β-Cell Dysfunction, Insulin Sensitivity, and Glycosuria Precede Diabetes in Hepatocyte Nuclear Factor-1α Mutation Carriers

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OBJECTIVE — Patients with diabetes due to hepatocyte nuclear factor (HNF)-1α mutations have β-cell deficiency, insulin sensitivity, altered proinsulin levels, and a low renal threshold for glucose. It is uncertain how many of these features precede the development of diabetes. The aim of our study was to test for these characteristics in young nondiabetic HNF-1α mutation carriers.

RESEARCH DESIGN AND METHODS — A total of 47 offspring from 19 extended families underwent genetic testing, a standard oral glucose tolerance test, and urine testing.

RESULTS — HNF-1α mutations were found in 20 offspring, 7 with diabetes and 13 without diabetes. The 13 nondiabetic mutation carriers were compared with 27 family control subjects, who were matched for age, sex, and BMI. There was marked β-cell deficiency with reduced insulinogenic index (53.5 [31.5–90.9] vs. 226.0 [126.0–407.1], SD [range], $P < 0.001$) and area under the curve for insulin ($P < 0.001$). Insulin sensitivity was increased in mutation carriers (homeostatic model assessment of insulin sensitivity 144.6 [82.7–252.7] vs. 100 [66.9–149.4], $P = 0.025$). A total of 38% of mutation carriers had glycosuria at 2 h compared with 0% of control subjects ($P = 0.0034$). Those with glycosuria had peak glucose values that were higher than the mutations carriers without glycosuria (range 8.1–11.8 vs. 6.2–8.4 mmol/l, $P = 0.002$). The seven subjects with diabetes all showed glycosuria.

CONCLUSIONS — We conclude that marked β-cell deficiency, increased insulin sensitivity, and a low renal threshold are present in young nondiabetic HNF-1α mutation carriers. The presence of glycosuria post–glucose load could be used to screen children of mutation carriers as it occurs in all mutation carriers with a peak glucose in the oral glucose tolerance test >8.4 mmol/l.

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Abbreviations: AUC, area under the curve; FPG, fasting plasma glucose; HNF, hepatocyte nuclear factor; HOMA, homeostatic model assessment; IGT, impaired glucose tolerance; MODY, maturity-onset diabetes of the young; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Maturity-onset diabetes of the young (MODY) results from mutations in at least six genes; the glycolytic enzyme glucokinase and the transcription factors hepatocyte nuclear factor (HNF)-1α, HNF-4α, HNF-1β, insulin promoter factor 1, and NeuroD1. The different genetic etiologies in MODY have distinct clinical phenotypes (1–3). Mutations in the gene (*TCF1*) encoding the transcription factor HNF-1α are the most common cause of MODY in Caucasians (4,5).

Patients with HNF-1α mutations have normal glucose tolerance in early childhood but typically present in their teens or early adult life with symptomatic diabetes and have increasing hyperglycemia and treatment requirements throughout life (6,7). Progressive β-cell failure leads to increasing hyperglycemia due to reduced insulin secretion in response to hyperglycemia (6,8–11). An increased proinsulin-to-insulin ratio was reported to be characteristic of the β-cell defect in HNF-1α subjects with diabetes in one study (6) but not in a second (9). A feature of the β-cell defects is that they are more sensitive to the hypoglycemic effects of sulfonylureas compared with patients with type 2 diabetes (12). There is controversy as to whether diabetic subjects with HNF-1α mutations show altered insulin sensitivity. Insulin sensitivity has been reported as increased (6,10), similar (9), and reduced (11). Marked insulin resistance and the associated dyslipidemia are not clinical features of subjects with diabetes due to an HNF-1α mutation (13).

A low renal threshold for glucose was first noted in MODY families by Tattersall in 1974 (14). Two of the families originally reported have since been shown to have HNF-1α mutations (15; A.S., S.E., A.T.H., personal communication). Glycosuria has since been reported in additional diabetic patients with HNF-1α mutations (16,17), and this is also a key feature of the HNF-1α–deficient

(HNF1$^{-/-}$) mouse (18). The mechanism has been shown to be reduced expression of the low-affinity/high-capacity glucose cotransporter (SGLT2) that is under direct control by HNF-1α (17).

Because the phenotype of HNF-1α patients may be complicated by hyperglycemia, the study of nondiabetic subjects is of interest and may assist in early detection of possible mutation carriers in families. Previous studies of nondiabetic mutation carriers in HNF-1α families have been on small numbers or older subjects. The largest study to date was performed on patients with a mean age of 29 years (9); nondiabetic subjects at this age may show a different phenotype from nondiabetic mutation carriers who go on to get diabetes at the usual age.

The studies performed on nondiabetic adult mutation carriers have clearly shown β-cell dysfunction with reduced first- and late-phase insulin secretion during an intravenous glucose tolerance test (9–11) and reduced insulin secretion in response to a hyperglycemic clamp and arginine (11). Contradictory results have been obtained with regard to an increased proinsulin-to-insulin ratio (6,9) and increased insulin sensitivity (6,9–11). Only a single nondiabetic mutation carrier has been reported to have glycosuria, corresponding to a blood glucose of 7.7 mmol/l (16).

The phenotype in young pre-diabetic mutation carriers has not been extensively studied. Young subjects under the mean age of diagnosis of diabetes are more likely to represent the common phenotype than subjects who appear protected from developing diabetes at the usual age. The aim of our study was to test for the presence of the phenotypes seen in diabetic HNF-1α gene mutation carriers (hyperglycemia, reduced insulin secretion, reduced proinsulin levels, increased insulin sensitivity, and glycosuria) in young nondiabetic mutation carriers from families with MODY.

RESEARCH DESIGN AND METHODS

— A total of 59 families with MODY due to HNF-1α mutations were identified from the Exeter Diabetes U.K. MODY collection. We were unable to contact 21 families, 11 did not have offspring of an appropriate age, and 8 declined to take part. A total of 47 subjects from 19 extended families agreed to undergo an oral glucose tolerance test (OGTT) either at their home or within our clinical investigations unit. Subjects with diabetes ($n = 7$), one previously not diagnosed, were excluded from analysis except as a reference group for the presence of glycosuria to allow assessment of glycosuria as a screening tool. The study was approved by a multicentered research ethics committee, and all subjects or their guardians gave written informed consent.

A detailed history was recorded from each subject. Height and body weight were measured with the subject in light clothing without shoes. Waist circumference was measured midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteal region. BMI and waist-to-hip ratio were then calculated.

A standard (75-g) OGTT was performed after an overnight fast. For subjects <42.9 kg, a glucose load of 1.75 g/kg was given. For subjects with known diabetes, oral hypoglycemic agents were omitted for 48 h before the study, and insulin was omitted on the morning of the study. Blood was collected at −5, 0, 30, 60, 90, and 120 min for plasma glucose and serum insulin and at −5 or 0 and 120 min for total proinsulin. Blood was collected from fasting subjects for HbA_{1c} (A1C), lipid profile, testosterone, estradiol, luteinizing hormone, follicle-stimulating hormone, and genetic screening. Samples for glucose, insulin, proinsulin, lipid profile, and sex hormones were separated in a portable centrifuge and kept on ice until being frozen before analysis.

Urine samples were collected at 0 and 120 min during the OGTT, and the presence of glucose was ascertained by dipstix [Combur test strips, glycosuria present if >2.8 mmol/l(+)].

Patients were assigned as pre- or postpubertal according to clinical criteria and testosterone levels in men and estradiol in women (19,20).

Measures of hyperglycemia

Fasting plasma glucose (FPG) was calculated from the mean of the samples taken at −5 and 0 min. Patients were classified as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and diabetes according to World Health Organization and American Diabetes Association criteria (21,22). Area under the curve (AUC) was calculated using the trapezoidal rule in subjects where all time points during OGTT were available. The peak glucose value was defined as the single highest plasma glucose during the OGTT.

Measures of insulin secretion and insulin sensitivity

Fasting serum insulin was calculated from the mean of the samples taken at −5 and 0 min. Incremental insulin (I_{30}) was the change in insulin concentration during the first 30 min of the OGTT (insulin 30 min − fasting insulin). β-Cell function was assessed using the insulinogenic index, which is defined as [(insulin 30 − fasting insulin)/(glucose 30 − fasting glucose)]. AUC for insulin was also calculated using the trapezoidal rule. Fasting intact serum proinsulin was calculated from the mean of samples taken at −5 and 0 min.

Insulin sensitivity was determined from the fasting glucose and specific insulin using homeostatic model assessment (HOMA). This is based on a model of the glucose-insulin feedback system in a homeostatic state (23). We used the modified version of this program (provided by Dr. J. Levy, OCDEM, Headington, Oxford, U.K.), which is adapted for specific insulin measurement, and assigned the value in control subjects as 100% (24).

Assays

Plasma glucose was measured by glucose oxidase–based methods (coefficient of variance [CV] 1.9%). A1C was measured by in-house high-performance liquid chromatography (CV 4.2%). Cholesterol, triglycerides, and HDL cholesterol were measured on Modular analyzers (Roche Diagnostics). LDL cholesterol was derived by calculation from the Friedewald formula. Estradiol, testosterone, follicle-stimulating hormone, and luteinizing hormone were measured on Immuno 1 analyzers (Bayer Healthcare). Insulin was measured using a specific immunoenzymometric assay (Medgenix, BioSource, U.K.) calibrated against International Reference Preparation 66/304 and with no detectable cross-reactivity with intact proinsulin or 32,33 split proinsulin (interassay CV <10% over range 95–1,038 pmol/l). Total proinsulin was measured by immunometric assays (antibodies 3B1/PEP001 and A6/3B1, respectively) with enzyme amplification as the detection system (Dako, Ely, U.K.). The assay was

Table 1—*Characteristics of nondiabetic mutation carriers and family members without a mutation*

	Nondiabetic mutation carriers	Family control subjects	*P*
n (M/F)	13 (10/3)	27 (17/10)	0.48
Age when tested (years)	15 (8–34)	17 (8–33)	0.69
BMI (kg/m^2)	22.21 ± 4.18	24.17 ± 7.06	0.36
WHR	0.84 ± 0.006	0.83 ± 0.009	0.93
Pubertal stage			
Male, pre/postpubertal	5/5	7/10	0.71
Female, pre/postpubertal	1/2	2/8	0.99
Lipid profile			
Cholesterol	4.3 (3.3–5.8)	4.2 (2.9–5.3)	0.32
Triglycerides	0.98 (0.54–2.84)	0.88 (0.43–1.32)	0.33
HDL	1.36 ± 0.38	1.28 ± 0.30	0.46
LDL	2.52 ± 0.79	2.38 ± 0.61	0.56
Total/HDL	3.20 (2.30–5.88)	3.21 (1.91–7.74)	0.89

Data are means ± SD or means (range), unless otherwise indicated. Age when tested was compared by the Mann-Whitney *U* test. BMI and WHR were compared by the *t* test. Categorical data were compared by the Fisher's exact test. Cholesterol, triglycerides, and total/HDL (total-to-HDL cholesterol ratio) were compared by the Mann-Whitney *U* test. HDL and LDL were compared by the unpaired *t* test. All units are given in millimoles per liter. WHR, waist-to-hip ratio.

calibrated against the First International Reference Reagent coded 84/611 (interassay CV <15% over range 16–76 pmol/l).

Mutation testing

DNA was extracted from peripheral lymphocytes using standard methods. Genomic DNA was amplified using primers previously described (25) with modifications to avoid allelic dropout (26). PCR products were purified and sequenced using BigDye Terminator chemistry (Applied Biosystems, Warrington, U.K.). Reactions were analyzed on an ABI Prism 377 DNA Sequencer (Applied Biosystems) and electropherograms compared with the index case and a normal reference sequence in order to assign the genotype.

Statistical analysis

Parametric data are presented as means ± SD and were compared by an unpaired *t* test. Insulin, total proinsulin, and HOMA data were log-transformed and are presented as the geometric means ± SD range. Other nonparametric data are presented as median (interquartile range) and compared by the Mann-Whitney *U* test. Categorical data were compared by Fisher's exact test. OGTT data were compared by repeated measures for ANOVA. Insulin sensitivity was compared by univariate ANOVA to allow for the potential confounders of age, BMI, sex, and pubertal stage. Data were analyzed using Microsoft Excel 2000 and SPSS for Windows (Version 11). All tests were two sided, and $P < 0.05$ was considered statistically significant. Our study had >80% power at $P < 0.05$ to detect a difference of 1.5 SD in the variables measured.

RESULTS

Clinical characteristics

A total of 47 subjects from 19 extended families agreed to undergo an OGTT. Ten subjects from these families were already known to have diabetes and a mutation in HNF-1α and did not take part. Twenty offspring were identified with a mutation (14 different mutations identified), of whom 11 had NGT, 2 had IGT (2-h plasma glucose 8.4 mmol/l and 9.4 mmol/l), and 7 had diabetes. Of the 27 nonmutation carriers (family control subjects), 26 had NGT and 1 had IGT (2-h plasma glucose 8.2 mmol/l).

The characteristics of the nondiabetic mutation carriers and family members without mutations (family control subjects) are shown in Table 1. The two groups were of similar age, pubertal stage, sex, BMI, and waist-to-hip ratio.

Hyperglycemia

There was no significant difference in A1C between mutation carriers and family control subjects (5.6 ± 0.6 vs. 5.3 ± 0.3%, $P = 0.10$). FPG was similar in the two groups (5.1 ± 0.5 vs. 5.0 ± 0.4 mmol/l, $P = 0.65$); however, the mutation carriers had a significantly higher 2-h plasma glucose compared with family control subjects (6.2 ± 1.7 vs. 5.3 ± 0.9 mmol/l, $P = 0.033$) (Fig. 1). In subjects where all time points during OGTT were available, mutation carriers ($n = 9$) had a higher AUC for glucose compared with family control subjects ($n = 26$) (839 ± 160 vs. 740 ± 115 mmol · l^{-1} · min^{-1}, $P = 0.05$). There was a significant difference during OGTT between the two groups on ANOVA for repeated measures ($P < 0.001$).

β-Cell deficiency

Mutation carriers had significantly lower fasting serum insulin (61 [35–106] vs. 88 [59–133] pmol/l, $P = 0.022$) and 2-h serum insulin (141 [57–350] vs. 295 [179–488] pmol/l, $P = 0.003$) compared with family control subjects (Fig. 1). Mutation carriers had a significantly lower early insulin response as shown by a significantly lower incremental insulin (125.2 [64.1–244.6]) vs. 428.3 [209.5–875.7] pmol/l, $P < 0.001$) and insulinogenic index (53.5 [31.5–90.9] vs. 226 [126.0–407.1], $P < 0.001$) compared with family control subjects. In subjects where all time points were available, mutation carriers ($n = 6$) had a significantly lower AUC for insulin compared with family control subjects ($n = 21$) (25,269 [17,726–36,021] vs. 53,309 [34,967–81,272] pmol · l^{-1} · min^{-1}, $P < 0.001$).

Proinsulin levels

There was no significant difference between mutation carriers and family control subjects in fasting total proinsulin concentrations (6.12 [2.97–12.64] vs. 40 [4.77–14.78] pmol/l, $P = 0.14$), 2-h total proinsulin concentrations (25.90 [12.2–54.8] vs. 32 [21.6–75.3] pmol/l, $P = 0.09$), or fasting total proinsulin-to-insulin ratio (0.09 [0.05–0.18] vs. 0.09 [0.06–0.16], $P = 0.86$).

Insulin sensitivity

In keeping with the similar fasting glucose but lower fasting insulin, insulin sensitivity as assessed by HOMA was significantly increased in mutation carriers compared with control subjects (144.6 [82.7–252.7] vs. 100 [66.9–149.4], $P = 0.025$). This remained significant when compared by univariate ANOVA, taking into

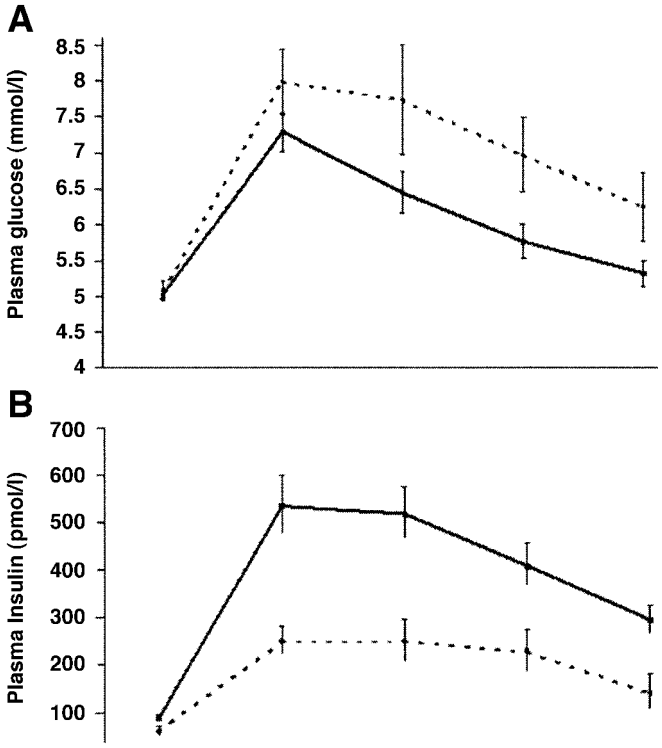

Figure 1—*Glucose and insulin values during an OGTT in nondiabetic HNF-1α mutation carriers and family control subjects without mutations.* A: *Means ± SE plasma glucose (mmol/l) (A) and geometric means ± SE serum insulin (pmol/l)* (B) *at fasting and 30, 60, 90, and 120 min during OGTT for mutation carriers (dashed line) and family control subjects (solid line).*

account age, BMI, sex, and pubertal stage ($P = 0.026$). There were no significant differences in serum HDL, total-to-HDL cholesterol ratios, or triglycerides between the two groups (Table 1).

Glycosuria

All seven diabetic subjects had glycosuria at 2 h, with a mean peak glucose of 20.4 ± 5.2 mmol/l (range 12.6–25.8). A total of 67% (4/6) also had glycosuria after fasting. A total of 38% (5/13) of the nondiabetic mutation carriers had glycosuria at 120 min compared with 0% (0/23) of the family control subjects ($P = 0.0034$) (Fig. 2). The nondiabetic mutation carriers who had glycosuria had a higher peak glucose value during OGTT than mutation carriers without glycosuria (10.2 ± 1.9 vs. 7.4 ± 0.7 mmol/l, $P = 0.002$). Mutation carriers with glycosuria had peak plasma glucose values ranging between 8.1 and 11.8 mmol/l compared with 6.2 and 8.4 mmol/l in individuals without glycosuria.

Limiting analysis to mutation carriers with NGT

To assess if the differences remained when the study was limited to mutation carriers with normoglycemia, we removed the two mutation carriers and the one family control with IGT and repeated the analysis. The only change in the results was that the difference in the 2-h glucose was no longer significant, but the AUC for glucose in the OGTT remained significantly higher in mutation carriers ($P = 0.04$). All other results remained significant, the insulinogenic index was reduced in mutation carriers (55.8 [32.5–95.7] vs. 229.3 [126.5–415.5], $P < 0.001$), insulin sensitivity (HOMA-S) was higher (148.2 [83.3–264.1] vs. 100 [66.4–150.7], $P = 0.024$), and glycosuria was present in mutation carriers (4/11 vs. 0/22, $P = 0.008$).

CONCLUSIONS

— We have shown that many of the phenotypes seen in subjects with diabetes due to a mutation in the HNF-1α gene are also seen in young mutation carriers before hyperglycemia develops. The key phenotypes present before hyperglycemia were marked β-cell deficiency, increased insulin sensitivity, and a low renal threshold for glucose.

Recruitment was difficult; even though this study was limited to OGTT (most often done at home), eight families were reluctant to take part. The high penetrance of HNF-1α mutations means it is difficult to find nondiabetic mutation carriers, especially when an OGTT is performed. Our study of 13 nondiabetic mutation carriers is second only to the Scandinavian cohort described by Groop and colleagues (9,10) and is bigger than other studies (8,11). Our subjects are considerably younger than previously reported series, with a median age of 15 years compared with 29 years (9,10). The

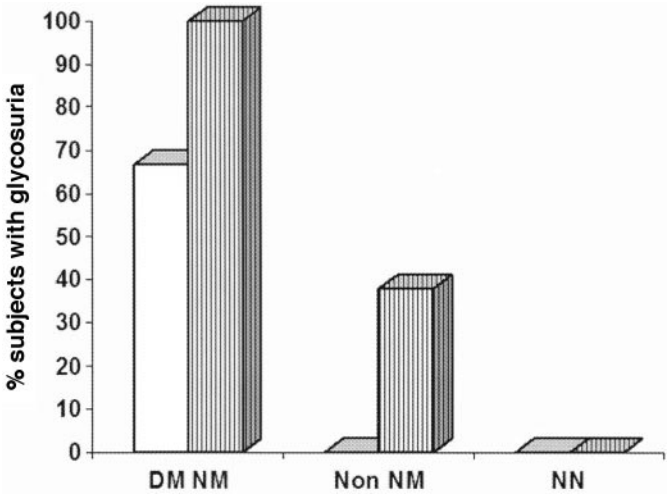

Figure 2—*Presence of glycosuria fasting and post-OGTT in subjects with and without HNF-1α mutations. Percentage of subjects with glycosuria is shown for subjects with diabetes due to HNF-1α mutations (DM NM), nondiabetic mutation carriers (non NM), and family control subjects (NN). Values are given for glycosuria at 0 min (□) and 120 min (▥) during the OGTT.*

mean age of diagnosis of diabetic subjects from the Exeter Diabetes U.K. MODY collection was 20.4 years (4), which is similar to other countries (9–11). Therefore, our cohort is more likely to include subjects who will go on to develop diabetes rather than representing a group in whom either genetic or environmental factors have reduced the penetrance of the mutations.

Although there was no difference in FPG, mutation carriers had a higher 2-h plasma glucose and AUC glucose compared with family control subjects. It has previously been shown that subjects with HNF-1α mutations have normal FPG and are classified as having diabetes by a raised 2-h plasma glucose during OGTT (27). This can be explained as the insulin secretion rate in HNF-1α mutation carriers is only reduced when plasma glucose levels exceed 8 mmol/l (8). This emphasizes the need to carry out OGTT when screening for diabetes in these families.

Mutation carriers had β-cell dysfunction and reduced insulin secretion compared with family control subjects even when the glucose tolerance was normal. This is in keeping with previous studies of nondiabetic mutant carriers that have used the OGTT, the intravenous glucose tolerance test, and hyperglycemic clamps (9–11).

There was increased insulin sensitivity by HOMA analysis of fasting glucose and insulin. This suggests normoglycemia may be maintained by increased insulin sensitivity despite reduced β-cell function. This is in keeping with a previous study that has shown increased insulin sensitivity in nondiabetic HNF-1α mutation carriers compared with NGT subjects from families with type 2 diabetes using an OGTT-derived insulin sensitivity index (10). Insulin sensitivity was not increased in glucose clamp studies of three normoglycemic nondiabetic mutation carriers (11). HOMA has limitations, because it is a fasting assessment rather than a dynamic one and therefore predominantly reflects hepatic rather than muscle insulin resistance, although it has been validated against the intravenous glucose tolerance test and euglycemic clamps in normoglycemic subjects (23,28). We cannot exclude the possibility that these mutation carriers have not developed diabetes as a result of their increased insulin sensitivity. In this case, it would reflect a characteristic of nondiabetic mutation carriers rather than of subjects with HNF-1α mutations per se. The finding in young subjects (mean 15 years) ~5 years below the mean age of diagnosis suggests insulin sensitivity is a characteristic of all mutation carriers.

We found no significant difference in fasting or 2-h total proinsulin and proinsulin-to-insulin ratio between mutation carriers and family control subjects. This contrasts previous studies that have shown a reduced fasting proinsulin (9) or an increased proinsulin-to-insulin ratio (6). The differences may reflect smaller numbers, or the difference in proinsulin-to-insulin ratio (6) may be characteristic of diabetic HNF-1α subjects. The previous study only classified diabetes on the basis of FPG, so some of the "nondiabetic" subjects may have been identified as having diabetes if an OGTT had been performed.

A total of 38% of subjects with mutations had glycosuria at 2 h post-OGTT despite 2-h plasma glucose values <11.1 mmol/l. This is explained by the low renal threshold (14,16,17), which in diabetic MODY subjects, has been shown to be due to reduced glucose reabsorption due to reduced expression of the low-affinity/high-capacity glucose cotransporter (SGLT2) that is under direct transcriptional control by HNF-1α (17). The peak values seen suggest glycosuria occurs if plasma glucose rises above ~8.4 mmol/l during OGTT. The renal threshold for HNF-1α subjects with diabetes has formally been measured at a whole-blood value of 6.5 mmol/l (equivalent to plasma glucose of 7.4 mmol/l). The previously described nondiabetic mutation carrier had glycosuria and a corresponding random plasma glucose of 7.7 mmol/l (16). The presence of 2-h glycosuria in all subjects with diabetes and a peak value in an OGTT of >8.4 mmol/l suggests that this could be used as a noninvasive screening tool in young children. A positive urine test for glycosuria after a large oral glucose load would guide the need for a formal OGTT and genetic testing.

In conclusion, we have shown that β-cell deficiency, increased insulin sensitivity, and glycosuria are all found in HNF-1α mutation carriers before the development of hyperglycemia. This study emphasizes the marked insulin deficiency seen in young subjects even before hyperglycemia develops. The presence of glycosuria at 2 h after an oral glucose load is of practical importance and should help to guide the need for an OGTT or genetic screening in young family members.

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