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Familial hyperinsulinemia due to a structurally abnormal insulin: Definition of an emerging new clinical syndrome.

Haneda, M. ; Polonsky, KS. ; Bergenstal, RM. ; Jaspan, JB ; Shoelson, SE, ; Blix, PM ; Chan, SJ ; Kwok, SCM ; Wishner, WB ; Zeidler; A. ; Olefsky, JM ; Freidenberg, G,: Tager, HS. ; Steiner, DF : Rubenstein, AH

FAMILIAL HYPERINSULINEMIA DUE TO A STRUCTURALLY ABNORMAL INSULIN

Definition of an Emerging New Clinical Syndrome

MASAKAZU HANEDA, KENNETH S. POLONSKY, RICHARD M. BERGENSTAL, JONATHAN B. JASPAN, STEVEN E. SHOELSON, PETRA M. BLIX, SHU J. CHAN, SIMON C. M. KWOK, WILLIAM B. WISHNER, ADINA ZEIDLER, JERROLD M. OLEFSKY, GARY FREIDENBERG, HOWARD S. TAGER, DONALD F. STEINER, AND ARTHUR H. RUBENSTEIN

Abstract We have identified a patient with mild diabetes, marked fasting hyperinsulinemia (89 to 130 μU of insulin per milliliter), and a reduced fasting C-peptide: insulin molar ratio of 1.11 to 1.50 (normal, >4). The patient responded normally to exogenous insulin. However, her endogenous immunoreactive insulin showed reduced biologic activity during a glucose-clamp study with hyperglycemia and a reduced ability to bind to the insulin receptor and stimulate glucose transport in vitro. Family studies showed that five additional relatives in three generations had variable degrees of glucose intolerance, marked hyperinsulinemia, and a reduced peripheral C-peptide:insulin molar ratio. Restriction-endonuclease cleavage of DNA isolated from circulating leukocytes in the patient and in family members with hyperinsulinemia revealed loss of the *Mbo*II recognition site in one allele of the insulin gene — consistent with a point mutation at position 24 or 25 in the insulin B chain. Other studies using high-pressure liquid chromatography and detailed gene analysis have identified the defect as a serine for phenylalanine substitution at position 24 of the insulin B chain. The secretion of a structurally abnormal insulin should be considered in patients with hyperinsulinemia who respond normally to exogenous insulin and have a reduced C-peptide:insulin molar ratio. Glucose tolerance may range from relatively normal to overtly diabetic. (N Engl J Med 1984; 310:1288-94.)

IT is well recognized that hyperinsulinemia may be present in some patients with diabetes. The majority of such patients have insulin resistance, demonstrated by the observation that exogenously administered insulin has reduced biologic activity.[1,2] It has recently become apparent, however, that hyperinsulinemia may occur in association with structural abnormalities in the insulin molecule. We have described a patient with diabetes who had marked hyperinsulinemia and relatively normal insulin sensitivity.[3,4] This patient was shown to secrete [Leu B^{25}] insulin, a variant form of insulin with reduced biologic activity, in addition to normal human insulin.[5-7] Two other patients with hyperinsulinemia in association with mutant forms of insulin have subsequently been identified.[5] The present report describes in detail the clinical characteristics of one of these patients and her family, delineating the emergence of a new clinical syndrome. We have demonstrated that a serine for phenylalanine substitution at position 24 of the insulin B chain is present in three successive generations of this family.[5,8]

METHODS

Clinical Information

The patient was a 28-year-old, nonobese, white woman who had been found to have asymptomatic glycosuria on routine urine testing at the age of 16. Mild hyperglycemia without ketonemia was present, and a diagnosis of diabetes mellitus was made. Since that time, she has received dietary advice and has been treated intermittently with a variety of oral hypoglycemic agents. Neutral Protamine Hagedorn insulin was administered for a few days when the patient was 26 but was discontinued because of local allergic reactions. In the three years before presentation the patient had two first-trimester miscarriages. Diabetes was diagnosed in the patient's father during a routine examination at the age of 40 years.

Physical examination of the patient was entirely normal. Her weight (48.7 kg) was 100 per cent of ideal for her height (165 cm, Metropolitan Life tables). Routine laboratory tests were negative except that the patient had fasting hyperglycemia (152 to 173 mg of glucose per 100 ml [8.4 to 9.5 mmol per liter]) and marked hyperinsulinemia (89 to 130 μU of insulin per milliliter [0.6 to 0.9 pmol per milliliter]). In order to elucidate the cause of the hyperinsulinemia, the patient was admitted to the Clinical Research Center at the University of Chicago. All studies were approved by the institutional review board, and written informed consent was obtained.

Clinical Studies

After an overnight fast venous blood was drawn for determination of plasma glucose, serum insulin, and plasma C-peptide levels before and at 30, 60, 120, and 180 minutes after the administration of 75 g of glucose by mouth. Insulin and C-peptide levels were also measured before and 6, 10, and 20 minutes after the administration of 1.0 mg of glucagon intravenously.

An insulin-induced–hypoglycemia test was carried out as previously described.[9]

In order to evaluate the patient's response to exogenous insulin, an insulin-clamp study with euglycemia was performed according to the method of Rizza et al.[10] Insulin was administered overnight by intravenous infusion to achieve a normal plasma glucose level. The following morning, steady-state insulin infusion was performed during three periods, each lasting two hours, with progressive increases in the infusion rate from 0.36 to 1 and then to 7.5 mU per kilogram of body weight per minute, respectively.

A glucose-clamp study with hyperglycemia was performed to evaluate the biologic activity of the patient's endogenously secreted insulin, according to the method of DeFronzo et al.[11] Glucose was administered intravenously, beginning with a priming dose and followed by a variable infusion adjusted at two- to five-minute intervals to maintain the plasma glucose level at 300 mg per 100 ml (16.5 mmol per liter). Four healthy volunteers were studied in the same way, with plasma glucose clamped at 163±12 mg per 100 ml (9.0±0.7 mmol per liter; mean ±S.E.M.).

Laboratory Studies

Assay Techniques

Plasma glucose was measured by the glucose oxidase method, using a glucose analyzer (Model 23, Yellow Springs Instruments, Yellow Springs, Ohio). Serum insulin,[12] proinsulin,[12] growth hormone,[13]

From the Departments of Medicine and Biochemistry, University of Chicago, Chicago; the Department of Medicine, University of Colorado, Denver; and the Department of Medicine, University of Southern California, Los Angeles. Address reprint requests to Dr. Rubenstein at the Department of Medicine, University of Chicago, 5841 South Maryland Ave., Chicago, IL 60637.

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plasma C-peptide,[14] and glucagon[15] concentrations were measured by radioimmunoassay, and cortisol levels were determined by a competitive-binding assay.[16] Insulin antibodies[17] and insulin-receptor antibodies[18] were measured as previously described.

Insulin receptors were measured on peripheral monocytes, according to the method of Bar et al.[19] Monocyte numbers were determined by esterase staining, and the results expressed per 10^6 monocytes.

Isolation of the Patient's Insulin and Evaluation of Its Biologic Activity

Immunoreactive insulin was isolated from the patient's serum by immunoaffinity chromatography, using cyanogen bromide–activated Sepharose 4B to which guinea pig antiporcine insulin antibodies had been coupled.[4,20] Immunoreactive insulin (110 pmol) was isolated from 257 ml of the patient's serum, with an overall recovery of 75 per cent.

In vitro binding of this purified immunoreactive insulin to insulin receptors was evaluated with the use of IM-9 lymphocytes and rat adipocytes.[21-24]

The biologic activity of immunoreactive insulin purified from the patient's serum was evaluated by measuring its ability to stimulate the transport of 2-deoxy-D-[1-^{14}C]glucose and oxidation of [1-^{14}C]glucose in isolated rat adipocytes, as previously described.[25]

Insulin-Gene Characterization

In order to determine whether a coding abnormality for the carboxyl terminal region of the B chain of the insulin molecule could be detected, DNA was isolated from peripheral leukocytes obtained from the patient and selected family members and was analyzed as previously described.[6,26,27]

Results

Screening for Insulin Resistance

No cause of insulin resistance could be demonstrated. Fasting levels of contrainsulin hormones were normal (growth hormone, 2.0 ng per milliliter; cortisol, 7.1 μg per 100 ml; and glucagon, 58 pg per milliliter). Occasional determinations of plasma immunoreactive glucagon showed slightly elevated levels, but the increase was due to an elevation of void-volume immunoreactive glucagon-like material with a consistently normal 3500-dalton glucagon component. Antiinsulin antibodies, insulin-receptor antibodies, and islet-cell surface antibodies were not detected. Binding of [^{125}I]insulin to receptors on the patient's monocytes was normal.

Oral Glucose and Intravenous Glucagon Tolerance Tests

The results of the oral glucose tolerance test are shown in Figure 1. Gel-filtration chromatography of serum immunoreactive insulin on Biogel P-30 revealed that 88 per cent of the material eluted from the column in the position of the human insulin standard, and 12 per cent coeluted with a human proinsulin marker (normal, 4 to 24 per cent). In response to intravenous glucagon, serum immunoreactive insulin increased from 99 μU per milliliter (0.7 pmol per milliliter) to a peak level of 255 μU per milliliter (1.76 pmol per milliliter) six minutes after the glucagon injection.

Insulin-Induced–Hypoglycemia Test

The patient responded normally to exogenous infusion of insulin. The basal plasma glucose level on the

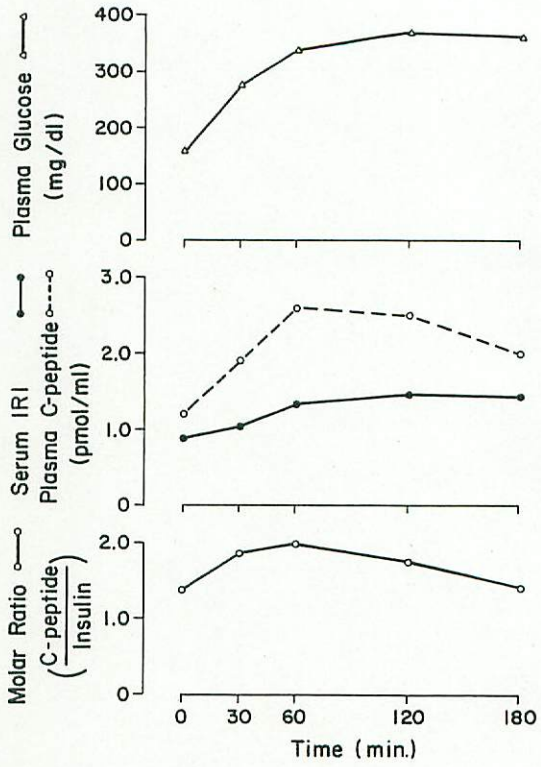

Figure 1. Changes in the Levels of Glucose (Upper Panel), Immunoreactive Insulin (IRI), and C Peptide (Middle Panel) and in the C-Peptide:Insulin Molar Ratio (Lower Panel) after Administration of 75 g of Glucose Orally to the Propositus.

To convert glucose values to millimoles per liter, multiply by 0.055.

day of this test (154 mg per 100 ml [8.5 mmol per liter]) fell at a rate of 2.32 mg per 100 ml (0.13 mmol per liter) per minute, reaching a nadir of 46 mg per 100 ml (2.5 mmol per liter) 30 minutes after the insulin infusion was started. Base-line rates of glucose appearance (2.29 mg per kilogram per minute) and disappearance (2.40 mg per kilogram per minute) were within the range we have previously found in normal subjects (2.08±0.19 and 2.14±0.22 mg per kilogram per minute, respectively).[9] Furthermore, as a result of the insulin infusion, the rate of glucose production was suppressed to 0.84 mg per kilogram per minute, which is similar to values that we have previously reported in normal controls (1.07±0.21 mg per kilogram per minute) under these conditions.[9]

Insulin-Clamp Study with Euglycemia

Steady-state plasma glucose levels of 88.2±2.8 mg per 100 ml (4.9±0.2 mmol per liter) were achieved after the overnight insulin infusion (coefficient of variation, 3.2 per cent), indicating the adequacy of the clamping technique. Suppression of endogenous insulin secretion was evaluated by measuring plasma C-peptide levels during each insulin-infusion period. Thus, there was 66 per cent suppression of endogenous C peptide during infusion of 0.36 mU per kilogram per minute, 86.5 per cent suppression during infusion of 1.0 mU per kilogram per minute, and 90.3 per cent suppression during infusion of 7.5 mU per

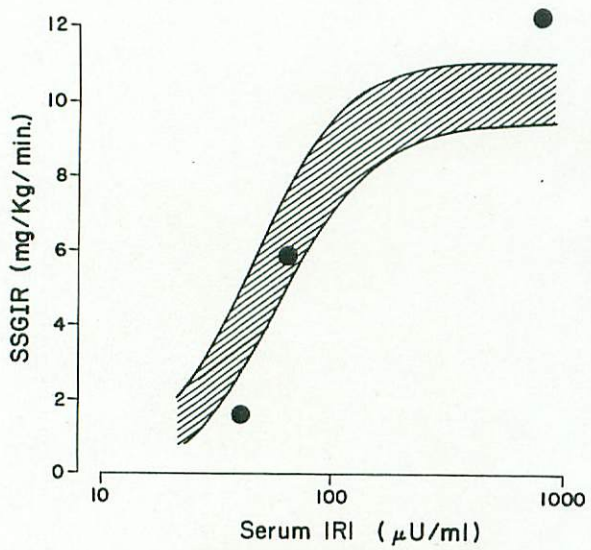

Figure 2. Steady-State Glucose-Infusion Rate (SSGIR) at Three Immunoreactive-Insulin (IRI) Levels during an Insulin-Clamp Study with Euglycemia Performed in the Propositus.

The hatched area represents the range of normal values (mean ±2 S.E.M.) reported by Rizza et al.[10] To convert insulin values to picomoles per milliliter, divide by 145.

kilogram per minute. Steady-state insulin levels measured during each of these three periods were 42, 65, and 766 μU per milliliter, respectively. The corresponding steady-state glucose-infusion rate during each period was 1.54, 5.77, and 12.07 mg per kilogram per minute, respectively. This dose–response curve was compared with data obtained in studies of normal subjects by Rizza et al.,[10] as shown in Figure 2. At the lowest insulin concentration, the steady-state glucose-infusion rate in the patient was below the normal range because of residual circulating endogenous insulin. The steady-state glucose-infusion rates required to maintain the clamp at the intermediate and high insulin-infusion rates clearly indicated a normal response to exogenous insulin. The metabolic clearance rate of infused insulin was calculated by assuming that endogenous insulin and C peptide were suppressed to the same degree. The value of residual endogenous immunoreactive insulin thus derived was subtracted from the total serum immunoreactive insulin. The resulting metabolic clearance rate of infused insulin was 20.4, 18.2, and 9.9 ml per kilogram per minute at the three insulin doses, respectively.

Glucose-Clamp Study with Hyperglycemia

There was no clearly identifiable first phase of insulin secretion in response to the intravenous glucose infusion. Steady-state plasma glucose levels of 300±1.9 mg per 100 ml (16.5±0.1 mmol per liter; coefficient of variation, 2.9 per cent) were achieved between 50 and 150 minutes, and values between these time points were used for the calculations. The M value was 4.06 mg per kilogram per minute. In a similar study performed in normal controls (n = 4) the M value was 6.19±1.12 mg per kilogram per minute at a plasma glucose level of 163±12 mg per 100 ml (9.0±0.7 mmol per liter). The mean plasma immunoreactive insulin and C-peptide values in the patient during the clamp study were 139.8 μU per milliliter (0.96 pmol per milliliter) and 1.29 pmol per milliliter, respectively, as compared with mean values of 38.8±6.0 μU per milliliter (0.27±0.04 pmol per milliliter) and 1.93±0.25 pmol per milliliter in the four controls. These results indicate a substantially reduced in vivo biologic activity of endogenously secreted insulin in the patient. In addition, the finding of lower C-peptide concentrations despite considerably higher plasma glucose levels in the patient, as compared with control values, suggests the presence of a defect in insulin secretion.

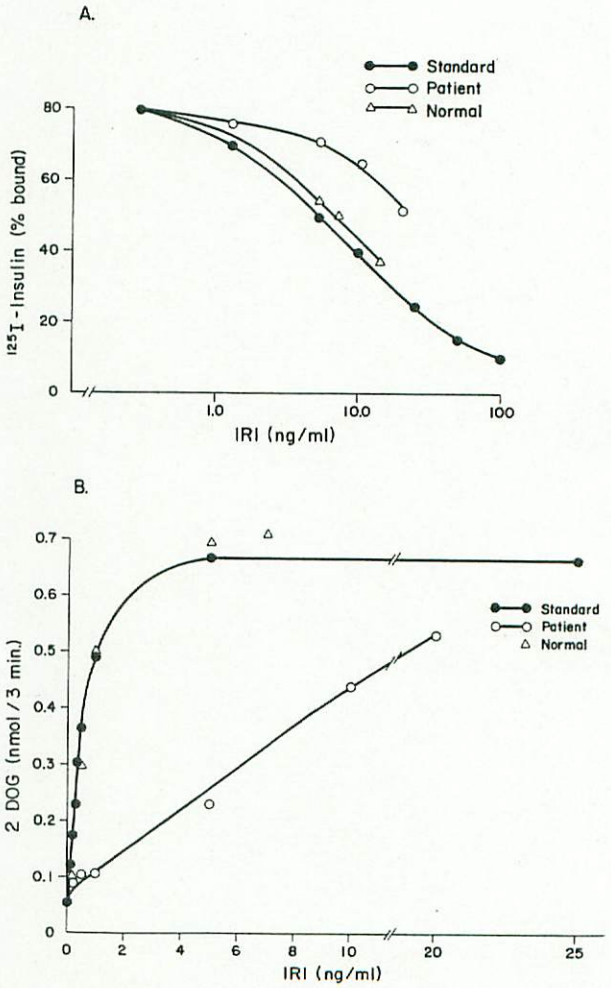

Figure 3. Assessment of Receptor Binding and Biologic Activity of Immunoreactive Insulin (IRI) Isolated and Purified from the Serum of the Propositus.

Panel A shows binding of [^{125}I]insulin to IM-9 lymphocytes in the presence of varying concentrations of porcine insulin standard and IRI isolated from the serum of the propositus and a normal subject. Results are expressed as the percentage of [^{125}I]insulin specifically bound per 10^7 lymphocytes. Panel B shows the ability of varying concentrations of the porcine insulin standard and of IRI isolated from the serum of the propositus (open circles) and a normal subject to stimulate 2-deoxyglucose (2 DOG) transport in rat adipocytes. To convert insulin values to picomoles per milliliter, divide by 5.8.

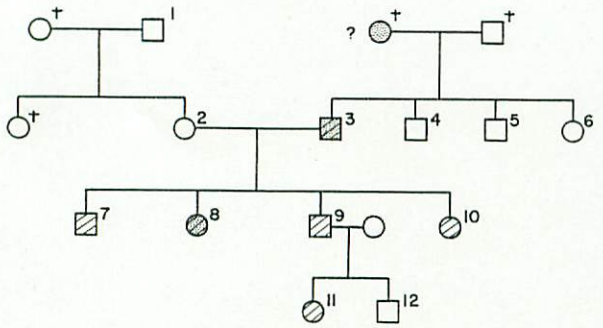

Figure 4. Pedigree of the Patient with Hyperinsulinemia and Selected Family Members.

The propositus is Subject 8. Family members with fasting hyperinsulinemia (diagonal lines), diabetes (dots), or both (dots) are shown. The dagger denotes deceased.

Biologic Activity of the Patient's Insulin

Immunoreactive insulin isolated from the patient's serum diluted in parallel with a human insulin standard in the insulin radioimmunoassay and comigrated with a porcine insulin standard on polyacrylamide-gel electrophoresis at pH 4.5 and 8.7. The ability of immunoreactive insulin isolated from the patient's serum, of porcine insulin standard, and of insulin isolated from a normal control to bind to IM-9 lymphocytes is shown in Figure 3A. The ability of the patient's insulin to stimulate 2-deoxyglucose transport is shown in Figure 3B. The results clearly indicate that immunoreactive insulin isolated from the patient's serum was less than 30 per cent as active as normal insulin in both these assay systems.

Family Pedigree

In order to determine whether the defect was hereditary, fasting glucose, insulin, and C-peptide levels were measured in other family members (Fig. 4 and Table 1). Six family members in three successive generations had hyperinsulinemia (fasting serum immunoreactive insulin levels, 78 to 139 μU per milliliter [0.54 to 0.96 pmol per milliliter]), with relatively normal fasting C-peptide concentrations (0.30 to 1.08 pmol per milliliter). The patient's father was overtly diabetic and was being treated with small doses of insulin. Two brothers and a sister had normal or slightly elevated fasting plasma glucose values (103 to 108 mg per 100 ml [5.7 to 5.9 mmol per liter]), and one of these brothers and the sister had impaired glucose tolerance. All three had marked fasting hyperinsulinemia (94 to 139 μU of insulin per milliliter [0.65 to 0.96 pmol per milliliter]). A three-year-old niece had a markedly elevated serum insulin level (78 μU per milliliter [0.54 pmol per milliliter]), with a normal fasting blood glucose level. The fasting C-peptide:insulin molar ratio was between 1.1 and 1.5 in the affected family members. This value is substantially lower than the fasting range in control subjects (normal, >4).

Insulin-Gene Studies

Results of the *Mbo*II cleavage analysis of DNA from a control subject, the patient, and members of the patient's family are shown in Figure 5. The normal subject's DNA (Lane N) yielded three fragments of 1600, 580, and 340 base pairs. The cleavage pattern of the mother's DNA (Lane A) was normal. In contrast, *Mbo*II digestion of DNA from the father (Lane B), the propositus (Lane C), a brother (Lane D), and a sister (Lane E) resulted in a band of 920 base pairs in addition to the three normal bands. The DNA fragment of 920 pairs corresponds to the sum of the two fragments of 580 and 340 base pairs and indicates a loss of the recognition site of *Mbo*II in one allele of the insulin gene. All these family members (Fig. 5B through 5E) had fasting hyperinsulinemia and low C-peptide:insulin molar ratios.

Discussion

We have identified a non-insulin-dependent diabetic patient who presented with glucose intolerance and marked hyperinsulinemia. No evidence for elevated circulating insulin antagonists was found, and the pa-

Table 1. Fasting Serum Values of Glucose, Insulin, and C Peptide and Oral Glucose Tolerance in a Patient with Mutant Insulin and in Selected Family Members.*

Subject No.	Age/Sex	Relationship	Plasma Glucose (mg/dl)	Serum Insulin (μU/ml)	Plasma C Peptide (pmol/ml)	C-Peptide: Insulin Molar Ratio	Oral Glucose Tolerance
1	89/M	Grandfather (maternal)	105	11	0.90	12.30	
2	59/F	Mother	98	8	0.54	10.10	Impaired
3	59/M	Father	207	Total, 270; Free, 92	0.30	0.48	Diabetic (receiving insulin)
4	61/M	Uncle (paternal)		Not tested			
5	66/M	Uncle		3	0.43	21.5	
6	68/F	Aunt (paternal)		8	0.57	10.7	
7	32/M	Brother	106	98	0.93	1.42	
8	28/F	Propositus	162	111	0.83	1.11	Diabetic
9	26/M	Brother	108	139	1.08	1.18	Impaired
10	23/F	Sister	103	94	0.72	1.15	Impaired
11	3/F	Niece	85	78	0.78	1.50	
12	7 mo/M	Nephew	65	4	0.30	12.0	

*The number assigned to each subject is the same as in Figure 4. To convert glucose values to millimoles per liter, multiply by 0.055. To convert insulin values to picomoles per milliliter, divide by 145.

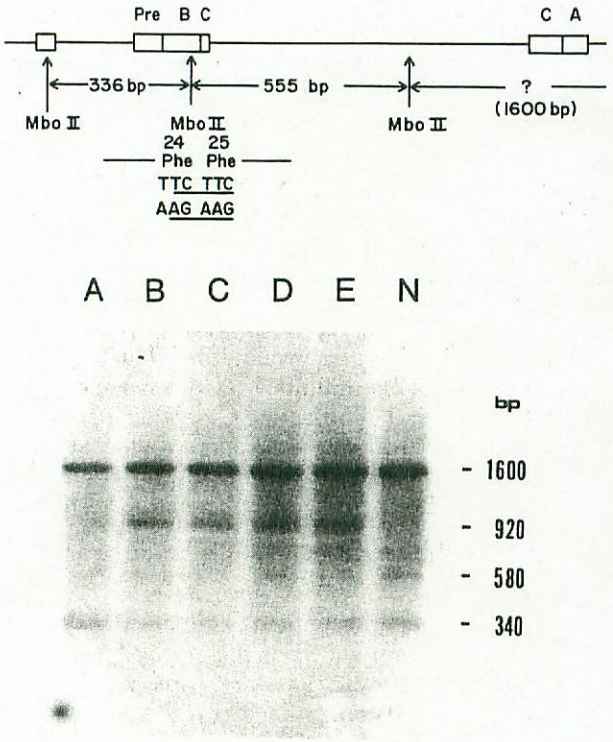

Figure 5. Restriction-Endonuclease *Mbo*II Cleavage Analysis of DNA from Selected Family Members.

As shown in the upper panel and in Lane N (from a control subject) in the lower panel, *Mbo*II endonuclease digestion of DNA normally yields three major bands representing fragments of 1600, 580, and 340 base pairs, respectively. The cleavage pattern of the mother's DNA (Lane A) is normal. The propositus (Lane C), her father (Lane B), a brother (Lane D, No. 9 in Fig. 4), and a sister (Lane E, No. 10 in Fig. 4) all have an extra band of 920 base pairs in addition to the three normal bands.

tient's insulin receptors on circulating monocytes were normal. She responded normally to exogenously administered insulin, as evidenced by normal stimulation of glucose use during an insulin-clamp study with euglycemia, as well as a rapid fall in the level of plasma glucose during an insulin-induced–hypoglycemia test. In addition, the metabolic clearance of infused insulin was similar to values previously reported in normal subjects.[28] On the other hand, the patient's own endogenous insulin was shown to have markedly reduced biologic activity during a glucose-clamp study with hyperglycemia.

Insulin isolated from the patient's serum by immunoaffinity chromatography was shown to migrate with normal human insulin on polyacrylamide-gel electrophoresis. Its ability to bind to IM-9 lymphocytes and stimulate 2-deoxyglucose transport in isolated adipocytes was less than 30 per cent of the binding ability of an insulin standard and of insulin isolated from the serum of control subjects. This combination of tests provided convincing in vivo and in vitro evidence that the insulin present in our patient had reduced biologic activity, presumably because of an abnormality in its structure.

Further evidence in favor of this hypothesis was obtained from restriction-endonuclease mapping of the patient's DNA, which indicated that one insulin-gene allele had undergone a mutation at the *Mbo*II-sensitive site coding for residues Phe^{B24} and Phe^{B25}(TTC-TTC). Of great interest is the finding that five additional members of the patient's family had marked hyperinsulinemia and a variable degree of glucose intolerance. *Mbo*II endonuclease mapping for the insulin genes in three of these subjects (we were unable to obtain DNA from the other two family members) also demonstrated an abnormality in the region coding for Phe^{B24} and Phe^{B25} — a result similar to that seen in the propositus. Using high-pressure liquid chromatography, we have recently been able to show that immunoreactive insulin isolated from the patient's serum consisted of both normal human insulin and an abnormal form, clearly distinct from human insulin.[5] Further studies involving solid-phase peptide synthesis of insulin analogues and high-pressure liquid chromatography,[5,8,29] as well as cloning of the patient's insulin genes,[7,30] have identified the defect in the propositus and her family as a serine for phenylalanine substitution at position 24 in the insulin B chain. The defect is present in three successive generations of the family — a finding consistent with an autosomal dominant mode of transmission.

In addition to the family described in the present report, two other patients have previously been shown to secrete structurally abnormal forms of insulin.[5] A pattern of characteristic clinical features appears to be emerging from studies in these patients (Table 2). Since the identification of additional patients with this syndrome depends on the ability of physicians to consider the diagnosis, these features merit a more detailed discussion. The extent of glucose intolerance in patients with mutant insulins may vary from mild impairment, occurring only after glucose loading, to overt diabetes. When marked hyperglycemia is present, however, the diabetes is still of the nonketotic type. The mild nature of the glucose intolerance in several affected subjects is presumably due to the fact that both normal and abnormal insulins are secreted as one would predict if both alleles of the insulin gene were codominantly expressed and only one was abnormal. Consistent with this formulation is the finding of approximately equal quantities of normal and abnormal insulin in the pancreas of one of the patients.[3,31] Furthermore, normal human insulin has been detected by high-pressure liquid chromatography in the peripheral circulation of the propositus in the present study, as well as in two additional patients with mutant insulins.[5]

It is interesting to consider the possible reasons why patients with mutant insulin have diabetes at all, since it should theoretically be possible for the beta cell to secrete sufficient quantities of the combination of normal and abnormal insulin to maintain normal glucose homeostasis. The propositus in the present study, who was overtly diabetic, had a marked defect in insulin

Table 2. Characteristics of Patients with Mutant Insulins.

(1) Hyperinsulinemia
(2) No evidence of insulin resistance
Normal contrainsulin hormones
Insulin antibodies absent
Insulin-receptor antibodies absent
Insulin receptors normal
(3) Normal response to exogenous insulin
Normal insulin tolerance test
Normal insulin-clamp study with euglycemia
(4) Reduced biologic activity of endogenous insulin
Abnormal glucose-clamp study with hyperglycemia
Reduced in vitro biologic activity of serum immunoreactive insulin
(5) Reduced C-peptide:insulin molar ratio
(6) Hyperglycemia/diabetes present or absent.

secretion in addition to a mutant insulin. Thus, although the patient had marked peripheral hyperinsulinemia, over 90 per cent of this circulating immunoreactive insulin was structurally abnormal by high-pressure liquid chromatography[5] and was functionally abnormal as demonstrated by the in vivo and in vitro tests described above. When combined with a defect in insulin secretion, a marked deficiency in circulating biologically normal insulin resulted, leading to overt diabetes. Since uncontrolled diabetes is invariably associated with reduced first-phase insulin secretion and beta-cell degranulation, it is not possible to determine with certainty whether the hyperglycemia observed in our patient was the cause or the result of the defect in insulin secretion. Clearly, further prospective studies of the other family members and of other such patients are required to determine the pathogenesis of diabetes in this syndrome. It is likely, however, that patients with inherited defects in the insulin molecule may be asymptomatic for many years, and diabetes may develop only when increased beta-cell secretion is no longer able to compensate for the reduced biologic activity of its secretory product. It is also conceivable that circulating abnormal insulin may act as an antagonist to normal insulin at the receptor level, but this situation has not yet been observed during in vivo testing. In this regard, although our original observations had suggested that [Leu B^{25}]insulin had antagonistic properties in vitro,[31] this finding has not been confirmed in subsequent experiments. Thus, it is still an open question whether a mutant insulin will be found that exerts antagonistic activity.

Hyperinsulinemia has been a feature of all patients with mutant insulins described to date. It is usually marked, and fasting values of approximately 100 μU of insulin per milliliter have been documented. It is this finding of marked hyperinsulinemia that is likely to bring patients with mutant-insulin syndromes to the attention of physicians. In the clinical evaluation of such patients other causes for hyperinsulinemia must be considered, including obesity and a variety of less common syndromes of insulin resistance. Specific tests aimed at excluding the major causes of insulin resistance should be performed, including measurement of contrainsulin hormones, insulin receptors on red cells, monocytes, or fat cells, and antibodies against insulin and insulin receptors. Furthermore, a normal response to exogenous insulin should be demonstrated during either an insulin tolerance test or an insulin-clamp study with euglycemia. Of course, a normal response would not be expected if a mutant insulin antagonized the biologic activity of normal insulin in vivo.

Another finding that appears to be characteristic of patients who secrete mutant forms of insulin is the presence of a low C-peptide:insulin molar ratio. In the peripheral blood this ratio is normally higher than 4 in the fasting state[32] in both healthy subjects and obese patients with hyperinsulinemia. All affected family members in the present study had ratios between 1 and 2. The reason for the reduced C-peptide:insulin molar ratio has not been definitively elucidated, although it is almost certainly due to diminished clearance of the structurally abnormal insulin. Since Terris and Steiner[33] have demonstrated that receptor binding is an important prerequisite for insulin degradation, a structurally abnormal insulin with reduced receptor-binding affinity would be expected to be cleared from the circulation more slowly than normal insulin.

If a patient is suspected of having an abnormal insulin on the basis of the above clinical picture and if the initial laboratory studies as outlined above are consistent with this diagnosis, further definitive tests to characterize the nature of the insulin should be performed. At present, the two most useful tests are high-pressure liquid chromatography of insulin isolated from serum and restriction-endonuclease mapping of DNA isolated from circulating leukocytes. It should be emphasized that two of the first three patients reported to have mutant insulins had amino acid substitutions in position 24 or 25 of the insulin B chain. Since this site is of central importance for receptor binding and biologic activity, it is not surprising that such defects have resulted in impaired glucose tolerance, diminished insulin clearance, and hyperinsulinemia. It is probable, however, that mutations at sites of the insulin molecule that are less critical for biologic activity will not be associated with diabetes, and if insulin clearance is normal, the patient may not even present with hyperinsulinemia. In some patients diagnosed as having maturity-onset diabetes of the young, a category of nonketotic diabetes transmitted in a dominant mode of inheritance, secretion of mutant insulins may be the basis of the disease.

The prevalence of genetic mutations in the insulin molecule among the general population is unknown. Our detection of such abnormalities in asymptomatic persons raises the possibility that similar defects may occur more frequently than has previously been realized. In fact, the situation may be analogous to that of

inherited defects in the hemoglobin molecule, which are relatively common. A detailed understanding of these conditions will provide valuable information about the structural determinants of normal insulin action, as well as about the cause and pathogenesis of diabetes.

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