Impaired Insulin Secretion and Increased Insulin Sensitivity in Familial Maturity-Onset Diabetes of the Young 4 (Insulin Promoter Factor 1 Gene)

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Diabetes resulting from heterozygosity for an inactivating mutation of the homeodomain transcription factor insulin promoter factor 1 (IPF-1) is due to a genetic defect of β-cell function referred to as maturity-onset diabetes of the young 4. IPF-1 is required for the development of the pancreas and mediates glucose-responsive stimulation of insulin gene transcription. To quantitate islet cell responses in a family harboring a Pro63fsdelIC mutation in IPF-1, we performed a five-step (1-h intervals) hyperglycemic clamp on seven heterozygous members (NM) and eight normal genotype members (NN). During the last 30 min of the fifth glucose step, glucagon-like peptide 1 (GLP-1) was also infused (1.5 pmol · kg⁻¹ · min⁻¹). Fasting plasma glucose levels were greater in the NM group than in the NN group (9.2 vs. 5.9 mmol/l, respectively; P < 0.05). Fasting insulin levels were similar in both groups (72 vs. 105 pmol/l for NN vs. NM, respectively). First-phase insulin and C-peptide responses were absent in individuals in the NM group, who had markedly attenuated insulin responses to glucose alone compared with the NN group. At a glucose level of 16.8 mmol/l above fasting level, GLP-1 augmented insulin secretion equivalently (fold increase) in both groups, but the insulin and C-peptide responses to GLP-1 were sevenfold less in the NM subjects than in the NN subjects. In both groups, glucagon levels fell during each glycemic plateau, and a further reduction occurred during the GLP-1 infusion. Sigmoidal dose-response curves of glucose clearance versus insulin levels during the hyperglycemic clamp in the two small groups showed both a left shift and a lower maximal response in the NM group compared with the NN group, which is consistent with an increased insulin sensitivity in the NM subjects. A sharp decline occurred in the dose-response curve for suppression of nonesterified fatty acids versus insulin levels in the NM group. We conclude that the Pro63fsdelIC IPF-1 mutation is associated with a severe impairment of β-cell sensitivity to glucose and an apparent increase in peripheral tissue sensitivity to insulin and is a genetically determined cause of β-cell dysfunction. Diabetes 49:1856–1864, 2000

Type 2 diabetes is a highly prevalent and heterogeneous disorder of glucose homeostasis characterized by an imbalance between insulin synthesis and secretion by the endocrine pancreatic β-cells and peripheral tissue sensitivity to insulin. Diabetes has long been recognized as a genetic disorder with complex modes of inheritance. Recent advances reveal the genetic basis for a monogenic type of diabetes referred to as maturity-onset diabetes of the young (MODY), which is characterized by diagnosis at <25 years of age, autosomal dominant inheritance, and lack of a requirement for insulin therapy for at least 5 years after initial diagnosis (1). To date, five MODY genes have been identified, four of which encode endocrine pancreatic transcription factors (2–5) and one that encodes the pancreatic and hepatic glycolytic enzyme glucokinase (6,7).

Insulin promoter factor 1 (IPF-1) (also known as STF-1, IDF-1, IDX-1, and PDX-1) is a homeodomain transcription factor that is absolutely required for the development of the pancreas (8,9) and also mediates glucose-responsive stimulation of insulin gene transcription (10–15). IPF-1 is also implicated in the transcriptional regulation of other key β-cell specific genes, including GLUT2 (16), islet amyloid polypeptide (17,18), and glucokinase (19). Earlier, we described a child born with pancreatic agenesis who is homozygous for an inactivating cytosine deletion mutation in the protein coding sequence of IPF-1 (Pro63fsdelIC) (8). Subsequently, we showed that the IPF-1 mutation is linked to autosomal dominant early-onset type 2 diabetes in heterozygous carriers of the mutant allele within both branches of the extended family of the proband (Fig. 1) (4). Three members of this pedigree (II-4, IV-7, and IV-8) satisfy the strictest criteria for the diagnosis of MODY4, and two additional subjects heterozygous for the Pro63fsdelIC mutation developed either diabetes or glucose intolerance before 30 years of age (IV-6 and IV-9), thus establishing IPF-1 as the MODY4 gene (4).
The relevance of these advances in genetic defects of the β-cell (MODY) to the genetics and pathophysiology of type 2 diabetes is demonstrated by the identification of mutations in these genes in subjects with more common late-onset type 2 diabetes (20,21). Seven new heterozygous IPF-1 mutations have been discovered in 10 French pedigrees, 4 British pedigrees, and 1 Japanese pedigree, all with late-onset type 2 diabetes. An examples of digenic inheritance has been reported in a family with late-onset type 2 diabetes in which the severity of the diabetic phenotype appears to be related to cosegregation of two distinct mutations in two different pancreatic transcription factor genes, one of which is IPF-1 (22).

An initial clinical evaluation of MODY subjects revealed a defect in glucose-induced insulin secretion, but the nature of the deficit varies among the MODY subtypes. The aim of this study was to define the pathophysiology of mono-genetic diabetes resulting from the Pro63fsdelC IPF-1 mutation (MODY4) and to characterize the pattern of the β-cell insulin secretory response across a wide range of hyperglycemia. Understanding the pathophysiology of diabetes resulting from IPF-1 mutations may have broad relevance to the diagnosis and treatment of a subset of late-onset type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**Subjects.** A total of 15 members (7 heterozygous for the Pro63fsdelC mutation in IPF-1 [NM] and 8 normal genotypes [NN]) of both branches of the MODY4 family (4) volunteered for participation in this study (Fig. 1). The clinical characteristics of the volunteers are described in Table 1. The Human Investigation Committee of the University of Virginia approved all methods and procedures. All volunteers gave their written informed consent.

**Body composition and pancreas size.** Total body fat mass, lean tissue mass, and bone mineral content were determined by dual-energy X-ray absorptiometry (DXA). DXA scans were performed in the pencil beam mode using the Hologic QDR 2000 (Waltham, MA) bone densitometer. All scans were analyzed by an expert technician using the Hologic Enhanced Whole-Body Software Version 7.2.

Computed tomography scans were performed with a Picker PQ 5000 (Cleveland, OH) and were analyzed with a tissue quantification analysis package on the Picker Voxel Q 3D imaging station. Subjects were examined in a supine position with their arms stretched above their heads. An abdominal scan at the level of the L4 to L5 intervertebral disc space was performed with no angulation using a lateral pilot for location, and abdominal visceral fat (AVF) and abdominal subcutaneous fat (ASF) cross-sectional areas were calculated as previously described (23). Fat content and muscle area of the thigh were assessed from a single slice taken at midpoint between the greater trochanter and the top of the patella (with the subject standing). To estimate the mass of the pancreas, a spiral series (pitch 1.5) of abdominal scans was taken to include the whole pancreas. The pancreatic area determined for each slice was multiplied by the slice interval thickness to provide a pancreatic tissue volume for each slice. The volume of pancreatic tissue for each slice was summed from all images.

**Five-step hyperglycemic clamp.** All subjects were asked to consume a weight-maintaining diet without carbohydrate restriction and to maintain their usual level of physical activity for 3 days before testing. All hypoglycemic medications were withheld for 18 h before the start of the clamp. Each volunteer was admitted to the General Clinical Research Center the evening before the test. All clamps were performed after an overnight fast, and testing began by 0730 as described previously (24). After determination of the stable fasting state, at time 0, a five-step hyperglycemic clamp was initiated. Each clamp step lasted 1 h. Initially, the fasting plasma glucose levels were increased by 5.6 mmol/l (100 mg/dl) for 1 h followed by 2.8 mmol/l (50 mg/dl) for each of the subsequent 4 h. During the last 30 min of the fifth glucose step, glucagon-like peptide 1 (GLP-1) (7-36 amide) was infused in a primed (10 min) constant (20 min, 1.5 pmol · kg⁻¹ · min⁻¹) manner as described previously (25). Synthesized GLP-1 (Boston Molecules, Woburn, MA) has a peptide content...
results

The two groups of volunteer subjects were similar regarding age, weight, and BMI (Table 1). However, the NM group was 10 years older \((P = 0.30)\), and six of the seven subjects in that group had diabetes. The ratio of men:women in the NM and NN groups was 5:2 and 2:6, respectively. Despite this difference, total percentages of fat \((P < 0.06)\), AVF \((121.1 \pm 20.4 \text{ vs. } 130.8 \pm 27.2 \text{ cm}^2)\), respectively; \(P = 0.78\), ASF \((287.8 \pm 37.3 \text{ vs. } 447.5 \pm 100.8 \text{ cm}^2)\), respectively; \(P = 0.14\), and thigh fat \((95.2 \pm 19.8 \text{ vs. } 162.3 \pm 38.6 \text{ cm}^2)\), respectively; \(P = 0.12\) were not different; thigh muscle area was also similar \((134.2 \pm 11.8 \text{ vs. } 109.7 \pm 9.5 \text{ cm}^2)\), respectively; \(P = 0.16\). Pancreas volume in the NM group was smaller \((62.2 \pm 7.9 \text{ vs. } 75.9 \pm 9.2 \text{ cm}^2)\), respectively; \(P = 0.28\).

Fasting plasma glucose levels in the NM and NN groups were \(9.2 \pm 1.6 \text{ and } 5.9 \pm 0.4 \text{ mmol/l}\), respectively. During each step of the clamp, plasma glucose was maintained at a stable level in each volunteer. The mean increase of plasma glucose plateau for each step of the clamp was \(5.4 \pm 0.05, 8.6 \pm 0.20, 10.8 \pm 0.27, 14.1 \pm 0.33, \text{ and } 15.9 \pm 0.28 \text{ mmol/l} \text{ above fasting level in the NM group. The corresponding levels for the NN group were } 5.5 \pm 0.21, 8.3 \pm 0.31, 10.7 \pm 0.28, 13.7 \pm 0.24, \text{ and } 16.6 \pm 0.35 \text{ mmol/l} \text{ (Fig. 2). Despite equivalent increases in the plasma glucose levels in the two groups, average glucose infusion rates necessary to maintain stable hyperglycemia during the last 30 min of each step were 126, 260, 260, 215, and 197% higher in the NN group compared with the NM group \((P < 0.01 \text{ for steps } 2-5)\) (Fig. 2).

Fasting plasma insulin levels in the NM and NN groups were \(72 \pm 7 \text{ and } 105 \pm 16 \text{ pmol/l} \text{, respectively. In response to the square wave of hyperglycemia, first-phase insulin response was absent in the NM group and was clearly evident in the NN group (Fig. 3). The peak levels at 4 min were } 84 \pm 12 \text{ and } 317 \pm 44 \text{ pmol/l in the NM and NN groups,}
respectively ($P < 0.01$). After the initial step of the clamp, a first-phase insulin response was not observed in the subsequent four steps in either group. In the NM group, second-phase insulin response changed little, but in the NN group, it increased progressively at each step of the clamp (Fig. 3).

The 30- to 60-min and the 240- to 270-min plasma insulin levels of the NM group averaged 122 ± 31 and 596 ± 238 pmol/l, respectively. The corresponding plasma insulin levels in the NN group were 487 ± 87 and 4,098 ± 908 pmol/l, respectively. During the 270- to 300-min period when GLP-1 was also

**FIG. 2.** Plasma glucose and glucose infusion during a five-step hyperglycemic clamp in subjects with (NM) and without (NN) the IPF-1 mutation (means ± SE). The letters indicate glucose levels: A, fasting glucose; B, A + 5.6; C, B + 2.8; D, C + 2.8; E, D + 2.8; F, E + 2.8 mmol/l; G, end of glucose infusion. GLP-1 is infused in a primed constant manner (1.5 pmol · kg⁻¹ · min⁻¹) from 270–300 min.
infused, insulin responses in the NM group were sevenfold less than in the NN group (1,481 ± 691 and 9,998 ± 1,703 pmol/l, respectively; \( P < 0.01 \)). However, the fold increase over glucose alone at the 240- to 270-min period was the same in both groups (~2.5-fold).

The time course of the C-peptide response to glucose and GLP-1 was similar to the time course of the insulin response (Fig. 3). The ratios of fasting plasma insulin to fasting C-peptide levels for the NM and NN groups were 0.14 and 0.14, respectively. The ratios of insulin to C-peptide for the last 30 min of each of the first four steps in the NM group were 0.12, 0.14, 0.12, and 0.14, respectively. The 240- to 270-min and the 270- to 300-min ratios were 0.15 and 0.20, respectively. The corresponding ratios for the NN group were 0.20, 0.25, 0.32, 0.38, 0.43, and 0.60, respectively (all \( P \leq 0.005 \)).

The lower limit of GLP-1 detection in our laboratory is 5 pmol/l. Endogenous plasma GLP-1 levels in both groups during the fasting state as well as during the first 270 min of the glucose infusion period were either below the level of detection or slightly >5 pmol/l. When a level was <3 pmol/l, the value 3 pmol/l was assigned to that sample. Fasting GLP-1 levels in the NM and NN groups were 3.3 ± 0.2 and

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**FIG. 3.** Plasma insulin, C-peptide, and GLP-1 levels during the five-step hyperglycemic clamps in subjects with (NM) and without (NN) the IPF-1 mutation (means ± SE). Inset in the first panel shows first-phase insulin response. A–G; see the legend for Fig. 2.
3.5 ± 0.2 pmol/l, respectively. The 240- to 270-min levels were 3.4 ± 0.3 and 3.5 ± 0.3 pmol/l, respectively. With the infusion of GLP-1 plasma, GLP-1 levels rose rapidly in both groups, and the 270- to 300-min concentrations were similar and averaged 155 ± 36 and 195 ± 27 pmol/l in the NM and NN groups, respectively (Fig. 3).

The fasting plasma PP levels in the NM and NN groups were similar and averaged 36 ± 9 and 51 ± 23 pmol/l, respectively. Plasma PP levels did not change significantly during either the glucose infusion alone or when GLP-1 was also infused (Fig. 4).

Fasting plasma glucagon levels in the NM and the NN groups differed and averaged 17.2 ± 1.4 and 14.4 ± 2.0 pmol/l, respectively (Fig. 4). With the start of the glucose infusion, plasma glucagon levels began to fall in both groups. The 240- to 270-min levels averaged 10.6 ± 1.3 and 7.1 ± 1.4 pmol/l in the NM and NN groups, respectively. The suppression is significant in both groups compared with the fasting level (P ≤ 0.01) but not between the two groups (P = 0.09). During the GLP-1 infusion period, despite vast differences in insulin response between the two groups, glucagon levels fell to 9.4 ± 1.0 and 6.7 ± 1.2 pmol/l in the NM and NN groups, respectively; these were not significantly different from each other or from the 240- to 270-min levels. However, the decrease in the glucagon levels during the entire clamp study was statistically significant (P < 0.0001). No statistically significant difference was evident in the pattern of decrease between the two groups (P = 0.87).

Fasting NEFA levels in the NM and the NN groups differed and averaged 0.74 ± 0.08 and 0.52 ± 0.08 mmol/l, respectively (P < 0.04). After the start of glucose infusion, NEFA levels began to decrease in both groups and reached a plateau by 120 min (Fig. 4). The 240- to 270-min levels were 0.26 ± 0.06 and 0.26 ± 0.08 mmol/l in the NM and the NN groups, respectively. Despite having a higher fasting NEFA level, the NM group suppressed to the same level as the NN group. During the GLP-1 infusion period, NEFA levels were 0.20 ± 0.05 and 0.24 ± 0.08 mmol/l in the NM and the NN groups, respectively. These levels were not different from each other or from the previous 30-min interval. The decrease in NEFA levels during the entire clamp was statistically significant (P < 0.0001), and a significant difference was evident in the pattern of decrease between the two groups (P < 0.04).

We also examined insulin sensitivity regarding both glucose uptake (Fig. 5) and suppression of NEFA (Fig. 6). Because fasting plasma glucose levels were significantly higher in the NM group, we calculated the MCR of glucose. A significant dose-response relationship was evident between glucose clearance and plasma insulin level. The NM group’s curve for glucose clearance was shifted to the left and had a lower maximal response compared with the
The variance (ence or absence of the mutation was related to the MCR of thigh muscle area, and pancreas volume, only the pres-BMI, fasting glucose, AVF, ASF, percentage of body fat, independent variables were sex, genotype (NM, NN), age, diabetes, fasting glucose, AVF, ASF, percentage of body fat, thigh muscle area, and pancreas volume, only the presence of diabetes, fasting glucose, AVF, ASF, percentage of body fat, degree of decrease in NEFA is evident in the NM group compared with the NN group. The ED50 values for insulin in the NM and NN groups were 142 and 384 pmol/l, respectively (24 and 64 µU/ml, respectively; P = 0.004). Thus, a maximal suppression in NEFA is achieved at a lower insulin level in the NM group. In stepwise regression analyses where the independent variables were sex, genotype (NM, NN), age, BMI, fasting glucose, AVF, ASF, percentage of body fat, thigh muscle area, and pancreas volume, only the presence of the mutation was related to the MCR of glucose, with the dependent variable accounting for 44% of the variance (P = 0.007).

**DISCUSSION**

The main focus of this study describes insulin release in response to changes in plasma glucose levels across a wide range of hyperglycemia in members of a family who have inherited an inactivating mutation within one of two alleles of the IPF-1 gene. A severe impairment of insulin release is demonstrated compared with members of the same family who do not harbor the mutation. The impairment appears to be specific for glucose-stimulated insulin release because no differences are apparent in the fold increase for GLP-1–stimulated insulin release (240- to 270-min interval/270- to 300-min interval). Furthermore, the impairment also appears to be β-cell specific because no differences in α-cell or PP cell responses were seen. However, specific provocative tests such as an arginine stimulation test and a meal tolerance test are necessary to establish the lack of differences in α- and PP cell sensitivities.

In type 2 diabetic volunteers and in contrast with nondiabetic volunteers, a first-phase insulin response does not occur during a hyperglycemic clamp at a glucose level of 5.6 mmol/l above fasting level (29). First-phase insulin response was also absent in each volunteer of the NM group during the first clamp step (5.6 mmol/l above fasting level). This circumstance was observed even in those individuals who were just diagnosed as having elevated fasting glucose levels consistent with the diagnosis of diabetes at the time of the study. The first-phase insulin responses were also absent in both groups during each of the subsequent four steps. Although, during the fifth step of the glucose clamp, plasma glucose levels were substantially elevated, the incremental step from the fourth to the fifth step was rather small (from 13.9 to 16.7 mmol/l). Thus, to obtain a second first-phase response during a stepped hyperglycemic clamp, a larger incremental increase in plasma glucose may be necessary than is required for the increment from a fasting plasma glucose level.

Recently, a two-step hyperglycemic clamp (each step 2.5 mmol/l above fasting level) was reported in five members of a MODY3 family (hepatocyte nuclear factor-1α gene mutation) and six nondiabetic nonrelated control volunteers (30). Similar to our study, first-phase insulin responses did not occur during either of the two steps in the MODY3 volunteers, and second-phase insulin responses were markedly attenuated during the first step. In the six nondiabetic volunteers, first- and second-phase insulin responses during the first step of the clamp were normal. During the second step, first-phase insulin response was absent, but an increase was evident in the second phase; those findings contrast with the findings of MODY3 volunteers in whom very little increase occurred in insulin secretion. Markedly attenuated insulin secretion rates (ISRs) (deconvolution of plasma C-peptide) in response to an increase in plasma glucose with a relatively constant slope (ramp) have also been reported in affected diabetic members of MODY3 families from France, Michigan, and New York pedigrees (31). Insulin responses and ISRs were also reduced in nondiabetic affected members compared with nondiabetic nonrelated control volunteers.

Diminished insulin responses (both phases) during hyperglycemic clamps (5.6 mmol/l above fasting) have been reported for both diabetic and nondiabetic members of a MODY1 pedigree compared with nonrelated control volunteers (32). Furthermore, diminished insulin and glucagon responses occur in response to an arginine infusion both before and during the clamp in the diabetic and nondiabetic MODY1 groups. Diminished ISR in response to ramp increases in plasma glucose levels have also been reported in affected members of the MODY1 Michigan family (R.W.) compared with nonmutated members (33). The reduction in ISR became more evident as plasma glucose levels increased. Because high blood glucose per se may cause a decrease in ISR, we separately analyzed subject II-4-M, who had a fasting glucose of 4.6 mmol/l. We found that he also had a severely deficient first-phase insulin response as well as a defective second-phase insulin response. Therefore, we believe that IPF-1 mutations cause a primary defect in insulin secretion.

In contrast with MODY1, MODY3, and MODY4, in which first-phase insulin responses similar to type 2 diabetes are absent, these responses are present in MODY2-affected members with the glucokinase mutation (34,35). However,
ISRs in response to ramp are lower than in control volunteers (35). Again, the reduction in insulin response, similar to MODY1 and MODY3, became more evident as plasma glucose levels increased to >9 mmol/L. Phenotypic evaluation of insulin response in MODY5 has not been reported (5). Thus, taken together, all types of MODY-affected members who have been examined up to this point display a marked reduction in insulin responses to glucose. However, MODY4-affected members do not have an impairment in GLP-1–stimulated insulin response, and this may prove to be a new and effective agent for controlling their glucose homeostasis.

We also examined peripheral tissue sensitivity to endogenously released insulin. We observed that the tissue sensitivity in the NM group was significantly higher compared with the NN group. However, we point out that the ranges of endogenously released insulin are different between the two small groups and that we used MCR as a measure of peripheral tissue sensitivity. Additionally, this apparent increase in peripheral tissue sensitivity to insulin was not determined with a hyperinsulinemic-euglycemic clamp in which plasma insulin levels are the same in each volunteer. In another study, glucose uptake in diabetic MODY3 volunteers during a euglycemic clamp was slightly higher than in nondiabetic MODY3 volunteers (55 vs. 43 pmol·kg\(^{-1}\)·fat-free mass·min\(^{-1}\); the difference was not significant (36). Thus, the peripheral tissue sensitivity to insulin of MODY3 individuals is not increased. However, MODY2 individuals appear to have a diminished peripheral tissue sensitivity to insulin accompanied by less reduction in hepatic glucose output during hyperinsulinemia (37). The improved peripheral tissue sensitivity to insulin in our study is not limited to glucose. We also observed improved fatty acid suppression in the NM group. The female:male ratio in the NM group (2:5) was opposite that of the NN group (6:2), and the NM group had a lower BMI and percentage of body fat. However, in stepwise regression analysis, only the presence or absence of the mutation accounted for the improvement in glucose homeostasis. Thus, this study provides preliminary evidence that a mechanism for maintaining fuel homeostasis in MODY4 individuals is an increased peripheral tissue sensitivity to insulin for the uptake of both glucose and fatty acids. Whether this enhanced sensitivity is also exhibited regarding protein and hepatic glucose output remains to be examined.

In conclusion, we demonstrate that, despite a markedly diminished β-cell response to hyperglycemia in MODY 4 individuals, the responses to glucose in the islet α-cells (glucagon) and PP cells are unaffected. Furthermore, the β-cell response to the insulinotropic hormone GLP-1 remains intact. An improved peripheral tissue sensitivity probably compensates at least in part for the diminished insulin secretion.

Since submitting our article for consideration, two articles have been published (20,21). Both of those showed that defective mutations in IPF-1 led to type 2 diabetes in heterozygotes. Han et al. (20) showed that carriers of the D76N IPF-1 mutation with perfectly normal glucose tolerance had significant and dramatic decreases in insulin response to an oral glucose tolerance test both at 30 min and throughout the rest of the test. Therefore, we believe that this indicates that IPF-1 mutations lead to a primary defect in insulin secretion.

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