Contrasting Diabetes Phenotypes Associated With Hepatocyte Nuclear Factor-1α and -1β Mutations

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OBJECTIVE — Mutations in the highly homologous transcription factors hepatocyte nuclear factor (HNF)-1α and -1β cause maturity-onset diabetes of the young types 3 and 5, respectively. Diabetes due to HNF-1α mutations is well characterized. However, physiological assessment of the HNF-1β phenotype is limited. We aimed to test the hypothesis that the diabetes phenotype due to HNF-1β mutations is similar to that in HNF-1α.

RESEARCH DESIGN AND METHODS — Fasting biochemistry and a tolbutamide-modified intravenous glucose tolerance test (IVGTT) were compared in matched HNF-1β, HNF-1α, type 2 diabetic, and control subjects. Homeostasis model assessment indexes were determined from fasting insulin and glucose. The peak measures for the insulin increment after tolbutamide and for the insulin increment after glucose were determined from the IVGTT.

RESULTS — The HNF-1β patients showed a 2.4-fold reduction in insulin sensitivity compared with the HNF-1α patients (P = 0.001) with fasting insulin concentrations 2.7-fold higher (P = 0.004). HNF-1β patients had lower HDL cholesterol (1.17 vs. 1.46 mmol/l, P = 0.009) and higher triglyceride (2.2 vs. 1.35 mmol/l, P = 0.015) levels than HNF-1α patients. The HNF-1β patients had similar β-cell responses to tolbutamide and glucose as the type 2 diabetic patients, but in the HNF-1α patients, the tolbutamide response was considerably increased relative to the response to glucose (P = 0.002).

CONCLUSIONS — HNF-1β patients have a different diabetes phenotype than HNF-1α patients. Those with HNF-1β mutations have hyperinsulinemia and associated dyslipidemia consistent with insulin resistance and may have a different β-cell defect. This suggests that despite considerable homology and a shared binding site, HNF-1α and HNF-1β have a different role in maintaining normal glucose homeostasis. This result suggests a new etiological pathway for insulin resistance involving HNF-1β.

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Heterozygous mutations in the transcription factor genes hepatocyte nuclear factor (HNF)-1α (TCF1) and HNF-1β (TCF2) cause maturity-onset diabetes of the young (MODY) types 3 and 5, respectively. The phenotype of HNF-1α mutations is primarily characterized by nondiabetic renal dysfunction, particularly renal cystic disease, diabetes, genitourinary tract malformation, abnormal liver function, and hyperuricemia (2–13).

HNF-1β is a homeodomain transcription factor, structurally related to HNF-1α. They share >90% sequence homology in their DNA binding domains and recognize the same DNA binding site. HNF-1α and HNF-1β function as homodimers or heterodimers (14). They are expressed in a number of tissues including liver, kidney, intestine, and pancreatic islets where they play a role in embryonic development of these organs and tissue-specific gene expression. It is not clear how the role of these transcription factors differs in the complex transcription factor network. However, they do differ both in the proportion at which they are expressed in tissues (e.g., HNF-1α predominates in the liver, whereas HNF-1β predominates in the kidney) and in the timing of expression during embryonic development with HNF-1β being expressed earlier than HNF-1α.

Diabetes due to HNF-1α mutations has been well characterized. Physiological studies in humans have identified a primary β-cell defect (15) with an elevated proinsulin-to-insulin ratio before the onset of diabetes (16). Animal and cell models of HNF-1α function in the β-cell suggest the main sites of action are in the glycolytic pathway (17,18).

The role of HNF-1β in the β-cell has not been determined, and the diabetes phenotype has only been described in a few individual families. Decreased insulin response to an oral glucose load has been described in three individuals from three families (5,7,11), consistent with reduced β-cell function. No assessment of insulin sensitivity has been described. There are no useful models of the role of HNF-1β in the β-cell because the HNF-1β−/− mouse dies at an early embryonic stage and there is no published literature on HNF-1β in pancreatic cell lines.

We hypothesize that because of the close homology of the HNF-1α and
HNF-1β transcription factors that the diabetes phenotype is similar. The aim of this study was to compare measures of β-cell function and insulin resistance in patients with HNF-1β mutations, HNF-1α mutations, type 2 diabetes, and normal control subjects.

**Research Design and Methods**

**Patients**

All U.K. subjects with known HNF-1β mutations who consented to at least fasting blood testing (10 subjects from six families: DUK 250, 298, 350, 448, 504, 507) were studied (7,9,10,19). Family 350 had a heterozygous mutation in the splice site of intron 2 (IVS2nt 507) were studied (7,9,10,19). Family 1103 with the insulin secretory response to a glycemic agent/insulin)

**Diabetes duration (years)**

2 (0–13) 7 (1–25)

**Sex (men/women)**

2/6 11/19 15/15

**BMI (kg/m²)**

26.8 (21.3–30.8) 25.2 (23.3–27.7) 25.5 (24.4–27.0)

**FPG (mmol/l)**

9.2 (5.0–16.7) 10.3 (5.5–12.8) 5.0 (4.6–5.2)

**IVGTT study**

HNF-1β HNF-1α Type 2 diabetes

<table>
<thead>
<tr>
<th>n</th>
<th>5</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24 (19–43)</td>
<td>38 (30–54)</td>
<td>68 (60–71)†</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>2/3</td>
<td>4/6</td>
<td>7/3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5 (22.7–30.8)</td>
<td>25.6 (23.1–27.2)</td>
<td>25.1 (22.6–29.0)</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>11.6 (6.35–18.9)</td>
<td>12.4 (8.0–15.5)</td>
<td>11.8 (6.8–14.6)</td>
</tr>
</tbody>
</table>

Data are medians (lower quartile – upper quartile). The three groups are compared by Kruskal-Wallis test.

*P < 0.05; †P < 0.001.

Insulin was measured by an immunoenzymometric assay (Insulin EASIA; Biosource, Belgium) calibrated against the International Reference Preparation 66/304 with no detectable cross-reactivity with intact proinsulin and 32-33 split proinsulin.

**Insulin sensitivity**

Insulin sensitivity was calculated from the FPG and specific insulin by homeostasis model assessment (HOMA) (22,23). Insulin sensitivity determined by HOMA has been validated by comparison with IVGTT and euglycemic-hyperinsulinemic clamp in normal subjects, impaired glucose tolerant, and type 2 diabetic patients (24).

**Statistical analysis**

Summary results are median (lower to upper quartile). Comparison across the groups (HNF-1α, HNF-1β, control or HNF-1α, HNF-1β, type 2 diabetes) was by a Kruskal-Wallis test. Where significant difference was demonstrated across the group, a Mann-Whitney U test was used to compare individual groups. As three separate groups were analyzed, a Bonferroni correction for this multiple comparison was applied, so a value of P < 0.017 was taken to be statistically significant.

**Results**

**Fasting analysis**

The three groups analyzed in the fasting state (HNF-1α, HNF-1β, and control) were well matched for BMI, and the HNF-1α and HNF-1β groups were well matched for FPG (Table 1). The fasting insulin concentration (Table 2) in HNF-1β patients was elevated 2.5-fold compared with the HNF-1α group (P = 0.004) and 1.8-fold compared with control subjects (P = 0.035). The fasting insulin concentration was similar in HNF-1α patients and control subjects (P = 0.12).

In keeping with the hyperinsulinemia, the insulin sensitivity determined by HOMA (Table 2) in HNF-1β was reduced 2.4-fold compared with HNF-1α patients (P = 0.001) and 2.2-fold compared with control subjects (P = 0.002). There was no difference in insulin sensitivity between HNF-1α and control subjects (P = 0.663).

**β-Cell function determined by HOMA analysis** was significantly reduced...
HNF-1α and -1β diabetes phenotype

Table 2—Fasting study: comparison of HNF-1α, HNF-1β, and control subjects

<table>
<thead>
<tr>
<th>Fasting study</th>
<th>HNF-1β</th>
<th>HNF-1α</th>
<th>Control</th>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>9.2 (5.0–16.7)</td>
<td>10.3 (5.5–12.8)</td>
<td>5.0 (4.6–5.2)</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>83 (50–101)</td>
<td>33 (17–60)</td>
<td>45 (29–68)</td>
</tr>
<tr>
<td>HOMA B (% normal)</td>
<td>56 (22–127)</td>
<td>27 (9–56)</td>
<td>101 (82–121)</td>
</tr>
<tr>
<td>HOMA S (% normal)</td>
<td>46 (25–57)</td>
<td>112 (70–190)</td>
<td>99 (67–171)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.6 (4.3–5.5)</td>
<td>4.9 (4.6–5.8)</td>
<td>5.5 (4.4–6.15)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.17 (0.82–1.23)</td>
<td>1.46 (1.23–1.64)</td>
<td>1.39 (1.08–1.66)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.2 (1.9–2.6)</td>
<td>1.35 (1.0–2.0)</td>
<td>1.3 (0.9–1.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P value (Mann-Whitney U)</th>
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<tbody>
<tr>
<td>Kruskal-Wallis</td>
</tr>
<tr>
<td>0.01</td>
</tr>
<tr>
<td>0.003</td>
</tr>
<tr>
<td>0.037</td>
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<tr>
<td>0.005</td>
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</table>

Data are medians (lower quartile – upper quartile). The three groups are compared by Kruskal-Wallis test, and when significant, comparison between groups is by Mann-Whitney U test. Bold values significant at P < 0.017

in HNF-1α subjects (21% of control values; P < 0.001). β-Cell function in HNF-1β subjects did not differ significantly from control subjects (55% of control values; P = 0.112) or HNF-1α subjects (P = 0.183), reflecting the wide range of β-cell function in the HNF-1β group (Table 2). The lipid changes in the HNF-1β subjects were consistent with the changes seen in insulin resistance. The total cholesterol did not differ between groups; however, the HDL cholesterol was lower in HNF-1β subjects compared with HNF-1α (P = 0.009) and control (P = 0.035) subjects. Triglyceride concentrations were elevated in HNF-1β subjects compared with HNF-1α (P = 0.015) and control (P < 0.001) subjects (Table 2).

IVGTT

The characteristics of the three patient groups (HNF-1α, HNF-1β, and type 2 diabetic) who had an IVGTT are shown in Table 1. The results of the IVGTT are shown in Fig. 1. Across the groups, the PEAKglu and PEAKtolb were similar (P = 0.185 and P = 0.592, respectively) (Table 3), although it is difficult to make direct comparisons in these groups because of their differing insulin sensitivity.

The ratio PEAKtolb to PEAKglu represents the response to tolbutamide relative to the response to glucose. This was markedly elevated in HNF-1α patients compared with type 2 diabetic patients (11.4 vs. 4.8; P = 0.002), reflecting a maintained response to tolbutamide in the face of a poor response to glucose. The HNF-1β response is similar to type 2 diabetic patients (5.8 vs. 4.8; P = 0.594) and reduced compared with HNF-1α patients, although this was not significantly different (P = 0.11).

In keeping with the fasting study, the fasting insulin concentration and insulin sensitivity was similar in the HNF-1β and type 2 diabetic patients (41 vs. 49 pmol/l; P = 0.594; 87 vs. 77% normal; P = 0.768).

CONCLUSIONS—We have described the pathophysiology of diabetes in patients with HNF-1β mutations and demonstrated that, despite considerable homology of HNF-1α and HNF-1β, there appear to be differences in the associated diabetes phenotypes in MODY3 and MODY5. HNF-1β patients have fasting

Figure 1—IVGTT. Glucose is intravenously given at 0 min, and tolbutamide is intravenously given at 19 min. Glucose results are presented as mean for HNF-1α (□), HNF-1β (○, dashed line), and type 2 diabetic (△) subjects. Insulin results are presented as geometric mean for HNF-1α (■), HNF-1β (○, dashed line) and type 2 diabetic (▲) subjects.
hyperinsulinemia and dyslipidemia characterized by a low HDL and raised triglycerides. The response to tolbutamide relative to glucose is increased in HNF-1α patients, whereas the response in HNF-1β patients is similar to type 2 diabetic patients. Although the interpretation is difficult due to the small number of HNF-1β subjects studied, it appears that HNF-1β patients have a phenotype much more similar to type 2 diabetes than to HNF-1α diabetic patients.

HNF-1β mutations are associated with hyperinsulinemia and insulin resistance determined by HOMA compared with HNF-1α mutations and control subjects. We were unable to calculate $S_f$ from the frequently sampled intravenous glucose tolerance test in three of the five HNF-1β patients due to the severity of their diabetes, meaning that there was an inadequate insulin response to allow modeling. It is not possible to draw a definitive conclusion from fasting analysis and HOMA-derived insulin resistance in this small group of patients. Euglycemic-hyperinsulinemic clamp studies are required to further define the etiology of the hyperinsulinemia. However, there is strong supporting evidence for insulin resistance in HNF-1β patients. First, the elevated triglycerides and low HDL that we have been shown in HNF-1β is typical of dyslipidemia associated with insulin resistance (25). The low HDL may be secondary to a direct loss of transcriptional regulation due to HNF-1β mutations; however, this would be unlikely as the HDL is normal or high in HNF-1α mutation carriers, and HNF-1α and HNF-1β share the same DNA binding site. Second, alanine aminotransferase was elevated above the laboratory reference range in four of seven HNF-1β patients. Elevated alanine aminotransferase is associated with hepatic steatosis and decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes (26). Finally, hyperuricemia, which is commonly seen in insulin-resistant states (27), has been described in HNF-1β mutation carriers (19).

Despite the evidence suggestive of insulin resistance, insulin requirements in patients with diabetes due to HNF-1β tend to be low. The mean insulin requirement in the patients whose insulin doses were known in our study was 0.45 U/kg, and the published insulin requirements are 0.26 U/kg (5), 34 U (8), and 27 U (8). These low requirements could reflect that patients still have considerable endogenous insulin secretion. C-peptide is predominantly renally cleared, so it could not be systematically studied in our patients. However, the two insulin-treated HNF-1β patients whose renal function was only mildly impaired (creatinine clearance 78 and 70 ml/min) had fasting C-peptides of 673 and 901 pmol/l, respectively, which was consistent with endogenous β-cell reserve.

An alternative explanation for hyperinsulinemia in HNF-1β patients could be impaired insulin clearance. The liver clears ~50% of endogenous portal insulin, and the kidneys clear 50% of peripheral insulin (28). HNF-1β mutations are associated with renal dysfunction, but insulin concentration is not increased in renal impairment until creatinine clearance falls <60 ml/min (29). We excluded patients from fasting analysis with a creatinine clearance <60 ml/min, and the creatinine clearance in our group was only mildly reduced, and so impaired renal clearance of insulin is unlikely to cause the hyperinsulinemia seen in this study. The main mechanism for insulin clearance in the liver and kidney is by internalization of insulin bound to the insulin receptor followed by degradation initiated by insulin-degrading enzyme (IDE) (28). One mechanism for the differences in insulin concentration in HNF-1α and HNF-1β could be differences in IDE expression in the liver or kidneys. There are no in vivo experimental data published on the role of the HNF-1 transcription factors in regulating IDE transcription. In silico analysis suggests that there is a putative HNF-1 binding site 739 bp upstream of the transcription start site of the human IDE gene ($P = 0.01$). However, a binding site could not be demonstrated upstream of the homologous rat IDE gene.

Whereas previous studies have shown reduced β-cell function after oral glucose tolerance test in individual patients (5,7,11), the β-cell function determined by HOMA in our study was not reduced in HNF-1β subjects compared with control subjects. This probably reflects the large variation in β-cell function in the group and the small number studied; however, it also highlights the considerable contribution of insulin resistance to the pathogenesis of diabetes in this group.

The PEAKtolb-to-PEAKglu ratio can be considered a simple measure of function of the β-cell pathway downstream to, and including, the sulfonylurea receptor relative to the presulfonylurea receptor pathway of glucose entry, glycolysis, and mitochondrial metabolism. The main metabolic defect in HNF-1α−/− mouse islets (31) and INS-1 cells overexpressing a dominant negative HNF-1α mutation (32) is in this presulfonylurea receptor pathway causing decreased intracellular

### Table 3—IVGTT study: comparison of HNF-1α, HNF-1β, and type 2 diabetic subjects

<table>
<thead>
<tr>
<th>IVGTT study</th>
<th>HNF-1β</th>
<th>HNF-1α</th>
<th>Type 2 diabetes</th>
<th>Kruskal-Wallis</th>
<th>HNF-1β vs. type 2 diabetes</th>
<th>HNF-1α vs. type 2 diabetes</th>
<th>HNF-1β vs. HNF-1α</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>11.6 (6.4–18.9)</td>
<td>12.4 (8.0–15.5)</td>
<td>11 (3.9)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PEAKglu (pmol/l)</td>
<td>36 (18–360)</td>
<td>22 (5–47)</td>
<td>54 (20–143)</td>
<td>0.185</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEAKtolb (pmol/l)</td>
<td>245 (129–685)</td>
<td>277 (108–453)</td>
<td>189 (92–389)</td>
<td>0.592</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEAKtolb-to-PEAKglu ratio</td>
<td>5.8 (1.3–15.7)</td>
<td>11.4 (8.9–42.0)</td>
<td>4.8 (2.5–5.9)</td>
<td>0.007</td>
<td>0.594</td>
<td>0.002</td>
<td>0.111</td>
</tr>
</tbody>
</table>

Data are medians (lower quartile – upper quartile). The three groups are compared by Kruskal-Wallis test, and when significant, comparison between groups is by Mann-Whitney $U$ test. Bold $P$ values significant at $P < 0.017$. 

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**Pearson and Associates**
HNF-1α and -1β diabetes phenotype

ATP. We have proposed that the high PEAKtolb-to-PEAKglu ratio in HNF-1α diabetes is due to the action of tolbutamide bypassing a severe defect in glucose metabolism with a relative preservation of the signaling pathway downstream of the sulfonylurea receptor (21). The PEAKtolb-to-PEAKglu ratio in HNF-1β subjects is similar to that of type 2 diabetic subjects and lower than in HNF-1α subjects, although this was not statistically significant. This suggests that the β-cell defects in diabetic subjects caused by HNF-1β mutations are qualitatively different from HNF-1α with different sites or severity of defects. A recent study has shown that HNF-1β is expressed very early in pancreatic development (HNF-1β-expressing ductal precursor cells) but are not present in mature β-cells (33). Diabetes may develop in HNF-1β mutation carriers from decreased generation of endocrine pancreatic cells during embryogenesis and consequently reduced β-cell mass. This contrasts with HNF-1α, which is highly expressed in differentiated β-cells, and HNF-1α haploinsufficiency causes diabetes primarily through a severe defect in glucose metabolism. The difference in insulin sensitivity and β-cell response to tolbutamide relative to glucose in HNF-1α and HNF-1β patients is likely to have implications for pharmacological treatment of these subjects. HNF-1α patients respond extremely well to sulfonylurea medication (21). In some cases, this has allowed the transfer of insulin (34). It is our clinical experience that HNF-1β patients do not respond particularly well to sulfonylureas, and this is consistent with the PEAKtolb-to-PEAKglu ratio being similar to type 2 diabetes. The low insulin sensitivity in HNF-1β subjects suggests that an insulin sensitizer such as metformin or a peroxisome proliferator–activated receptor-γ agonist would be the oral agent of choice.

In summary, HNF-1β patients have a different diabetes phenotype than HNF-1α patients. The differences in both pancreatic and extrapancreatic phenotypes of HNF-1α and HNF-1β mutations is striking, particularly in view of the close homology of these transcription factors and their shared binding site. Our work suggests that HNF-1α and HNF-1β have a different role in maintaining normal glucose homeostasis. Further studies are needed to explain these differences that may relate to specific actions of the two transcription factors, as well as differing tissue expression at different stages of fetal growth. This work suggests a novel etiology for insulin resistance involving the transcription factor HNF-1β.

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References