	DM	↓	↓↓	↓↓	↓↓	↓
M3#4	DM	↓	↓↓	↓↓	↓↓	↓
M3#5	DM	—	↓↓	↓↓	↓↓	—
M3#6	IGT	↓	↓↓	↓↓	↓↓	N
M3#7	IGT	N	↓↓	↓↓	N, D	N
M3#8	NGT	N	N	↓↓	N, D	N
M3#9	NGT	—	N	N	—	↓
M3#10	NGT	N	N	N	N, D	N

^aISI computed from euglycemic hyperinsulinemic clamp data, except for subjects M3#9 and M3#10, for whom it was computed from hyperglycemic clamp data.

hyperglycemic clamp by the three unaffected MODY3 subjects was 40, 69 and 79% of the amount secreted by the control subjects. Insulin secretion in response to arginine in the two unaffected MODY3 subjects who were tested, although quantitatively similar to that observed in control subjects, presented an abnormal temporal pattern. As shown in Fig. 2, the insulin secretion response was delayed and the return to basal (pre-arginine) levels was slow. This pattern of sustained response with slow return to basal levels was also observed in the affected MODY3 subjects with IGT and to a lesser extent in the affected MODY3 subjects with DM (data not shown). It is noteworthy that the response to the IVGTT was not consistently delayed in these subjects (Fig. 1).

The pathophysiological mechanisms leading to these defects remain unknown. It is interesting that severely impaired insulin secretory responses to glucose and arginine were also observed in perfused pancreas and perfused islets from HNF-1 α -deficient mice (34). However, these animals also suffer from severe energy and water wasting due to renal proximal tubular dysfunctions, with massive urinary glucose and amino acid loss (35) while these renal defects are either absent or much milder in MODY3 subjects (36). Impaired insulin secretion in response to arginine in mice and man would be consistent with a defect distal to the mechanisms of glucose sensing of beta-cells (37), and/or with a reduction in the beta-cell mass (38, 39). Pancreatic histology shows reduced beta-cell mass in HNF-1 α -deficient mice, which is, however, proportional to the reduction in mouse total BW (34). Thus, it is unclear whether the decreased insulin secretory response to arginine observed in mice and man is related to a reduction in beta-cell mass. It is noteworthy that insulin mRNA levels in the beta-cells of HNF-1 α -deficient mice were normal (34), suggesting that the secretory defects in response to glucose and arginine are not related to a direct effect of HNF-1 α on the insulin gene.

Our results are in agreement with those observed in response to graded i.v. glucose infusions in a group of American, English and French diabetic and non-diabetic MODY3 subjects (18). ISRs were decreased in the diabetic subjects over a broad range of glucose concentration. In non-diabetic MODY3 subjects, ISRs were similar to those of control subjects at plasma glucose levels lower than 8 mmol/l, but were significantly less than in controls at higher glucose levels, although individual results were heterogeneous. Interestingly, this pattern of decreased insulin secretion at high but not at low glucose levels was also observed in non-diabetic MODY1 subjects (40). As HNF-4 α is an upstream regulator of HNF-1 α expression (41), these two forms of MODY might share pathophysiological mechanisms. Decreased insulin secretion in response to OGTT and IVGTT were also reported in diabetic and non-diabetic Finnish MODY3 subjects (19). Interestingly, a 2- to 4-fold increase in insulin secretion, as compared with controls, in response to i.v. loads of glucose (IVGTT), tolbutamide or glucagon was reported by Hansen and co-workers in a non-diabetic Danish carrier of the P447L mutation (42). The authors suggest that hyperexcitability of beta-cells to i.v. secretagogues may be associated with the pre-diabetic stages of the disease. We have not observed such hyperexcitability of beta-cells in the three unaffected MODY3 subjects with NGT that we have studied (Table 3), who all carry different mutations.

The results observed in affected and unaffected MODY3 subjects contrast with those observed in diabetic MODY2 subjects, where first-phase insulin secretion and response to arginine are usually well preserved (Figs 1 and 2). Indeed, the beta-cell defect of MODY2 subjects seems to be primarily related to a glucose-sensing defect leading to an increase in the blood glucose threshold that triggers insulin secretion (12), and a right shift in the dose–response curve of glucose-induced insulin secretion (11), with an average 60% reduction in insulin secretion for a given glucose level.

The insulin secretion defect associated with GCK mutations is much less severe than that associated with *HNF-1 α* mutations. Diabetic MODY2 subjects secrete as much insulin as affected MODY3 subjects with IGT during a hyperglycemic clamp (Table 3), which supports the view that the GCK defect in the liver is an important contributor to the chronic hyperglycemia of MODY2 (13).

Insulin sensitivity was decreased in affected MODY3 subjects with DM and in one unaffected MODY3 subject with NGT who also presented with obesity (Table 4). A primary defect in insulin sensitivity associated with the *HNF-1 α* mutations should be detected before the development of chronic hyperglycemia, which was not the case in four MODY3 subjects with IGT or NGT. Insulin sensitivity was not decreased either in six lean Finish non-diabetic MODY3 subjects (19). Moreover, the study of five diabetic relatives of subject M3#9 from the Swiss family FS4 showed decreased stimulation of glucose utilization, oxidation and non-oxidative glucose disposal, as well as blunted suppression of endogenous production (43); these defects were shown to be related to the endogenous insulinopenia. Taken together these data are most consistent with the conclusion that the insulin resistance observed in diabetic MODY3 subjects is secondary to the pancreatic deficiency and/or resulting hyperglycemia. In this regard, Herman and co-workers assessed insulin secretion and insulin sensitivity in diabetic and non-diabetic MODY1 subjects (44). Similar insulin secretion abnormalities were observed in diabetic and non-diabetic subjects, while only some of the diabetic subjects had decreased insulin sensitivity. Polonsky and co-workers (45) used a mathematical model of the insulin–glucose feedback mechanisms and the data of the dose–response curve glucose/ISR to simulate the effects of variations in insulin sensitivity on glucose tolerance in non-diabetic MODY1 subjects and in their mutation-negative relatives. They observed that a moderate decrease in insulin sensitivity (40%) was associated with transition from NGT to overt diabetes in non-diabetic MODY1 subjects, given their insulin secretion defect, while no such effect was observed in the mutation-negative relatives. Studies of insulin sensitivity in a cohort of 125 MODY2 subjects showed that insulin resistance was associated with the deterioration of the glucose tolerance status (46): insulin sensitivity was higher in NGT, intermediate in IGT, and lower in diabetic MODY2 subjects. Taken together, these data suggest that, although not a primary event in the development of hyperglycemia of MODY, insulin resistance modulates the glucose tolerance and contributes as an additional mechanism for the chronic hyperglycemia.

In summary, affected MODY3 subjects with DM or IGT present a severe defect of insulin secretion in response to glucose and arginine. A less severe defect was also observed in unaffected MODY3 subjects with NGT, suggesting that these individuals carry a high risk for

developing NIDDM. The pathophysiological mechanisms and the target genes of *HNF-1 α* responsible for this defect remain unknown. They might be related to the glucose metabolism, to mechanisms that are distal to those of glucose sensing in beta-cells, and/or to a decrease in beta-cell mass. The MODY3 and MODY2 subtypes present different clinical and insulin secretion profiles, suggesting that even in relatively homogenous subtypes of NIDDM such as MODY, the genetic characterization may reveal specific metabolic profiles.

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