Hepatic Function in a Family with a Nonsense Mutation (R154X) in the Hepatocyte Nuclear Factor-4α/MODY1 Gene

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Abstract

Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous monogenic disorder characterized by autosomal dominant inheritance, onset usually before 25 yr of age, and abnormal pancreatic β-cell function. Mutations in the hepatocyte nuclear factor(HNF)-4α/MODY1, glucokinase/MODY2, and HNF-1α/MODY3 genes can cause this form of diabetes. In contrast to the glucokinase and HNF-1α genes, mutations in the HNF-4α gene are a relatively uncommon cause of MODY, and our understanding of the MODY1 form of diabetes is based on studies of only a single family, the R-W pedigree. Here we report the identification of a second family with MODY1 and the first in which there has been a detailed characterization of hepatic function. The affected members of this family, Dresden-11, have inherited a nonsense mutation, R154X, in the HNF-4α gene, and are predicted to have reduced levels of this transcription factor in the tissues in which it is expressed, including pancreatic islets, liver, kidney, and intestine. Subjects with the R154X mutation exhibited a diminished insulin secretory response to oral glucose. HNF-4α plays a central role in tissue-specific regulation of gene expression in the liver, including the control of synthesis of proteins involved in cholesterol and lipoprotein metabolism and the coagulation cascade. Subjects with the R154X mutation, however, showed no abnormalities in lipid metabolism or coagulation except for a paradoxical 3.3-fold increase in serum lipoprotein(a) levels, nor was there any evidence of renal dysfunction in these subjects. The results suggest that MODY1 is primarily a disorder of β-cell function. (*J. Clin. Invest.* 1997. 100:1400–1405.) Key words: maturity-onset diabetes of the young • diabetes mellitus • MODY1 • insulin deficiency • liver function

Introduction

A recent study has shown that mutations in the hepatocyte nuclear factor (HNF)[1]-4α gene can cause maturity-onset diabetes of the young (MODY), a monogenic form of diabetes mellitus characterized by autosomal dominant inheritance and early onset, usually before 25 yr of age (1, 2). The subtype of MODY resulting from mutations in the HNF-4α gene is termed MODY1. HNF-4α is a member of the nuclear receptor superfamily, a family of transcription factors that plays an important role in cellular regulation linking extracellular signals and transcriptional responses (3, 4). HNF-4α was identified during the course of characterizing the transcription factors that were responsible for the tissue-specific regulation of gene expression in adult liver (3). It is not restricted to liver, however, and is also found in other tissues, including kidney, intestine, and pancreatic β-cells (2–4).

Our understanding of the pathophysiology of MODY1 is based entirely on clinical studies in a single family, the R-W pedigree, a white family of German ancestry in which MODY is due to a nonsense mutation, Q268X, in the HNF-4α gene (1, 2, 5, 6). These studies showed that prediabetic subjects with the Q268X mutation had normal insulin sensitivity, but abnormal β-cell response to glucose, suggesting that MODY1 was primarily a disorder of β-cell function (5, 6). These studies, however, predated the identification of the MODY1 gene as the liver-enriched transcription factor HNF-4α, and it is not known whether alterations in hepatic or renal function are also a feature of HNF-4α deficiency.

In this study, we report the phenotypic characterization of β-cell, hepatic, and renal function in members of a family with a nonsense mutation (R154X) in the HNF-4α gene, identified during a systematic search for mutations in known MODY genes in subjects of German ancestry (7).

Methods

Subjects. The study population consisted of members of twelve unrelated families with early-onset non–insulin-dependent diabetes mellitus (NIDDM) ascertained through the Department of Internal Medicine III (University Clinic Carl Gustav Carus of the Technical University, Dresden, Germany). Families were selected based on the presence of NIDDM (type 2) in two or more generations with diagnosis before 35 yr of age in at least one subject. Sufficient family data were available to suggest a diagnosis of MODY in nine of these families (i.e., NIDDM in three generations with autosomal dominant inheritance and onset before 25 yr of age in at least one affected subject) (1). The remaining three families were classified as having early-onset NIDDM. The average age at diagnosis of diabetes in affected members of these twelve families was 29.9±2.8 yr (range, 14–60 yr) (mean±SEM) and included 18 men and 13 women of whom 12, 12,

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1. *Abbreviations used in this paper:* HNF, hepatocyte nuclear factor; MODY, maturity-onset diabetes of the young; NIDDM, non-insulin-dependent diabetes mellitus; PAI-1, plasminogen activator inhibitor-1; tPA, tissue-type plasminogen activator.

and 7 were being treated with insulin, oral hypoglycemic agents, and diet, respectively. At the time of recruitment, informed consent was obtained from each subject, and blood and urine samples were obtained for DNA isolation and clinical testing.

Screening HNF-4α gene for mutations. The minimal promoter region (nucleotides −21 to −459) (8) and 10 exons encoding the HNF-4α2 form (9) of HNF-4α were screened for mutations by PCR amplification and direct sequencing of both strands of the amplified PCR product as described previously (2). Sequence changes were confirmed by cloning the PCR product into pGEM-4Z (Promega Corp., Madison, WI) and sequencing clones derived from both alleles. The sequences of the primers for the amplification and sequencing of the minimal promoter region are P1 (5′-CAAGGATCCAGAAGATTGGC-3′) and P2 (5′-CGTCCTCTGGGAAGATCTGC-3′); the size of the PCR product is 479 bp. The sequence of the promoter of the human HNF-4α gene has been deposited in the GenBank database with accession number U72959.

Linkage analysis. Family members were typed with the markers D20S43, D20S89, D20S96, D20S119, D20S169, and D20S424, all of which are tightly linked to the HNF-4α gene (10). Tests for linkage were carried out using the haplotype formed from these markers, and assuming a recombination frequency between adjacent markers of 0.001 with the computer program ILINK (11, 12). The frequencies of the haplotypes were estimated from the data. The analysis assumed a disease allele frequency of 0.001 and two liability classes. Liability class 1 included individuals who were 25 yr of age with penetrances of 0.00, 0.95, and 0.95 for the normal homozygote, heterozygote, and susceptible homozygote, respectively. Liability class 2 included individuals who were less than 25 yr of age with penetrances of 0.00, 0.60, and 0.95 for the normal homozygote, heterozygote, and susceptible homozygote, respectively. The affection status of the one subject with impaired glucose tolerance was coded as affected. The maximum expected lod score was determined using the computer program SLINK (13, 14).

Clinical studies. A standard 75-g oral glucose tolerance test was given to subjects after a 12 h overnight fast. Treatment with insulin and oral hypoglycemic agents was discontinued 12 h and 24 h, respectively, before testing. Blood samples for glucose, insulin, C-peptide, and proinsulin were drawn at 0, 30, 60, 90, and 120 min. Fasting blood samples were also drawn for the measurement of insulin, islet cell, and glutamic acid decarboxylase (GAD) antibodies, glycosylated hemoglobin (HbA_{1c}), lipoprotein(a), apolipoproteins AI, AII, B, CII, CIII, and E, cholesterol (total and in VLDL, LDL, HDL, HDL2, and HDL3), triglycerides (total and in VLDL and LDL+HDL), thromboplastin time and partial thromboplastin time, fibrinogen, von Willebrand factor antigen (vWFr:Ag), plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (tPA), alanine aminotransferase, γ-glutamyl transferase, bilirubin, albumin, total protein, hemoglobin, creatinine, urea, amylase, lipase, and uric acid. A urine sample (from a 24-h collection of urine) was taken for measurements of creatinine and microalbumin.

Assays. Blood glucose was measured with a hexokinase method (Boehringer-Mannheim, Mannheim, Germany), plasma insulin, and C-peptide by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA; and C-peptide RIA, Diagnostic Systems Laboratories, Webster, TX, respectively), plasma proinsulin by ELISA (DRG Instruments GmbH, Marburg, Germany), HbA_{1c} by HPLC (DIAMAT Analyzer; Bio-Rad Laboratories, Hercules, CA), fibrinogen by the Clauss method (Fibrinogen A Kit; Boehringer Mannheim), PAI-1 by bioimmunoassay and ELISA (TC® Actibind PAI-1 and TC® PAI-1 ELISA; Technoclone/Immuno GmbH Deutschland, Heidelberg, Germany), tPA by ELISA (TintElize® tPA; Biopool AB, Umeå, Sweden), vWFr:Ag enzymatically (ELISA Asserachrom® vWF; Boehringer Mannheim), insulin- and GAD-Ab by ELISA and radioimmunoassay (ELIAS GmbH, Freiburg, Germany), islet cell-Ab by an immunofluorescence assay (using a positive sample from EUROIMMUN Immunologie GmbH, Groß Grönau, Germany), thromboplastin and partial thromboplastin time by the AMAX Analyzer (Munich, Germany). Total cholesterol, cholesterol in VLDL, HDL, LDL+HDL, and HDL3 were measured by the CHOD-PAP, total triglycerides and triglycerides in VLDL and LDL+HDL by the GPO-PAP method using the Ciba Corning 550 Express Clinical Chemistry Analyzer (Boehringer Mannheim). HDL2-cholesterol was calculated using the formula HDL2 = HDL − HDL3. Samples for the measurement of cholesterol, triglycerides in VLDL, HDL, and LDL+HDL, were prepared by preparative ultracentrifugation using an Optima tabletop TLX ultracentrifuge with a TLA-120.2 rotor (Beckman Instruments, Inc., Fullerton, CA). Serum creatinine, urea, uric acid, total protein, alanine aminotransferase, γ-glutamyl transferase, bilirubin, amylase, and urine creatinine were measured using the BM Hitachi 717 Chemistry Analyzer (Boehringer Mannheim). Lipase was measured using the Monarch System (Sigma Chemical Co., St. Louis, MO). Apolipoproteins AI, AII, B, and urine microalbumin were measured using the Behring-Nephelometer BN II (Behringwerke AG, Marburg, Germany). Apolipoproteins CIII and E were measured using the Sebia System (Fulda, Germany), apolipoprotein CII using the RID System (WAK, Bad Homburg, Germany).

Results

Identification of a nonsense mutation in the HNF-4α gene. Twelve families with early-onset NIDDM/MODY were ascertained for genetic studies of MODY in subjects of German ancestry. Mutations in the HNF-1α/MODY3 gene (15) were

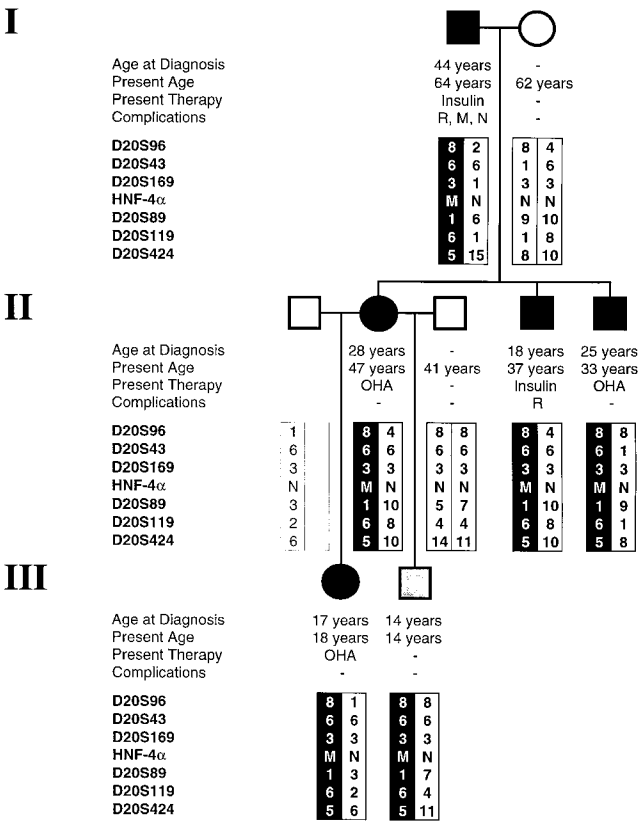

Figure 1. Dresden-11 pedigree. The members of this family with MODY and impaired glucose tolerance are indicated with *black and shaded symbols*, respectively. The age at diagnosis of diabetes mellitus, present age and therapy, and nature of complications are indicated. The haplotype associated with MODY in this family is shown. OHA, oral hypoglycemic agents; M, macrovascular disease; R, retinopathy; N, peripheral polyneuropathy.

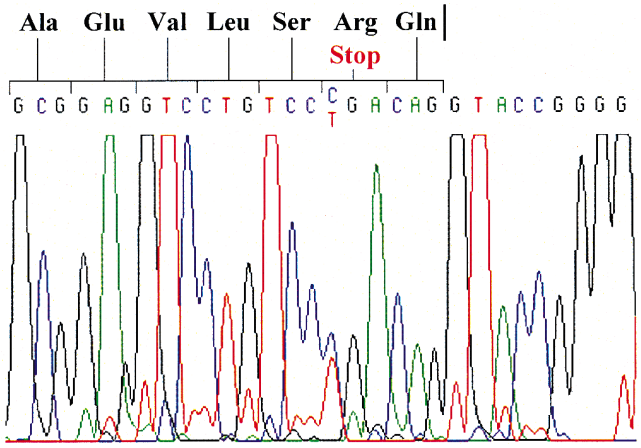

Figure 2. Partial sequence of exon 4 of the HNF-4α gene of subject II-4. The R154X mutation is indicated. Intron 4 follows the Gln codon, CAG.

found in three of these families (7). The HNF-4α gene was screened for mutations in one affected subject from the remaining nine families. There was a C→T substitution in codon 154 of exon 4 in the proband (II-4) of family Dresden-11 (Fig. 1), which generated a nonsense mutation CGA (Arg)→TGA (OP) (R154X, Fig. 2). The R154X mutation would result in the synthesis of a truncated protein of 153 amino acids with an intact DNA-binding domain, but lacking the ligand binding and transactivation domain (3). In addition to this mutation, there was a silent C→T substitution in the codon for Ala58 (GCC/GCT) in one subject that did not cosegregate with MODY/early-onset NIDDM.

The presence of the R154X mutation in other members of the Dresden-11 family was determined by PCR amplification and direct sequencing of exon 4. The R154X mutation cosegregated with MODY in the Dresden-11 family (Fig. 1). All diabetic subjects had the R154X mutation as did a 14-yr-old male (III-2) with impaired glucose tolerance. The at-risk haplotype showed some evidence for linkage with MODY with a lod score of 1.20 at a recombination of 0.00 (the maximum expected lod score in this pedigree is 1.20).

Age at diagnosis. Three subjects were diagnosed with NIDDM between 15–25 yr of age, and two others at 28 and 44 yr (Fig. 1). The subject, I-1, diagnosed with diabetes at 44 yr of age, had proliferative retinopathy at the time of diagnosis, suggesting that the onset of diabetes had been many years earlier.

Clinical severity of diabetes. The diabetes in the Dresden-11 family was severe, and all the diabetic subjects were treated with either insulin or oral hypoglycemic agents. Subjects with diabetes of long duration (e.g., I-1, II-4) had diabetic complications including proliferative retinopathy, macrovascular disease (coronary heart disease), and peripheral polyneuropathy. Surprisingly, none of the subjects with the R154X mutation had evidence of nephropathy. Thus, the diabetic phenotype of the Dresden-11 family is very similar to that seen in the R-W pedigree (1). None of the subjects in the Dresden-11 family were positive for islet, insulin, or GAD antibodies.

Insulin-secretory response. Previous studies have shown that prediabetic subjects with a mutation in HNF-4α exhibit a characteristic defect in the normal pattern of glucose-stimulated insulin secretion as well as abnormalities in other measures of normal β-cell function (5, 6). The OGTT studies showed a profound reduction in insulin secretion accompanied by diminished C-peptide and proinsulin levels in subjects with the R154X mutation (Fig. 3).

Lipid levels. None of the subjects with the R154X mutation showed evidence of secondary hypertriglyceridemia, even though several (I-1, II-4, and III-1) had poor metabolic control with HbA_{1c} levels of 10.6, 8.8, and 10.1, respectively (Table I).

Hepatic and renal function. HNF-4α is expressed in the liver and kidney, and as such, mutations in HNF-4α might be expected to affect the normal function of these tissues (3, 4). In this regard, HNF-4α regulates the expression of a number of apolipoproteins including AI, AIV, B, and CIII (4). The serum apolipoprotein levels and lipoprotein fractions were normal in the subjects with the R154X mutation except for lipoprotein(a) levels, which were elevated 3.3-fold (Table I). Lipoprotein(a) levels have been reported to be elevated in subjects with NIDDM in some studies (16, 17) but not others (18, 19). An elevation in lipoprotein(a) levels in subjects with HNF-4α deficiency, however, appears paradoxical as expression of lipoprotein(a) is controlled by HNF-1α (20), which is in turn regulated by HNF-4α (4). Thus, lower lipoprotein(a) levels, not higher, would be expected in subjects with the R154X mutation. Further studies will be necessary to determine the relationship between lipoprotein(a) levels and mutations in HNF-4α.

HNF-4α also regulates the expression of albumin, fibrinogen, and the coagulation factors VII, VIII, IX, and X (4, 21–24). The serum levels of albumin and fibrinogen and measurements of coagulation time were normal in subjects with the R154X mutation (Table I). HNF-4α is also expressed in the kidney, although the identity of the target genes in this organ are unknown (3, 4). The urinary creatinine and microalbumin levels were normal in subjects with the R154X mutation (Table I) suggesting that renal function was not impaired in subjects with mutations in the HNF-4α gene.

Discussion

MODY is a phenotypically and genetically heterogeneous form of diabetes mellitus (1). Mutations in the glucokinase/MODY2 gene are associated with a mild form of diabetes characterized by plasma glucose levels that are 20–30 mg/dl greater than normal in the fasting and postprandial state; a condition that is usually treated with diet (25). This mild hyperglycemic state is generally not associated with diabetic complications (25). In contrast, mutations in the HNF-1α/MODY3 and HNF-4α/MODY1 genes cause a severe form of diabetes mellitus that is usually treated with insulin and oral hypoglycemic agents, and is frequently associated with diabetic complications (1, 7, 26). Clinical and biochemical studies have provided a clear understanding of the molecular mechanism by which mutations in the glucokinase gene impair β-cell and hepatic function, and cause a physiological condition characterized by chronic mild hyperglycemia (27, 28). The precise mechanism by which mutations in the HNF-1α and HNF-4α genes result in β-cell dysfunction, however, is unclear (5, 6, 29, 30). Deficiency of these two functionally related transcription factors may result in the decreased expression of a protein(s) that plays a key role in determining the functional response of the β-cell to glucose, or perhaps affect β-cell/islet development.

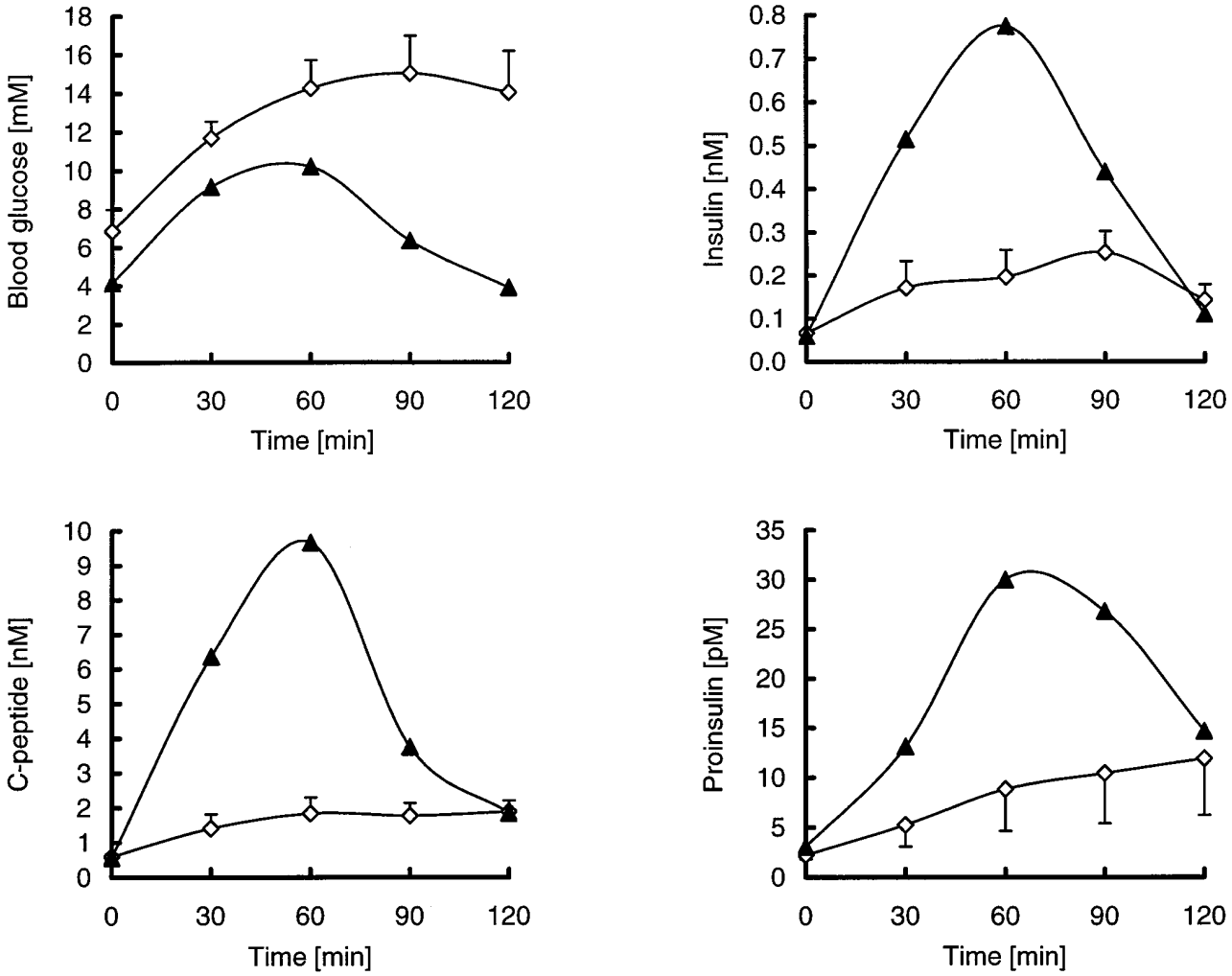

Figure 3. Oral glucose tolerance testing in the Dresden-11 family. The blood glucose, insulin, C-peptide and proinsulin levels during the course of the glucose tolerance test are shown. The *open symbols* are the means±SEM for subjects with the R154X mutation, including those with diabetes and impaired glucose tolerance, and the *filled symbols* are the means for the two normal subjects.

The prevalence of the different forms of MODY appears to differ among ethnic and racial groups. Mutations in the glucokinase and HNF-1α genes are the most common causes of MODY in France, with frequencies of 50% and 25%, respectively (25, 26). Whereas glucokinase mutations are very common in France, they appear to be less common in other populations, possibly because of ascertainment bias (31). Mutations in the HNF-1α gene are the most common cause of MODY identified to date in the United Kingdom, Germany, and Japan, with frequencies in subjects with early-onset NIDDM/MODY of 73%, 25–35% and 8%, respectively (this report, 7, 31, 32). By contrast, mutations in the HNF-4α gene appear to be much less common than mutations in either the glucokinase or HNF-1α genes. In this regard, the R154X mutation reported here is just the second such mutation found in the HNF-4α gene. Our study suggests that the frequency of HNF-4α mutations may be one-third that of HNF-1α mutations, at least in MODY subjects of German ancestry.

Although HNF-1α and HNF-4α are expressed in a number of adult tissues including liver, intestine, kidney, and pancreatic islets, mutations in these genes appear to affect only the pancreatic β-cell. The reason for the differential effects of HNF-4β deficiency is unclear, but may reflect more redundancy with respect to the function of the HNF-family of transcription factors in the liver compared with the β-cell.

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Table I. Clinical Parameters of the Dresden-11 Family

Parameter	Genotype		Reference values
	Normal/mutant	Normal/normal	
		female/male	
Age at diagnosis (yr)	26.40±3.47	—	—
Current age (yr)	35.50±7.58	62/41	—
n (females/males)	2/4	1/1	—
BMI (kg/m^2)	25.21±1.15	41.08/22.86	< 25.00
HbA_{1c} (%)	8.13±0.78	5.60/5.30	< 6.50
Basal insulin (nM)	0.067±0.005	0.080/0.040	0.059–0.253
Basal C-peptide (nM)	0.60±0.08	0.68/0.45	< 1.06
Cholesterol (mM), total	4.72±0.41	5.03/5.01	< 5.20
in VLDL (mM)	0.79±0.31	0.21/0.70	0.10–1.40
in LDL (mM)	2.86±0.25	3.62/3.34	1.80–5.10
in HDL (mM)	1.17±0.18	1.32/1.26	0.80–2.50
in HDL2 (mM)	0.31±0.06	0.44/0.27	0.10–0.60
in HDL3 (mM)	0.86±0.12	0.88/0.99	0.80–1.90
Triglycerides (mM), total	0.70±0.13	0.65/1.45	0.40–2.80
in VLDL (mM)	0.43±0.13	0.34/1.06	0.10–2.10
in LDL+HDL (mM)	0.28±0.02	0.33/0.47	0.20–0.80
Lipoprotein (a) (mg/liter)	816.0±90.4	3.0/6.0	< 250.0
ApoB (g/liter)	1.38±0.22	1.33/1.38	0.72–1.50
ApoAI (g/liter)	1.66±0.16	1.89/2.00	1.12–1.75
ApoAII (g/liter)	0.32±0.02	0.29/0.53	0.30–0.70
ApoE (mg/liter)	61.2±12.2	65.0/55.0	13.0–76.0
ApoCII (mg/liter)	36.0±5.3	36.0/61.0	7.0–63.0
ApoCIII (mg/liter)	26.7±3.7	23.0/36.0	16.0–45.0
General liver and kidney function			
Hemoglobin (mM)	9.7±0.4	9.2/10.8	8.6–12.1
Creatinine (μM)	91.5±5.6	73.0/80.0	< 124.0
Urea (mM)	5.6±0.8	6.6/1.0	3.6–8.9
Total protein (g/liter)	72.7±1.7	77.2/84.0	65.0–85.0
Albumin (g/liter)	38.6±1.0	38.5/43.5	37.0–53.0
Alanine aminotransferase (μmol/[liter·s])	0.39±0.06	0.39/0.91	0.10–0.67
γ-glutamyl transferase (μmol/[liter·s])	0.54±0.12	0.55/1.11	0.18–0.83
Bilirubin (μM), total	16.7±5.2	13.7/24.3	1.0–16.0
Uric acid (μM)	249±28	317/359	208–416
Exocrine pancreatic function			
Amylase (U/liter)	56.8±6.7	30.0/58.0	17.0–115.0
Lipase (μmol/[liter·s])	1.22±0.40	0.20/3.00	0.38–3.40
Coagulation parameters			
Thromboplastin time (%)	117±6	108/125	70–120
Partial thromboplastin time (s)	33±1	29/35	30–40
Fibrinogen (g/liter)	3.54±0.23	2.89/3.69	1.50–4.00
Von Willebrand Factor Antigen (%)	103±11	145/115	70–200
PAI-1 (ng/ml), total	36±8	102/40	30–80
tPA (ng/ml)	10.6±1.5	17.2/16.0	2.0–10.0
Urine analysis			
Creatinine (mM)	8.36±0.88	7.96/2.86	4.66–18.00
Microalbumin (mg/24 h)	< 2.2	13.5/< 2.2	2.2–18.0

Values are means±SEM. The two normal subjects are shown with the single values. Reference values are those from the Institute of Clinical Laboratory Diagnostics, University Clinic Carl Gustav Carus, Dresden.

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