

Contrasting Insulin Sensitivity of Endogenous Glucose Production Rate in Subjects With Hepatocyte Nuclear Factor-1 β and -1 α Mutations

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Heterozygous mutations in the transcription factors hepatocyte nuclear factor (HNF)-1 α and -1 β result in MODY (maturity-onset diabetes of the young). Despite structural similarity between HNF-1 α and -1 β , HNF-1 β mutation carriers have hyperinsulinemia, whereas HNF-1 α mutation carriers have normal or reduced insulin concentrations. We examined whether HNF-1 β mutation carriers are insulin resistant. The endogenous glucose production rate and rate of glucose uptake were measured with a two-step, low-dose (0.3 mU \cdot kg⁻¹ \cdot min⁻¹) and high-dose (1.5 mU \cdot kg⁻¹ \cdot min⁻¹) hyperinsulinemic-euglycemic clamp, with an infusion of [6,6-²H₂]glucose, in six subjects with HNF-1 α mutations, six subjects with HNF-1 β mutations, and six control subjects, matched for age, sex, and BMI. Endogenous glucose production rate was not suppressed by low-dose insulin in HNF-1 β subjects but was suppressed by 89% in HNF-1 α subjects ($P = 0.004$) and 80% in control subjects ($P < 0.001$). Insulin-stimulated glucose uptake and suppression of lipolysis were similar in all groups at low- and high-dose insulin. Subjects with HNF-1 β mutations have reduced insulin sensitivity of endogenous glucose production but normal peripheral insulin sensitivity. This is likely to reflect reduced action of HNF-1 β in the liver and possibly the kidney. This may be mediated through regulation by HNF-1 β of the key gluconeogenic enzymes glucose-6-phosphatase or PEPCK. *Diabetes* 55:405–411, 2006

Heterozygous mutations in hepatocyte nuclear factor (HNF)-1 α and -1 β can cause maturity-onset diabetes of the young (MODY) (1,2). HNF-1 α and -1 β are homeodomain-containing transcription factors that share >90% sequence homology in their DNA binding sites and can function as homo-

heterodimers (3). They are expressed in a number of tissues, primarily the liver, kidney, and pancreas. They are important in embryonic organ development and tissue-specific gene expression.

Although HNF-1 α and -1 β are structurally similar, the phenotypes associated with their mutations differ. HNF-1 β mutation carriers are characterized by renal abnormalities, particularly renal cystic disease (2,4–8). Other features include pancreatic atrophy, exocrine dysfunction, hyperuricemia, genital tract malformations, and abnormal liver function tests (4,9–11). In contrast, HNF-1 α mutations are primarily characterized by diabetes. This may reflect differential expression of these transcription factors, with HNF-1 α predominately expressed in the liver and HNF-1 β in the kidney, and differences in timing of expression of HNF-1 β and -1 α in embryonic development.

The primary pathology in MODY is β -cell dysfunction. This has been well documented in MODY because of mutations in HNF-1 α and -4 α , using graded glucose infusions (12,13). Although such elegant studies have not been carried out in patients with diabetes because of HNF-1 β mutations, oral and intravenous glucose tolerance tests in individual patients have shown reduced insulin response to glucose (4,5,7). In a recent study, subjects with HNF-1 β mutations did not show the large response to tolbutamide relative to glucose seen in HNF-1 α mutation carriers, suggesting a difference in the etiology of the β -cell defect in these two forms of MODY (14).

Studies of subjects with HNF-1 α mutations suggest that insulin sensitivity is not decreased; it has been reported to be normal compared with control subjects (14,15) and increased compared with first-degree relatives of patients with type 2 diabetes (16). Subjects with HNF-1 β mutations were recently shown to have fasting insulin levels 2.5 times those of subjects with HNF-1 α mutations and 1.8 times those of control subjects matched for BMI (14). Insulin sensitivity determined by homeostasis model assessment (HOMA) was reduced in subjects with HNF-1 β compared with normal control subjects and subjects with HNF-1 α mutations. HNF-1 β subjects also showed other markers of insulin resistance more commonly associated with type 2 diabetes, such as dyslipidemia with raised triglycerides and decreased HDL cholesterol (14).

We hypothesized that HNF-1 β mutations may be associated with insulin resistance, and this would not be a feature of HNF-1 α mutations. Because HNF-1 β is a liver transcription factor and has not been described in muscle or fat, we hypothesized that the site of insulin resistance in HNF-1 β would be the liver and/or the kidney. We carried

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G6Pase, glucose-6-phosphatase; HNF, hepatocyte nuclear factor; HOMA, homeostasis model assessment; MCR, metabolic clearance rate; MODY, maturity-onset diabetes of the young; NEFA, nonesterified fatty acid.

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out a two-step, euglycemic-hyperinsulinemic clamp combined with an infusion of a glucose tracer to measure the insulin sensitivity of endogenous glucose production rate and glucose uptake (17) in HNF-1 β and -1 α mutation carriers and matched control subjects.

RESEARCH DESIGN AND METHODS

Six subjects matched for age, sex, and BMI were recruited into three groups: HNF-1 α mutation carriers, HNF-1 β mutation carriers, and healthy control subjects with no first degree-relatives with diabetes. The HNF-1 α and -1 β mutation carriers were recruited from the Diabetes UK MODY collection based in Exeter, U.K. The mutations in HNF-1 β in these subjects were: S151P (6), R181X, P159fsdelT (9), R295P, P328L329fsdelCCTCT (5), and IVS2 + 1G>T (18). Three of the six subjects had diabetes, and all three were treated with insulin. The mutations in HNF-1 α in these subjects were E132K, IVS8 + 1G>A, R229P, R272H, and two subjects with P291fsinsC. One of these subjects had diabetes and was treated with a sulfonylurea. All participants gave written informed consent, and the study was approved by the South West Surrey and East Devon research ethics committees.

Study protocol. Subjects were asked not to undertake any vigorous physical activity for 72 h and to fast overnight before the study day. On the day of the study, subjects were weighed and intravenous cannulae inserted into the antecubital fossa of each arm: one for drawing blood and the other for infusion of glucose isotope, insulin, and exogenous glucose. Subjects treated with insulin were changed to an injection of soluble (Actrapid) insulin the evening before the study, and the normal dose was halved. Subjects treated with oral hypoglycaemic agents omitted their medication for 2 days before the study. After taking baseline (unenriched) blood samples, a primed constant infusion of [6,6-²H₂]glucose (170 mg, 1.7 mg/min) was commenced. In subjects with diabetes, a fasting glucose measurement was taken on admission, and if necessary they were commenced on a variable infusion of soluble insulin to lower their glucose to 5 mmol/l. The isotope infusion was commenced once this target was reached. After achieving a steady state with the stable isotope, a further five baseline samples were taken between 100 and 120 min to measure the enrichment of glucose and concentration of insulin, glucose, nonesterified fatty acids (NEFAs), and glucagon. Samples were also taken for the measurement of renal function, liver function, and lipid profile at baseline (100–120 min).

At 120 min, a two-step, hyperinsulinemic-euglycemic clamp was started: step 1 insulin infusion 0.3 mU · kg⁻¹ · min⁻¹, low dose for 120 min; and step 2 insulin infusion 1.5 mU · kg⁻¹ · min⁻¹, high dose for 180 min. Blood glucose concentration was measured every 5 min during the insulin infusion, and glucose concentration was maintained within ± 1 mmol/l of the baseline glucose concentration, using dextrose spiked with [6,6-²H₂]glucose (7 mg/g glucose for step 1 and 10 mg/g glucose for step 2). Previous studies (F.S.-M., A.M.U., unpublished data) have shown that this is the optimal spiking needed to maintain constant enrichment of plasma glucose during the high-dose insulin infusion. Blood samples were taken every 30 min except between 210 and 240 min (steady-state measurements with low-dose insulin infusion) and between 390 and 420 min (steady-state measurements with high-dose insulin infusion) when samples were taken every 5 min. Two patients with HNF-1 β mutations required an initial insulin infusion before commencing the clamp. Their fasting glucoses were 12.4 and 10.6 mmol/l; the former patient did not progress from low-dose infusion to high-dose infusion because of venous access difficulties. One patient with HNF-1 α mutation had a fasting glucose of 16.3 mmol/l and required an initial insulin infusion before commencing the clamp. One control subject did not progress from low- to high-dose insulin infusion because of venous access difficulties.

Body composition. Height, weight, and waist-to-hip ratio were measured before each study. Bioelectrical impedance analysis was measured in the erect position, after voiding, using a body fat analyzer (TBF-305; Tanita, Arlington Heights, IL).

Analytical methods. All isotopic enrichments were measured by gas chromatography–mass spectrometry on an HP 5971A mass selective detector (Agilent Technologies, Berkshire, U.K.). The isotopic enrichment of glucose was determined using a penta-*O*-trimethylsilyl-D-glucose-*O*-methoxime derivative analyzed by selected ion monitoring of the ions at *m/z* (charge/mass ratio) 319 and 321 (19). Glucose concentration in the plasma samples was determined by a glucose oxidase method using a Clandon Scientific glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Insulin was measured, using an in-house double-antibody radioimmunoassay (intra-assay coefficient of variation [CV] 6%) (20). Serum NEFA concentration was determined using a commercially available kit (interassay CV 3.0%; Wako Chemicals, Neuss, Germany). Glucagon was measured using a commercially available kit (interassay CV 5%; Linco Research, St. Louis, MO).

TABLE 1
Biochemical data of the experimental mice (16 weeks)

Baseline characteristics	HNF-1 β	HNF-1 α	Control
<i>n</i>	6	6	6
Age (years)	34.5 \pm 4.05	35.5 \pm 4.63	34.3 \pm 4.55
Sex (M/F)	2/4	2/4	2/4
BMI (kg/m ²)	26.2 \pm 0.88	27.1 \pm 1.07	26.0 \pm 1.30
Waist-to-hip ratio	0.86 \pm 0.02	0.88 \pm 0.03	0.85 \pm 0.04
Body fat (%)	31.4 \pm 4.2	32.1 \pm 5.2	33.2 \pm 4.7
Fasting plasma glucose (mmol/l)	6.9 (4.9–12.4)	7.1 (4.8–16.3)	5.1 (4.4–5.7)
Creatinine clearance (ml/min)	56 \pm 7*	93 \pm 9	100 \pm 8.8
HbA _{1c} (%)	6.7 \pm 1.0	6.2 \pm 0.7	5.0 \pm 0.06
Baseline insulin (pmol/l)	103.67 \pm 15.94†	56.5 \pm 7.86	58.51 \pm 4.79

Data are the means \pm SE, *n*, and the means (range). **P* = 0.005 vs. control, *P* = 0.017 vs. HNF-1 α ; †*P* = 0.023 vs. control, *P* = 0.017 vs. HNF-1 α .

Calculations. Endogenous glucose appearance rate (*R_a*) and glucose disposal rate (*R_d*) were calculated, using the model proposed by Steele et al. (21) modified for stable isotopes. The volume of distribution was assumed to be 22% of body weight. The calculation was also modified for inclusion of [6,6-²H₂]glucose in the dextrose infusion (22). Before calculation of glucose turnover, plasma glucose concentration and glucose enrichment time courses were smoothed, using optimal segments technique analysis (23). Calculation of the metabolic clearance rate (MCR) of glucose (ml · kg⁻¹ · min⁻¹) at each steady state was model independent (glucose tracer infusion rate/glucose tracer concentration). For each time point, the mean endogenous glucose *R_a* and *R_d* were calculated. In addition to each steady state (basal, low dose, and high dose), the mean of the five steady-state sample values was calculated for endogenous glucose *R_a*, *R_d*, and MCR.

Statistical analysis. The results are presented as the means \pm SE. Characteristics at baseline and the change in glucose *R_a* and *R_d* from the basal steady state to the low- and high-dose steady state were compared between groups, using ANOVA. When *P* < 0.05, Tukey's post hoc analysis was performed. Glucose *R_a*, *R_d*, MCR, insulin, NEFAs, and glucagon concentration during the basal, low-dose and high-dose steady state were compared across each group by repeated-measures ANOVA with a post hoc Tukey's test.

RESULTS

Baseline characteristics. Subjects with HNF-1 β mutations were well matched to both HNF-1 α and control subjects (Table 1). In keeping with renal abnormalities associated with HNF-1 β mutations, creatinine clearance estimated using the Cockcroft and Gault equation (24) was lower in this group than in the control group (*P* = 0.005) and the HNF-1 α mutation carriers (*P* = 0.017). Insulin concentration was significantly higher at baseline in subjects with HNF-1 β mutations than in subjects with HNF-1 α mutations and control subjects.

Hyperinsulinemic-euglycemic clamp. During the clamp there was no significant difference in the glucose and insulin concentrations of HNF-1 α subjects, HNF-1 β subjects, and control subjects at low- and high-dose steady state (Fig. 1). There was no difference in the calculated MCR for insulin (MCR_{insulin}) at low-dose steady state (8.51 \pm 0.54, 10.2 \pm 0.89, and 9.55 \pm 0.59 ml · kg⁻¹ · min⁻¹ for HNF-1 β , HNF-1 α , and control, respectively; *P* = 0.25) or high-dose steady state (9.99 \pm 0.63, 9.7 \pm 0.73, and 9.6 \pm 0.57, respectively; *P* = 0.67).

There was no significant difference in basal endogenous glucose *R_a* and glucose *R_d* between the three groups (Table 2). There was no significant difference in the exogenous glucose infusion rate required to maintain euglycemia between the three groups at low-dose steady state (1.56 \pm 0.40, 2.53 \pm 0.44, and 2.31 \pm 0.26 mg · kg⁻¹ · min⁻¹ for HNF-1 β , HNF-1 α , and control, respectively; *P* = 0.18) or high-dose steady state (10.57 \pm 1.58, 10.28 \pm

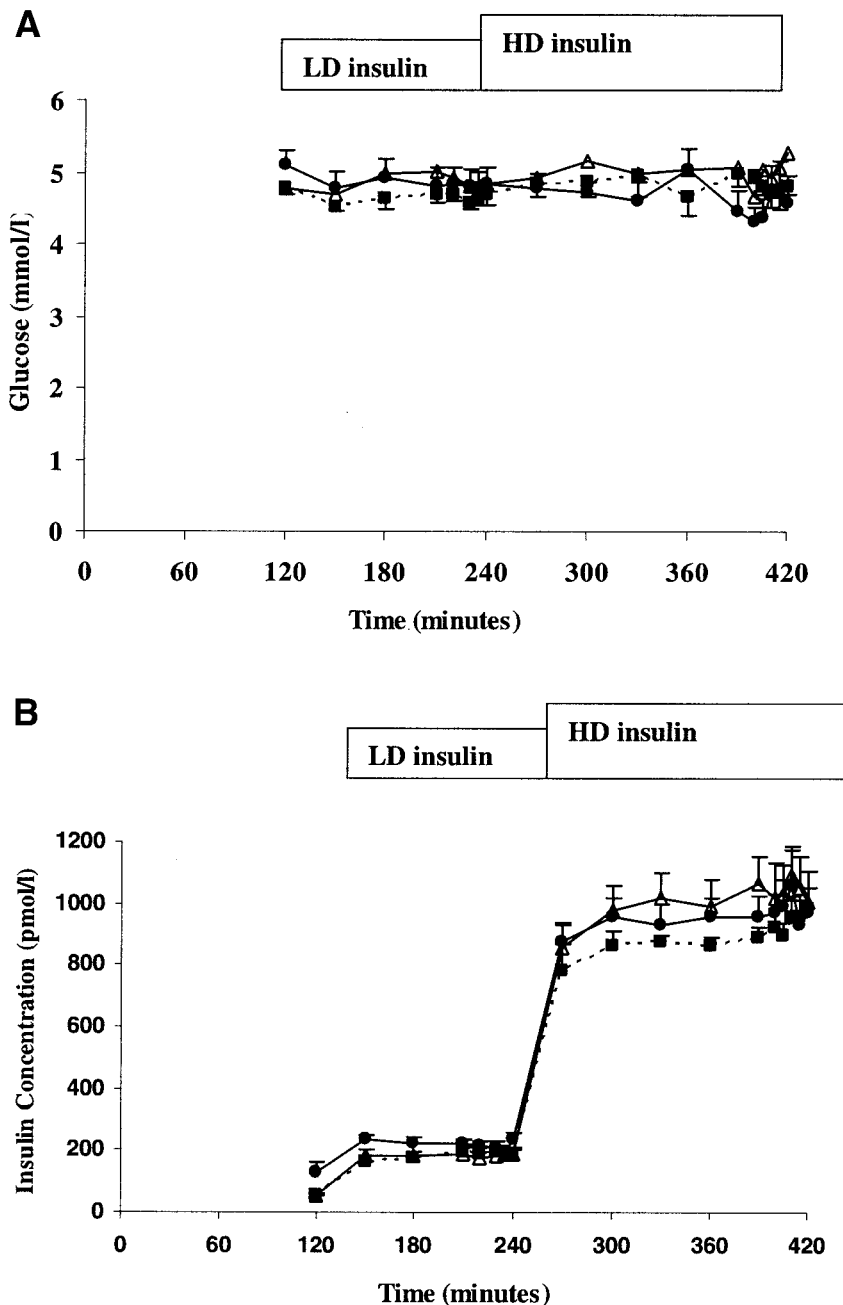


FIG. 1. Glucose (A) and insulin (B) concentrations during low-dose (LD) and high-dose (HD) insulin infusion in the hyperinsulinemic-euglycemic clamp. Data are the means \pm SE. ● and solid line, HNF-1 β mutation carriers; \triangle and solid line, HNF-1 α subjects; ■ and dashed line, control subjects.

1.22, and 9.96 ± 0.62 , respectively; $P = 0.95$). The HNF-1 β group showed less suppression of endogenous glucose R_a with low-dose insulin infusion than the HNF-1 α group ($P = 0.004$) and the control group ($P < 0.04$) (Table 2, Fig. 2). Compared with the basal steady state, low-dose insulin infusion suppressed endogenous glucose R_a by 80% ($P < 0.001$) in control subjects and by 89% in HNF-1 α subjects ($P = 0.004$), but it had no significant effect in HNF-1 β mutation carriers ($P = 0.53$). All groups showed complete suppression of endogenous glucose R_a with high-dose insulin compared with basal ($P < 0.01$). When only those not requiring an insulin infusion to achieve euglycemia before the clamp were analyzed (HNF-1 β : $n = 4$; HNF-1 α : $n = 5$; and control subjects: $n = 6$), a similar result was seen, with endogenous glucose production suppressed less by low-dose insulin in the HNF-1 β patients (change from basal: $1.86 \pm 2.34 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) compared

with HNF-1 α patients (9.66 ± 1.69 , $P = 0.011$) and control subjects (7.46 ± 0.48 , $P = 0.05$).

In all groups there was an increase in glucose R_d and MCR with the high-dose insulin infusion compared with basal ($P < 0.01$ for all groups). Comparing between groups, there was no significant difference between the change in glucose R_d and MCR from basal to low-dose insulin infusion and from basal to high-dose insulin infusion (Table 2, Fig. 3).

NEFA concentrations. All groups showed suppression of NEFA with low-dose ($P = 0.05$, $P < 0.001$, and $P < 0.001$ for HNF-1 β , HNF-1 α , and control subjects, respectively) and high-dose insulin infusion ($P = 0.01$, $P < 0.001$, and $P < 0.001$, respectively) (Table 3).

Glucagon concentrations. Basal glucagon concentrations were lower in the control group (49.8 ± 8.99 ng/ml) than the HNF-1 α (83.7 ± 27.7 ng/ml) and HNF-1 β group

TABLE 2
Glucose metabolism during the hyperinsulinemic-euglycemic clamp

	HNF-1 β		HNF-1 α		Control subjects	
	Change from basal		Change from basal		Change from basal	
Endogenous glucose R_a (basal) ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	9.49 \pm 1.65	—	10.84 \pm 1.2	—	9.28 \pm 0.43	—
Endogenous glucose R_a (LD) ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	7.11 \pm 1.64	2.38 \pm 1.73*	1.09 \pm 0.85†	9.63 \pm 1.39	1.81 \pm 0.6†	7.46 \pm 0.48
Endogenous glucose R_a (HD)‡ ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	-0.08 \pm 1.32†	10.65 \pm 0.75	0.9 \pm 2.65†	10.66 \pm 1.79	-2.04 \pm 2.07†	11.3 \pm 2.09
Glucose R_d (basal) ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	9.33 \pm 1.25	—	11.46 \pm 0.74	—	9.40 \pm 0.57	—
Glucose R_d (LD) ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	15.95 \pm 3.00	6.63 \pm 2.49	16.08 \pm 2.26	4.63 \pm 1.95	14.99 \pm 1.72	5.60 \pm 1.34
Glucose R_d (HD)* ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	58.0 \pm 10.67†	47.7 \pm 10.21	56.53 \pm 7.47†	45.1 \pm 6.96	54.4 \pm 5.18†	45.12 \pm 4.94
Glucose MCR (basal) ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	2.36 \pm 0.24	—	2.91 \pm 0.33	—	2.14 \pm 0.11	—
Glucose MCR (LD) ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	3.48 \pm 0.81	1.12 \pm 0.81	3.5 \pm 0.48	0.59 \pm 0.39	3.28 \pm 0.37	1.14 \pm 0.29
Glucose MCR (HD)* ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	13.5 \pm 2.86†	11.11 \pm 2.86	11.66 \pm 1.61†	8.74 \pm 1.50	11.94 \pm 0.74†	9.78 \pm 0.64

Data are the means \pm SE. * $P = 0.004$ vs. HNF-1 α ; $P < 0.04$ vs. control subjects; † $P < 0.01$ vs. basal; ‡ $n = 5$ HNF-1 β mutation carriers and control subjects in high-dose insulin infusion. HD, high-dose insulin infusion; LD, low-dose insulin infusion.

(89.4 \pm 13.6), although this was not significant ($P = 0.31$). There was no significant difference in glucagon concentration across the three groups with low- and high-dose insulin infusion.

DISCUSSION

This study has demonstrated that subjects with HNF-1 β mutations show reduced insulin sensitivity of endogenous glucose production compared with subjects with HNF-1 α mutations and healthy control subjects. In contrast, there was no difference in peripheral insulin sensitivity (insulin-mediated glucose disposal) between the three groups of subjects. This is the first time that insulin sensitivity has been studied in subjects with HNF-1 β mutations using the hyperinsulinemic-euglycemic clamp and the first comparison of insulin sensitivity in subjects with HNF-1 α and -1 β mutations. Impaired insulin sensitivity of endogenous glucose production rate without any impairment of peripheral glucose uptake has not previously been described in any other type of diabetes.

The finding of insulin resistance in HNF-1 β is in keeping with a recent study (14) showing that subjects with HNF-1 β mutations have features of insulin resistance, such as high fasting insulin levels and dyslipidemia, and

decreased insulin sensitivity when determined by HOMA. In the current study, using euglycemic-hyperinsulinemic clamps with a glucose tracer, we have confirmed the finding of insulin resistance in HNF-1 β mutation carriers and identified the site of this resistance to insulin action as failure to suppress endogenous glucose production. Insulin-induced peripheral glucose uptake and suppression of lipolysis was normal. Because HOMA is a measure of insulin sensitivity derived from fasting measures, it reflects glucose production more than peripheral insulin action, so the results are consistent in both studies. Endogenous glucose production (R_a) is primarily a measure of hepatic insulin sensitivity. Glucose production also occurs in the kidneys, although the contribution to total glucose production is controversial. One study has reported that renal glucose output is responsible for only 5% of glucose production in the fasting state (25), whereas Stumvoll et al. (26) suggest that 25% of total glucose production is from the kidney. Therefore, the contribution from the kidney cannot be overlooked, particularly when studying patients with the HNF-1 β mutation because HNF-1 β is strongly expressed in both the liver and kidneys.

In patients with HNF-1 α mutations, insulin sensitivity

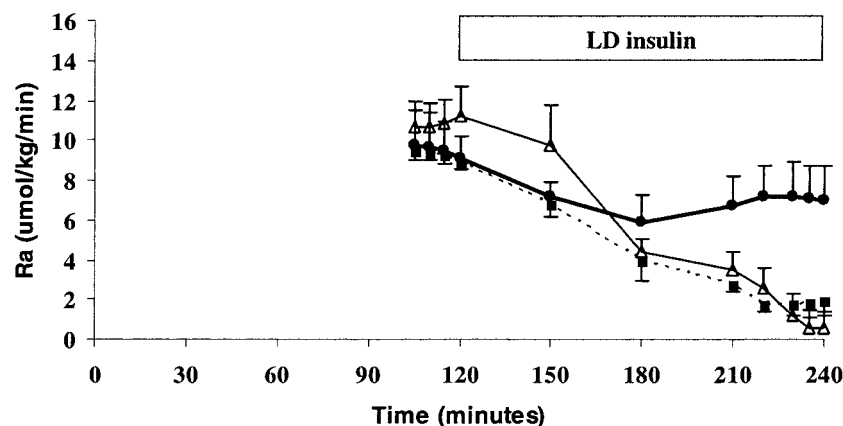


FIG. 2. Endogenous glucose R_a during the hyperinsulinemic-euglycemic clamp. Data are the means \pm SE. ● and solid line, HNF-1 β mutation carriers; △ and solid line, HNF-1 α subjects; ■ and dashed line, control subjects. LD, low-dose insulin infusion.

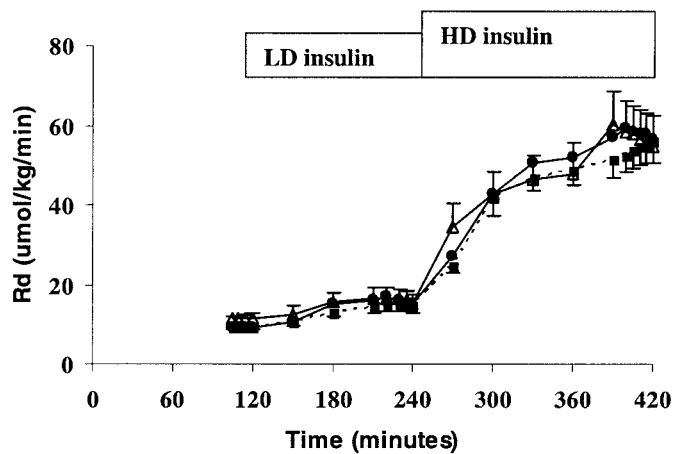


FIG. 3. Glucose R_d during the hyperinsulinemic-euglycemic clamp. Data are the means \pm SE. \bullet and solid line, HNF-1 β mutation carriers; Δ and solid line, HNF-1 α subjects; \blacksquare and dashed line, control subjects. HD, high-dose insulin infusion; LD, low-dose insulin infusion.

has been measured previously with a hyperinsulinemic-euglycemic clamp, without an isotope of glucose, with insulin doses of 1–1.2 mU \cdot kg $^{-1}$ \cdot min $^{-1}$. This dose would be expected to suppress endogenous glucose production and would thus be a measure of the insulin sensitivity of peripheral glucose uptake. In these studies, insulin sensitivity was reported to be similar to control subjects (15,27) and increased compared with first-degree relatives of patients with type 2 diabetes (16), who are known to be an insulin-resistant group (28). Our results are consistent with these previous studies that suggest peripheral insulin sensitivity is normal in HNF-1 α patients.

One previous study measured endogenous glucose output in HNF-1 α mutation carriers using a low-dose (0.3 mU \cdot kg $^{-1}$ \cdot min $^{-1}$) hyperinsulinemic-euglycemic clamp with infusion of glucose isotope (29). Endogenous glucose production was suppressed by this dose of insulin to 41% of basal values in the HNF-1 α patients, which was less than in the normal control subjects, who suppressed it to 20% of their basal values ($P < 0.05$). In the current study, subjects with HNF-1 α mutations showed $>75\%$ suppression of endogenous glucose production with low-dose insulin, which was not different from the control patients. The differences between our study and the previous study probably reflect methodological differences; the previous study used basal values determined on a separate day, and glucose concentration was only clamped at the end of the insulin infusion in the HNF-1 α mutation carriers.

Euglycemic-hyperinsulinemic clamps have shown insulin resistance both peripherally and at the level of the liver in patients with established type 2 diabetes (17,30), although in impaired glucose tolerance, peripheral insulin sensitivity alone is reduced (31). Studies in patients with type 1 diabetes have shown decreased peripheral insulin

sensitivity (32,33) and decreased hepatic insulin sensitivity (17).

Patients with HNF-1 β mutations have impairment in renal function, which itself is associated with insulin resistance. However, we believe there are two reasons why this does not explain the finding in this patient group. First, creatinine clearance was only moderately reduced in our HNF-1 β patients (mean 58.2 ml/min), and the MCR of insulin was unaltered in the HNF-1 β mutation carriers compared with control subjects. Second, patients with renal failure show impaired peripheral glucose utilization in addition to increased endogenous glucose output (34).

This study has several limitations. HNF-1 β mutations are rare, and therefore we were only able to study a small number of subjects. However, this is the only study that has used the hyperinsulinemic-euglycemic clamp in this group, and the study was adequately powered to detect the marked differences in insulin response that were anticipated from previous studies. In addition, both HNF groups were heterogeneous, containing patients with and without diabetes and various degrees of diabetic control. Despite the heterogeneity of the groups, the differences in the response of endogenous glucose production to low-dose insulin was consistent within groups. This is reassuring, suggesting that the effect seen was a result of the genotypic differences rather than diabetes itself. Poor glycemic control is associated with insulin resistance (35), but this is secondary to impaired stimulation of peripheral glucose uptake rather than suppression of endogenous glucose production. Therefore, glucotoxicity is unlikely to account for our findings. An insulin infusion was required in two subjects with HNF-1 β mutations and one subject with HNF-1 α mutations before commencement of the clamp. This may have adversely affected the endogenous glucose R_a in these patients. However, we showed consistent results within groups and consistent differences across groups, suggesting that prior insulin infusion was not a confounding factor.

Insulin acts at the liver and kidney to suppress gluconeogenesis and glycogenolysis by inhibiting two rate-limiting enzymes, PEPCK and glucose-6-phosphatase (G6Pase). In type 2 diabetes, it has been proposed that increased endogenous glucose production may reflect increased expression of these enzymes (36). G6Pase is increased in animal models of diabetes (37,38), and over-expression of G6Pase in rat liver resulted in glucose intolerance and hyperinsulinemia (39). HNF-1 binding sites are present in both promoter regions of PEPCK and G6Pase genes, suggesting a regulatory role (40,41). In the G6Pase promoter, two regions have been described that form an insulin response element (42). The forkhead transcription factor Foxo1 is phosphorylated by protein kinase B/Akt, and this is required for insulin suppression of G6Pase expression (43). HNF-1 also binds to this insulin response element and acts to enhance this effect (44).

TABLE 3

NEFA concentrations during the basal and low- and high-dose insulin infusion steady state

	HNF-1 β *	HNF-1 α	Control subjects
Basal (mmol/l)	0.69 \pm 0.19	0.62 \pm 0.13	0.73 \pm 0.091
Low-dose insulin infusion (mmol/l)	0.21 \pm 0.10 \dagger	0.09 \pm 0.03 \ddagger	0.08 \pm 0.02 \ddagger
High-dose insulin infusion (mmol/l)	0.03 \pm 0.016 \S	0.17 \pm 0.003 \ddagger	0.03 \pm 0.02 \ddagger

Data are the means \pm SE. * $n = 5$ in high-dose insulin infusion; $\dagger P = 0.05$ vs. basal; $\ddagger P < 0.001$ vs. basal; $\S P = 0.01$ vs. basal.

From this, it can be seen that HNF1 transcription factors have a regulatory role in insulin action on gluconeogenesis. However, the differential effects on insulin sensitivity by mutations in HNF-1 α and -1 β is intriguing. Differential regulation of G6Pase by HNF-1 β and -1 α has been previously described (45), but it could also reflect differences in tissue expression, or differences in embryological development.

In summary, patients with HNF-1 α and -1 β mutations have important differences in insulin sensitivity. HNF-1 β mutation carriers do not suppress endogenous glucose production in response to a low-dose insulin infusion, but they have normal insulin-stimulated glucose uptake, in contrast to HNF-1 α mutation carriers, where both insulin suppression of endogenous glucose production and insulin-mediated glucose uptake are normal. This finding highlights the importance of the HNF-1 transcription factors in mediating insulin action and suggests that studying patients with monogenic defects (HNF-1 β mutations) may provide insight into the mechanism and potential therapy of hepatic insulin resistance in complex polygenic type 2 diabetes.

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