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A New Form of Insulin Resistance with Growth Retardation, Fatty Liver, and Hypogonadotropic Hypogonadism

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Summary

A 17-year-old boy presented with growth retardation, marked hepatomegaly, and sexual infantilism. Elevated fasting serum insulin levels and a blunted hypoglycemic response to exogenous insulin (up to 0.35 unit/kg) demonstrated severe insulin resistance. Neither anti-insulin nor anti-insulin receptor antibodies were present. The molecular size of his circulating insulin and its binding to IM-9 lymphocytes was normal. Despite high circulating insulin values, both erythrocytes and cultured skin fibroblasts showed normal insulin binding capacity and affinity. Tissue responsiveness was examined by measuring the insulin-induced increase in 2-deoxyglucose uptake into fibroblasts. Although the basal glucose transport rate was slightly lower than that of controls, the insulin-induced increase was normal. However, the normal increase in thymidine incorporation in response to insulin was blunted, as were the thymidine incorporation responses to epidermal growth factor and fibroblast growth factor. These studies demonstrate the possible existence of a new form of post-insulin receptor defect as a cause of insulin resistance, but underscore the difficulty that exists in defining the exact nature of the defect in these disorders.

Abbreviations

IRI, immunoreactive insulin
G, gonads
PH, pubic hair
EGF, epidermal growth factor
hFGF, human fibroblast growth factor
Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
2-DG, 2-deoxyglucose

Insulin resistance, a clinical syndrome characterized by a blunted response to endogenous insulin, with or without a similar poor response to administered insulin, causes some degree of hyperglycemia as well as other more variable clinical features (2, 26). Its etiology may include production of an abnormal insulin molecule (6, 33), the presence of circulating antagonists to insulin such as anti-insulin or anti-insulin receptor antibodies and contra-insulin factors (5, 25, 36), abnormally rapid degradation of insulin (27), quantitative (32) and qualitative (18, 35) abnormalities of the insulin receptors (18), and poorly defined defects in post-receptor mechanisms (19, 22).

This report describes a 17-year-old boy with an unusual form of insulin resistance in whom extensive studies point to a post-receptor defect but have failed to define the exact nature of the abnormality.

CASE REPORT

A 17-year-old Caucasian boy was referred for evaluation of hepatomegaly and growth retardation. He was the product of an uneventful 37-week pregnancy with a birth weight of 2.2 kg. He showed no dysmorphic features other than bilateral cryptorchidism. There was no family history of consanguinity or diabetes. The midparental stature was 161.3 cm. His height and weight growth was below but parallel to the third percentile throughout childhood. Orchidopexy was carried out at 6 years of age; at age 8, his serum follicle-stimulating hormone, luteinizing hormone, and testosterone values were in the normal prepubertal range. At age 15, hepatomegaly was first noticed. Biopsy of the liver demonstrated severe fatty metamorphosis; there was appreciable glycogen content and moderate periportal fibrosis with some bile

duct reduplication and mild mixed inflammatory cell infiltration. The exact diagnosis was uncertain, but chronic active hepatitis was considered. Prednisone (7.5–60 mg/day) was administered for 1 year with no effect on liver size. Other studies had excluded α-1 antitrypsin deficiency, Wilson's disease, glycogen storage disease, galactosemia, fructose intolerance, cystic fibrosis, chronic viral infection, or an immunodeficiency disorder.

On physical examination at age 17 4/12 years, he was short (height, 146.5 cm; weight, 41.9 kg), and had a distended abdomen and thin extremities. His blood pressure was 110/60 mm Hg and pulse was 84 beats/min. A firm liver edge was palpable 9 cm below the right costal margin. There was no ascites and the spleen was not palpable. His penis and testes were of infantile size but there was a small amount of pubic hair (Tanner stage G_1 PH_2). There was no evidence of acanthosis nigricans or lipodystrophy. His bone age was 14.5 years. Routine blood counts, serum electrolytes, creatinine, urea nitrogen, and serum lipoproteins were within normal limits. Blood glucose was 129 mg/dl. Liver function tests showed abnormal enzyme values (serum aspartate transaminase, 435 U/liter; normal range, 0–50 U/liter; alanine transaminase, 685 U/liter; normal range, 10–40 U/liter; lactate dehydrogenase, 455 U/liter; normal range, 100–225 U/liter), but his serum bilirubin was normal. Anti-DNA antibody was in the normal range and antinuclear factor was negative. The urinalysis was normal except for a small amount of glucose; there was no ketonuria.

Insulin resistance became apparent during testing for possible growth hormone deficiency. In response to IV insulin (doses as large as 0.2 U/kg), no effect on blood glucose was observed; an additional injection of 0.15 U/kg of insulin 30 min after the first elicited only a slight decline of blood glucose from 100 to 73 mg/dl. His fasting blood glucose ranged from 93–120 mg/dl with corresponding fasting serum IRI levels from 84–214 μU/ml (normal less than 15 μU/ml). Following oral administration of glucose (70 g), serum glucose values were 429 mg/dl at 30 min, 394 mg/dl at 60 min, 318 mg/dl at 120 min, and 220 mg/dl at 180 min. Serum IRI levels remained above 240 μU/ml throughout the oral glucose tolerance test. The results of other endocrine studies are listed in Table 1. Samples of serum, red blood cells, and skin were taken for further evaluation of insulin responses. During 4 years of follow-up, there was no significant change in his fasting serum IRI levels, glucose tolerance, or liver size. Depotestosterone, 200 mg IM monthly, has resulted in adequate development of secondary sex characteristics (Tanner stage G_4 PH_4) and maturation of his skeletal age to 17 years.

MATERIALS AND METHODS

Materials included: [*methyl*-^{3}H]thymidine (49 Ci/mmol) and 2-deoxy-D-[1-^{14}C]glucose (57 mCi/mmol, Amersham); 2-deoxy-D-glucose (Sigma); fetal bovine serum, Dulbecco's modified Eagle's medium and penicillin-streptomycin solution (Gibco); and highly purified crystalline porcine insulin (24.4 U/mg, Connaught Laboratories). Mouse EGF was prepared from male mouse submaxillary glands according to the method of Savage and Cohen (30), and hFGF was prepared from human brain (21).

Studies of the Circulating Insulin Molecule and of Insulin Antagonists. The ratio of proinsulin to insulin in the patient's serum was evaluated by radioimmunoassay following separation of the two components by Sephadex G-50 superfine gel filtration. The ability of the insulin in the peak insulin fraction from this column to bind to a known effective insulin receptor was assessed using IM-9 lymphocytes (8). Anti-insulin receptor antibodies in serum were measured by the method of Flier *et al.* (5).

Studies of Insulin Receptor. ^{125}I-insulin binding to erythrocytes. Insulin was iodinated by the chloramine T method (12) to a specific activity of 100–140 μCi/μg. ^{125}I-Insulin binding to erythrocytes was determined in two separate blood samples from the patient, and six control samples from healthy male adults and two samples from other patients with insulin resistance by the method of Gambhir *et al.* (7). The erythrocytes (1.6×10^9) were incubated with 2×10^4 cpm ^{125}I-insulin (0.1 ng) and varying amounts of cold insulin in a total volume of 0.5 ml.

^{125}I-insulin binding to cultured fibroblasts. Fibroblast cultures were established using skin from the patient's abdomen and from skin biopsies of the same region taken during surgery from four normal male control subjects of a similar age. The explants were grown in Dulbecco's modified Eagle's medium supplemented with 50 U/ml of penicillin, 50 μg/ml of streptomycin, and 10% fetal bovine serum in an atmosphere of 5% CO_2, 95% air at 37°C. Medium was changed twice a week and the cultured fibroblasts were used at confluency after 6–12 passages. It was noted that the doubling time of the patient's fibroblasts was 150% that of the control fibroblasts. Eighteen hours prior to assay, the medium was replaced with new medium containing 0.1% fetal bovine serum.

^{125}I-Insulin binding to cultured fibroblasts *in situ* was determined by the method of Schilling *et al.* (32) with minor modifications. Confluent fibroblasts ($7–9 \times 10^5$ cells/35-mm dish) were incubated with 4×10^5 cpm of ^{125}I-insulin and varying amounts of cold hormone in 1 ml buffer (0.1 M Hepes, 0.12 M NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 10 mM glucose, 10 mg bovine serum albumin, pH 8.0) at 15°C for 5 h. After incubation, cells were washed three times with cold buffer and dissolved with 0.1 N NaOH containing 0.1% Triton X-100. Radioactivity of the lysates was counted in a scintillation counter.

Studies of Post-receptor Insulin Action. 2-Deoxyglucose incorporation into fibroblasts. 2-DG incorporation into fibroblasts was studied by the method of Schilling *et al.* (32), but using confluent

Table 1. *Serum hormone concentrations and responses to stimulation tests**

	Basal value	Peak value	Stimulation by	Interpretation
Thyroxine (μg/dl)	9.9			Normal
Triiodothyronine (ng/dl)	8.3			Normal
TSH (μU/ml)	1	10	TRH (200 μg)	Blunted
Prolactin (ng/ml)	3.4	10	TRH	Normal
Growth hormone (ng/ml)	1.0	2.6	Arginine (0.5 g/kg)	Blunted
	0.8	5.5	l-dopa Propranolol	Blunted
FSH (μg LER907/dl)	19	31	LH-RH (100 μg)	Normal basal; normal response
LH (μg LER907/dl)	18	21	LH-RH	Elevated basal; blunted response
Testosterone (ng/ml)	24	77	hCG (2000 IU IM) daily ×3	Prepubertal
Cortisol (μg/dl)	19			Normal
Glucagon (pg/ml)	36			Normal
Basic somatomedin† (U/ml)	0.81			Normal

* TSH, thyroid-stimulating hormone; LH-RH, luteinizing hormone-releasing hormone; TRH, thyrotropin-releasing hormone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin. Normal basal range for male age 16–18: FSH, 11.5–27.0 μg/dl; and LH, 2.4–5.8 μg/dl.

† Serum basic somatomedin level was determined by Dr. R. M. Bala (University of Saskatchewan) (1).

cell cultures instead of trypsinized cell suspension. The fibroblasts were preincubated in Krebs-Ringer phosphate buffer in the presence or absence of insulin (100 ng/ml) at 37°C for 20 min; 50 μl of 2 mM [^{14}C]2-DG (2 μCi/μmol) was then added and further incubation was carried out for 5 or 10 min. The cells were washed rapidly in cold buffer three times to terminate the reaction, and solubilized in 0.1 N NaOH containing 0.1% Triton X-100 and Biofluor, and the lysate was counted in a scintillation counter.

Thymidine incorporation into fibroblasts. Fibroblasts were grown to confluency and then replated in 35-mm dishes at a concentration of 0.4–0.8 × 10^5 cells/dish. After 24 h, the maintenance growth medium containing 10% fetal bovine serum was replaced with medium containing 0.05% fetal bovine serum; the cells were incubated in this medium for 48 h prior to assay to reduce DNA synthesis and cell replication to a minimum. Thymidine incorporation into fibroblasts was determined by the method of Harley *et al.* (14). In a preliminary study, maximum thymidine incorporation was found after the patient's fibroblasts and control fibroblasts had been incubated with medium containing 10% fetal bovine serum for 24 and 20 h, respectively. Therefore, the cells were incubated for time periods ranging from 5–29 h with 0.1 serum-free medium containing 0.1% bovine serum albumin, and varying concentrations of dialyzed fetal bovine serum, EGF, or human FGF-like substance. A small volume of serum-free medium containing [^{3}H]thymidine (0.2 μCi/dish) was then added and incubation continued for 30 min. The assay was terminated by washing the cells once in cold saline and twice in 5% trichloroacetic acid. The cells were dissolved in 0.1 N NaOH and counted in a scintillation counter.

RESULTS

Studies of the Circulating Insulin Molecule and of Potential Insulin Antagonists. Proinsulin represented approximately 25% of the total circulating insulin. Radioreceptor assay of the peak insulin fraction showed activity comparable to that determined by radioimmunoassay. No circulating anti-insulin or anti-insulin receptor antibodies were found.

Studies of the Insulin Receptor on Erythrocytes and Cultured Fibroblasts. Scatchard plot analysis (31), shown in Figure 1, demonstrated that, despite the high circulating insulin levels, the insulin-binding capacity and affinity of the erythrocyte receptors were within the normal adult range. In contrast, two other patients with insulin resistance (cases 1 and 2) showed reduced insulin-binding capacity.

The results of ^{125}I-insulin binding to the cultured fibroblasts are shown in Figure 2. These studies indicate that the ability of the patient's fibroblasts to bind insulin was also normal.

Studies on Post-receptor Insulin Action. Insulin effect of 2-deoxyglucose uptake in cultured fibroblasts. The effect of insulin upon the ability of fibroblasts from the patient and one of the normal control subjects to take up [^{14}C]2-DG is shown in Figure 3. Basal 2-DG uptake (without insulin) in the patient's fibroblasts was somewhat lower than in control fibroblasts, but insulin (100 ng/ml) enhanced uptake to a degree comparable to the increase in control fibroblasts. The patient's fibroblasts also demonstrated a variable increase in 2-deoxyglucose uptake in response to insulin at concentrations lower than 100 ng/ml. Maximal acceleration of 2-DG uptake in both the patient's and control fibroblasts was found at concentrations between 100 ng/ml and 1 μg/ml of insulin. This experiment was repeated four times with separate passages of the patient's cells and control fibroblasts from different subjects. The mean (±SD) basal uptake of the patient's cells was 1.50 ± 0.16 nmol 2-DG/10^6 cells/10 min, while that of the controls was 2.46 ± 0.54. After exposure to insulin (100 ng/ml), the uptake was 2.16 ± 0.14 in the patient's cells and 3.64 ± 0.44 in the controls.

Thymidine incorporation into fibroblasts. The effects of dialyzed fetal bovine serum, insulin, EGF, and hFGF-like substance on thymidine uptake in the patient's fibroblasts and control fibroblasts is shown in Figure 4. A high concentration of insulin (10 μg/ml) increased thymidine incorporation into the control fibroblasts 9-fold, but elicited only a 2.4-fold rise in the patient's fibroblasts. A lesser response to [^{3}H]thymidine incor-

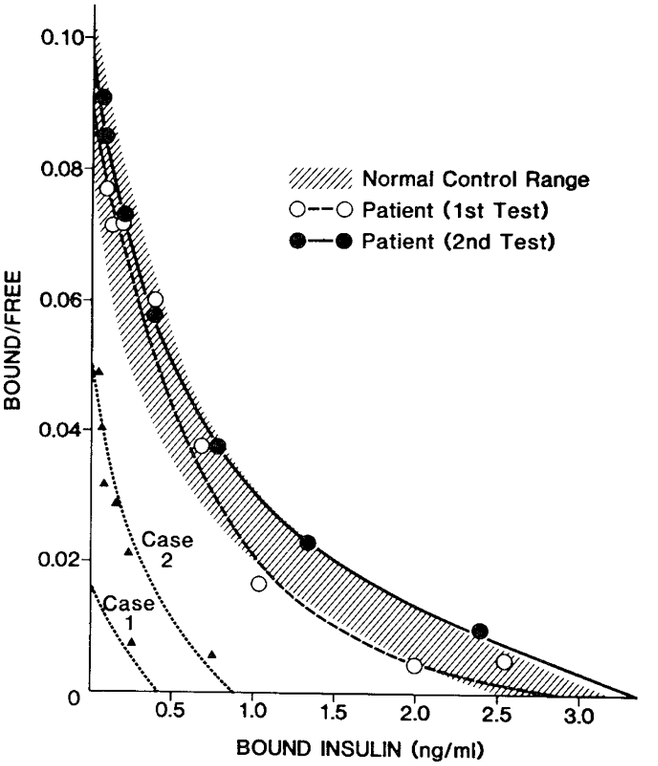

Fig. 1. Scatchard plot analysis of insulin binding to erythrocytes. ^{125}I-Insulin binding to circulating erythrocytes (specific binding for 3.2 × 10^4 cells) was determined using the method of Gambhir *et al.* (7). The *shaded area* represents the normal range derived from six healthy adult males. Case 1 is a patient with acanthosis nigricans and case 2 is a 14-year-old prepubertal obese female (150% of ideal weight), both of whom are insulin-resistant. ●——● and O---O represent results obtained on patient's erythrocytes in two separate experiments.

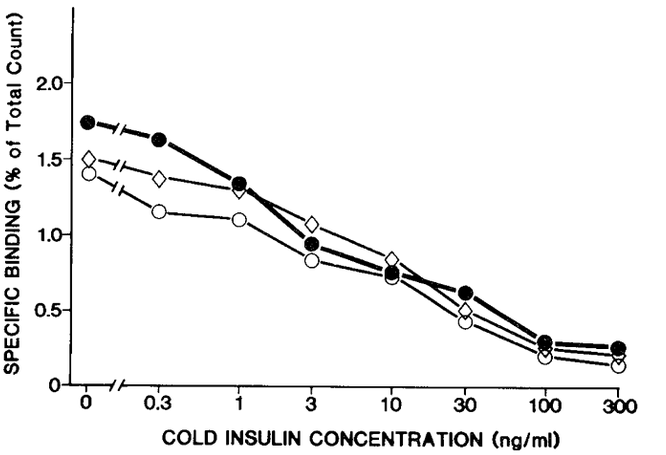

Fig. 2. ^{125}I-insulin binding to cultured fibroblasts. The results are expressed as specific binding for 1 × 10^6 cells. Each *point* represents mean of triplicate assays. Specific binding was calculated as the difference between total radioactivity bound and nonspecific binding in the presence of 5 μg/ml insulin (results not shown). ●——●, index case; ◇——◇ and O——O, two control subjects.

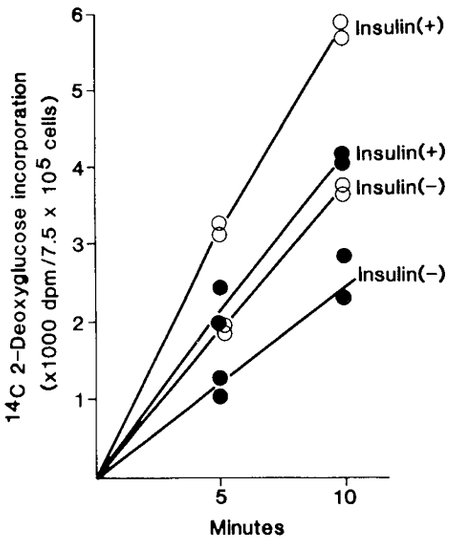

Fig. 3. Insulin effect of 2-deoxyglucose uptake by fibroblasts. The uptake of [^{14}C]2-deoxyglucose with or without insulin (100 ng/ml) by fibroblasts from the patient (●——●) and a control subject (○——○) is shown.

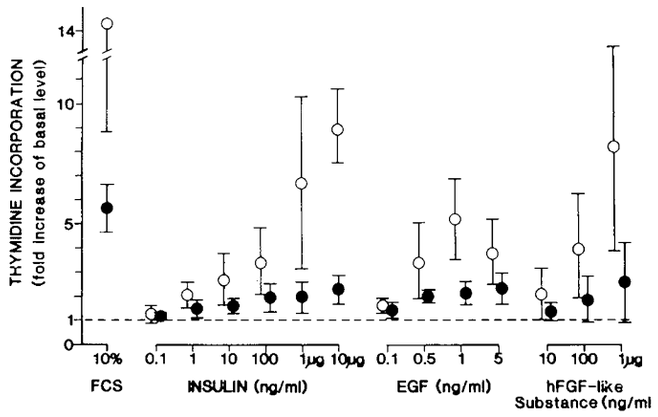

Fig. 4. The effects of fetal bovine serum, insulin, EGF, and hFGF-like substance on [^{3}H]thymidine incorporation into fibroblasts. Each *point* represents the mean ± SD of three assays: each assay included fibroblasts from four control subjects (○) and from the patient at three different generations (●). The response of the patient's fibroblasts to insulin at 10μg/ml is significantly lower than in control fibroblasts ($P < 0.01$).

poration in the patient's fibroblasts was also observed in response to dialyzed fetal bovine serum, EGF, and hFGF.

DISCUSSION

The clinical features of this patient differ from those seen in other reported forms of insulin resistance. No clinical evidence of hypersecretion of any hormonal insulin antagonist such as growth hormone, thyroxine, cortisol, or glucagon was found. There were no features of congenital lipodystrophy (16) or acanthosis nigricans (5, 18) and no evidence of any other disease known to be associated with insulin resistance such as obesity, leukemia, collagen vascular disease, ataxia telangiectasia, or uremia (2). Although his liver showed some cirrhotic changes, the degree of insulin resistance was not in keeping with that observed in cirrhosis (11, 17). Some patients with liver disease may show impaired degradation of circulating insulin (17) or a decreased number of insulin receptors (11) but these were not present in our patient. The fatty infiltration of his liver was most likely a result of abnormal glucose metabolism, as is seen in congenital lipodystrophy (28) and poorly controlled insulin-dependent diabetes mellitus (13), rather than the cause of his insulin resistance.

Since we were unable to define any clinical syndrome known to be associated with insulin resistance, we attempted to describe it in molecular terms. There was no biochemical evidence of any circulating hormonal or immunological insulin or insulin receptor antagonists. We evaluated the structural integrity of the patient's insulin molecule by fractionating his serum by gel filtration. Since the fraction of proinsulin present in his serum was normal (6), we concluded that there was no intracellular abnormality of processing of proinsulin to insulin. The amount of immunoassayable insulin in the insulin peak was comparable to the insulin capable of competing for binding sites on human IM-9 lymphocytes. Thus, on the basis of normal molecular size and binding in two unique binding assays, we have inferred normal structure and bioactivity of his circulating insulin.

We observed normal insulin-binding capacity and affinity of his erythrocytes and cultured fibroblasts. It is of interest that receptor capacity in this prepubertal boy was the same as in adult controls despite circulating insulin levels 10-fold higher than normal since both childhood and hyperinsulinism are normally associated with decreased receptor number. This finding suggests a failure of down-regulation of the insulin receptor and is compatible with a post-receptor defect (12).

The observation that our patient's fibroblasts demonstrated normal insulin-stimulated glucose uptake provides further evidence that the defect is not an intrinsic receptor abnormality but rather a defect distal to the coupling between the insulin-receptor complex and the glucose transport system. We did however find some impairment of glucose transport since both the absolute levels of basal and insulin-stimulated glucose uptake were lower than in the controls, suggesting a defect in the glucose transport mechanism itself. Our findings are similar to those described recently by Kaplowitz and d'Ercole (19) in an infant with leprechaunism, but are in direct contrast to those described in two other infants with leprechaunism (23, 24) in whom the insulin resistance was also thought originally to be due to a post-receptor defect on the basis of normal insulin binding to fibroblasts, normal basal glucose transport, but absent insulin-stimulated glucose uptake. Further study in one of these patients (35) has revealed a qualitative defect of the insulin receptor characterized by temperature instability. In view of the normal acceleration of glucose uptake in response to insulin in our patient, a qualitative intrinsic receptor defect seems unlikely.

It also appears that our patient has a more general defect of cellular metabolism, since the normal increases in DNA synthesis (as measured by thymidine uptake into fibroblasts) elicited by insulin and also by exposure to fetal bovine serum, EGF, or hFGF were impaired in his fibroblasts. Kaplowitz and d'Ercole (19) have reported decreased thymidine uptake in response to insulin, EGF, FGF, and somatomedin-C as well as prolonged cell doubling time in the fibroblasts of an infant with leprechaunism who, as described in the preceding paragraph, had the same abnormality of glucose transport as our patient. In addition, the fibroblasts from their patient exhibited impaired uptake of the nonmetabolizable amino acid analogue aminoisobutyric acid in response to the growth factors insulin, EGF, somatomedin-C and multiple stimulating activity. With the exception of multiple stimulating activity, each of these growth factors has its own unique receptor. They postulated, therefore, a post-receptor defect involving a metabolic pathway affected in common by these growth factors and which may be expressed in multiple tissues to explain the prenatal growth retardation. It is possible that our patient has a similar post-receptor defect that is expressed as decreased growth factor-stimulated thymidine uptake and a prolonged doubling time. However, similarly altered growth properties have been observed in cells of diabetic patients (10) and may therefore represent a defect due to *in vivo* environmental factors rather than an intrinsic genetic defect. Impaired DNA

synthesis occurs also in Werner's syndrome (34), in which insulin resistance is accompanied by growth retardation, hypogonadism, cataracts, and premature senescence (4). In this syndrome, there is evidence for a post-receptor metabolic defect (22) as well as an intracellular senescent factor responsible for abnormal cell growth (34). Thus, it remains unclear whether the altered growth properties of our patient's fibroblasts represent an intrinsic post-receptor defect or simply an acquired abnormality related to the diabetic state. These patients represent a formidable diagnostic challenge and, as in our case, pathogenesis is not always easily defined. This case report also underscores the need for further understanding of the heterogeneous group of clinical disorders recognized with insulin resistance due to receptor or post-receptor defects including leprechaunism, Werner's syndrome, lipoatrophic diabetes, and acanthosis nigricans (type A). There appears to be a continuum in phenotypic severity ranging from the serious life-threatening form of leprechaunism in the newborn period as we have previously reported (29, 32) to milder defects manifested by prenatal and postnatal growth retardation, hypogonadism, and secondary hepatomegaly as in the present case.

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