Insulin Resistance and Acanthosis Nigricans

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SUMMARY

Studies are reported of a twenty-five-year-old woman with insulin resistance. Daily insulin dosage fluctuated, ranging from zero to a maximum of 177,500 units on one day. Associated conditions were acanthosis nigricans, multiple infections, cerebellar infarction, renal vein thrombosis, and chronic nephritis. Plasma obtained during resistance to large insulin dosage yielded high insulin content by immunoassay, high insulin-like activity by rat diaphragm, fat pad and mouse hypoglycemia assays, and no excessive binding capacity by the cellulose-trapping method. Plasma obtained after forty-two days without insulin treatment caused glycogen synthesis in the rat diaphragm. The cause of the resistance was unknown.

Insulin resistance has attracted much attention both at the bedside and in the laboratory. Recently Field and co-workers described a remarkable young woman with impermanent diabetes, absence of hypoglycemia despite very high insulin dosage, and acanthosis nigricans.[1] The present report describes the course, complications, and certain characteristics of insulin resistance in a patient similar in many respects to the one of Field et al.

CASE REPORT

A twenty-five-year-old Negro woman, patient M.P. (CGH 348340), was admitted to the Cincinnati General Hospital on March 19, 1961, with diabetic ketoacidosis and pregnancy of estimated twenty-five weeks' duration. Her past history was unremarkable except for mild toxemia during the third of three previous pregnancies. The maternal grandmother had diabetes. Physical examination was normal except for the findings of diabetic acidosis and pregnancy. The urine was strongly positive for acetone and sugar, contained 3+ protein, and had an unremarkable sediment. The blood glucose was 235 mg. per 100 ml., the serum CO_2 content 9 mEq./L., and urea nitrogen 9 mg. per 100 ml. Conventional therapy for diabetic acidosis was carried out, and ketosis remitted in twenty-four hours after the administration of 1,900 units of crystalline zinc insulin.

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Subsequently the amount of insulin necessary to maintain gross diabetic control remained high with 3,000 units being given in the remainder of the first hospital week, and 1,105 units in the second week. In the third week acetonuria appeared, and insulin was increased progressively to 6,400 units on the twenty-sixth hospital day with little effect on acetonuria or glycosuria. Because of the possible development of acidosis with adverse affect on the fetus, corticosteroid therapy was instituted in hopes of alleviating the resistance. Over twenty-four hours, 62.5 mg. of prednisone were given along with 6,000 units of insulin. The next morning the patient was comatose in ketoacidosis, and prednisone was discontinued. In the first twenty-four hours of treatment of acidosis 80,000 units of insulin were given with no change in plasma ketone concentration (estimated by the acetest tablet) or physical status. In the next twelve hours, 54,000 units were given with some decrease in ketonemia, but again no change in physical status. Six hours later, coma had changed to stupor, and cesarean section was made on speculation that pregnancy was causing or at least contributing to the insulin resistance. A stillborn, 1,150-gm., male infant was delivered. In the six hours following operation 39,500 units were administered and ketonuria disappeared. The total insulin dosage in forty-eight hours was 194,100 units administered intravenously, 5,000 to 15,000 units hourly in U-5,000 concentration.* Convalescence was uneventful.

The subsequent course of the patient was characterized by long periods of severe insulin resistance and uncontrolled diabetes interrupted by shorter periods without need for insulin. In addition there were multiple infections, acanthosis nigricans, nephrosis and renal failure, and an ill-defined neurologic syndrome. She died suddenly without apparent cause 622 days after her initial admission in acidosis. The pertinent aspects of her course were as follows:

Course of diabetes. In the management of her diabetes, insulin was given in amounts aimed to prevent excessive glycosuria. Sometimes she required from 5,000 to 12,000 units daily for as long as 160 days, and at other times no insulin for up to forty-four days. The greatest dose was 177,500 units administered during one day on which appendectomy was performed. It was never known if an optimum amount had been given. Many times greater dosage might have improved glucose utilization, but there was always fear of producing severe hypoglycemia since entrance into a less resistant phase could not be predicted. On the other hand, it is also possible that much of the insulin was ineffectual. At one period the patient excreted on the average 300 gm.

*U-5,000 crystalline zinc insulin supplied through the courtesy of Dr. W. R. Kirtley of the Eli Lilly Company.

of glucose in 8 L. daily when receiving 1,500 units of insulin. When the dose was raised to 12,000 units, the urine volume and glucose content decreased four days later to 5 L. and 190 gm. of glucose, respectively. This was interpreted as possibly indicating insulin effect. Chlorpropamide and phenformin were given alone and in combination with insulin, but no effects on glucose utilization were apparent. Dietary regulation was never satisfactory because of the many intercurrent illnesses and the unwillingness of the patient to follow a prescribed diet when she could eat.

Daily urine volumes ranged usually from 3 to 8 L. with glucose content of 100 to 360 gm. Only occasionally was the volume under 2 L. with less than 100 gm. of glucose. Of 104 blood glucose determinations (AutoAnalyzer), twenty-nine were less than 200 mg., the lowest being 54 mg. per 100 ml. Of particular interest were the absence of symptomatic hypoglycemia at any time, and the absence of ketonuria after the first ninety days of observation despite grossly inadequate carbohydrate utilization. At a time when glucose excretion exceeded 300 gm. daily and the blood sugar concentration was 306 mg. per 100 ml., the blood ketone concentration was 1.2 mg. per 100 ml. and the plasma free fatty acid concentration 0.58 mEq./L.

At autopsy the lesions associated with diabetes were sought. A normal pancreas was found except for absent beta cell granules by aldehyde fuchsin stain. Arteriosclerosis was present in the circle of Willis and kidney, and there was no evidence of diabetic glomerulosclerosis.

Infections. The patient acquired numerous infections during the time of observation. Those requiring surgical treatment included subcutaneous (four), wound (three), intra-abdominal (two), gluteal (two), tubo-ovarian (one), and dental abscess (one). Organisms recovered individually or in combination included Staph aureus (six), E. coli (three), and klebsiella, clostridia, pseudomonas, and proteus (one). In addition there were appendicitis, pharyngitis and esophagitis due to monilia, and urinary infection due to E. coli, aerobactor, and proteus. Laparotomy was performed three times in addition to the cesarean section.

*Acanthosis nigricans.** This condition probably was present prior to the onset of diabetic symptoms but was not diagnosed until six months after initial hospitalization. One skin biopsy was interpreted as compatible with, and a second diagnostic of, acanthosis. The lesion was diffuse, severe, and included most of the external body surface. The tongue and lips were enlarged, and a tongue biopsy revealed chronic inflammation. Minimal, if any, regression occurred with time. In spite of extensive search, no evidence of malignancy was found during life or at autopsy.

Renal disease. Persistent proteinuria with excretion up to 18.0 gm. of protein daily appeared six months after initial hospitalization. This was followed in order by hypoalbuminemia, edema, hyperchloremic acidosis, and moderate renal failure before death. At autopsy there was found thrombosis of the inferior vena cava and renal veins, chronic pyelonephritis, and chronic membranous and lobular glomerulonephritis.

Neurologic disease. The patient experienced two convulsions followed by stupor early in her course. Blood glucose concentrations were above 200 mg. per 100 ml. After the second episode she developed lower motor paralysis of the right arm lasting five weeks. Spinal fluid and electroencephalographic examination were normal. At autopsy infarction and calcification of the right cerebellar lobe were found, cause unknown. It is not certain if the neurologic symptoms were related to this lesion.

Miscellaneous tests. Miscellaneous examinations other than those associated with above conditions included unremarkable electrocardiograms and X Rays of chest, gastro-intestinal tract, skull, and bones. Concentrations of plasma calcium, phosphorus, urate, amylase, cholesterol, phospholipid, triglyceride, free fatty acids, and blood ketones were normal. Also normal were plasma 17-OH steroids and urinary outputs of 17-keto- and 17-OH steroids. Electrophoretic analysis of serum proteins revealed a consistently high globulin ranging from 4.2 to 6.1 gm. per cent with a gamma fraction of 2.6 to 2.8 gm.

INSULIN ACTIVITY

Attempts to characterize the resistance to administered insulin were made both at the bedside and in the laboratory.

Type of diabetes. The family history of diabetes, the development of diabetes during pregnancy, and the absence of pancreatic disease or other endocrinopathy indicate that the patient had genetic or essential diabetes. The failure to find any increase in plasma lipids including ketones in the face of markedly decreased glucose utilization in the last year and a half of her life was unusual for her age. The patient was malnourished but not sufficiently to consider lipoatrophic diabetes as a possibility.

Pattern of insulin requirements. It was not possible to determine any patterns or cycles of insulin need. Fluctuations in requirements occurred unpredictably, and sometimes glucose excretion was less than 100 gm. when only small amounts or no insulin were given. The absence of known hypoglycemia at any time suggests, however, that resistance factors were always present and somehow prevented lowering of the blood glucose below normal.

Conditions associated with resistance. Early in the course, pregnancy and infections were thought to be causal factors because of rising insulin requirements in their presence. Later events suggest, however, that, if anything, the infections probably aggravated pre-existing resistance. There was no evidence of abnormality of the thyroid, adrenal, and pituitary glands. Skin sensitizing antibodies to insulin were not present by direct or passive transfer skin tests.

It is not known whether the acanthosis nigricans was related in some manner to the insulin resistance, or whether its presence was coincidental. An association of acanthosis with diabetes in young persons has been reported.[2] The similarity of the present patient to the one reported by Field et al.[1] does suggest, however, that acanthosis nigricans and insulin resistance may co-exist by other than chance alone.

Plasma insulin. Determinations of plasma insulin concentration by immunoassay[3] and estimates of plasma insulin-like activity (ILA) by the rat diaphragm[4] and mouse hypogly-

*The authors are indebted to Dr. Helen Curth of the Department of Dermatology, Columbia University, College of Physicians and Surgeons, who visited Cincinnati to examine the patient and give consultation.

cemia assays[5] were made. Samples were kept frozen until assay. Glucose was determined by the glucose oxidase method (Worthington Glucostat) and diaphragm glycogen by the method of Seifter et al.[6] In addition, determinations in other laboratories were made of ILA by the rat epididymal fat pad assay by Dr. Jack Leonards of Western Reserve Medical School,[7] of ILA by the rat diaphragm assay before and after incubation with adipose tissue extract by Dr. Walter Shaw of the Eli Lilly Company[8] and of insulin binding capacity (IBC) by the cellulose trapping method by Dr. Thaddeus Prout of Johns Hopkins.[9]

The findings are given in table 1. Plasma obtained on the twenty-eighth hospital day, during acidosis in the first episode of severe resistance, contained in excess of 100 mu./ml. of ILA by the rat diaphragm assay and 6,700 mu./ml. by the mouse hypoglycemia assay. The latter value is not unreasonable in view of the massive insulin dosage at the time. On the same plasma specimen the IBC was 8 mu./ml., the level found in a person who has been treated with insulin. The next specimen was obtained on the 130th day when she had received no insulin for forty-two days but was taking 100 mg. of phenformin daily. By the rat diaphragm and mouse hypoglycemia assays ILA of 10 and 12 mu./ml., respectively, were found. That the activity was due to insulin is suggested by two observations. First, in the rat diaphragm there was an increase in glycogen of 2.6 μg./mg. of fresh diaphragm when the medium contained the patient's plasma, and there was no change in glycogen when the medium contained phenformin at concentration of 50 μg. per cent, though the expected increase in glucose uptake occurred. Second, incubation of the patient's plasma with cysteine reduced the ILA by the mouse hypoglycemia assay from 12 to 1 mu./ml., a level found in the normal state. Whether the activity represented endogenous, remaining exogenous insulin, or both, cannot be determined. If anything, the higher than normal levels of ILA suggest that large amounts of exogenous insulin still were present.

A plasma sample was obtained on the 161st day when the patient had been receiving no insulin or other hypoglycemic drugs for twelve days. By the mouse hypoglycemia assay the ILA was 6 mu./ml., and the IBC was 10 mu./ml. Another sample was obtained on the 213th day when the patient had been receiving an insulin dose of from 6,000 to 12,000 units daily. At this time the ILA by the rat diaphragm assay was in excess of 100 mu./ml., and by the mouse hypoglycemia method 132 mu./ml.

The final plasma sample of the 379th day was tested by all cooperating investigators. The patient was receiving chlorpropamide 500 mg., phenformin 200 mg., and insulin of 2,000 to 4,000 units daily. The values obtained by the authors using the immunoassay and rat diaphragm and mouse hypoglycemia assays, and that obtained by Dr. Jack Leonards using the fat pad assay were all elevated and remarkably comparable considering the diversity of methods. Dr. Walter Shaw did find a much lower value, however, with the rat diaphragm method. Of particular interest is his demonstration of large relative proportion of "bound" insulin which was freed after incubation with adipose tissue extract. In contrast, Dr. Thaddeus Prout found the insulin-binding capacity by his method still in the range of a diabetic patient treated with insulin.

Urinary insulin-like activity. In view of the heavy proteinuria, investigations were carried out to determine if urinary loss of insulin might account for at least part of the insulin resistance. Zero-point-two-eight (0.28) units of insulin labeled with 75 microcuries of I-131 (Abbott) were injected intravenously on a day when the patient received 4,000 units of insulin and the urinary protein was 6.45 gm. At twenty-four hours, 57 per cent of the label had appeared in the urine in trichloracetic acid-precipitable form. This is in contrast to the usual 2 to 5 per cent appearing in the precipitable form in twenty-four hours in a normal person.[10] On another occasion, when she was receiving 7,200 units daily, the excretion of urinary insulin-like activity was estimated to be in twenty-four hours: 150 units by the rat diaphragm method, 170 units by the mouse hypoglycemia method, and 120 units by immunoassay. Control urine containing protein from nondiabetics and diabetics receiving therapeutic doses of insulin

TABLE 1

Estimates of plasma insulin-like activity

Day	Insulin dose (U)	Rat diaphragm (Tucker)	Rat fat pad (Leonards)	Mouse hypoglycemia (Goetz)	Rat diaphragm ±ATE* (Shaw) Bound	Rat diaphragm ±ATE* (Shaw) Free	Rat diaphragm ±ATE* (Shaw) Total	Immunoassay (Klink)	Insulin binding capacity (Prout)
				Milliunits/ml.					
28	103,600	>100		6,700					8
130	0(42 d)†	10		12					
				<1‡					
161	0(12 d)			6					10
213	12,000	>100		132					
379	4,000	10-100	200	77	13.4	0.72	14.2	80	10
	Normal fasting state	<1	<1	<1	0.6	0.05	0.65	<0.1	0

*Assay before and after incubation with adipose tissue extract.
†Patient had received no insulin for forty-two days but was taking 100 mg. phenformin daily.
‡Assay of plasma after incubation with cysteine.

yielded less than 2 units per twenty-four hours by either method. These findings suggest that considerable amounts of insulin or its degradation products were being excreted. Even if insulin were present in the urine the rate of excretion would not have been sufficient to account for the inadequacy of the plasma insulin effect.

DISCUSSION

The early proposal by Martin et al. that chronic insulin resistance exists when the insulin requirement exceeds 200 units per day, continues to be accepted.[11] Such a definition is wholly arbitrary but quite satisfactory as a descriptive term since the fraction of diabetics requiring this dose or greater is exceedingly small. Extraordinarily large amounts of insulin were given to the present patient, 194,100 units during forty-eight hours treatment of acidosis and 177,500 during twenty-four hours at a later date without acidosis. It is not known if a peak of insulin effect was ever achieved, and her maximum tolerable dose may have been much greater. Previously reported high dosages given in acidosis have been 56,080 units in twenty-six hours with recovery,[12] and 97,740 units in fifty-six hours without recovery.[13] In chronic resistance up to 38,000 units per day have been given.[1]

At present an etiological classification of insulin resistance is not available, and patients have been categorized instead by clinical course and associated conditions.[14] In most situations no associated condition is found, and the resistance simply is designated as chronic idiopathic insulin resistance. There have been numerous studies of such patients with demonstration of plasma antibodies and antagonists.[14] There have also been cases reported wherein large amounts of insulin appeared to have little or no effect in the resistant patient, but plasma obtained after administration of the insulin yielded high ILA. In these instances, ILA has been shown in vitro by the fat pad assay[13,15,16] and rat diaphragm assay,[1,17,18] and in vivo in the mouse,[1,19] rat,[15,17] and man.[16] The finding of high ILA by in vitro and in vivo assay in the present patient places her in this group.

In her clinical course the present patient was similar to that of Field et al. in the remarkable degree of insulin resistance, absence of hypoglycemia, and the presence of acanthosis nigricans. There were differences in that ketonuria persisted in Field's case and only occurred early in the present case, and that remission of both diabetes and acanthosis took place in Field's patient. In the laboratory the patients were similar in the findings of high plasma ILA when there was little effect from the administered insulin. It is of particular interest that in both cases plasma obtained after cessation of insulin therapy brought about glycogen synthesis in the rat diaphragm. Field et al. were of the opinion that the activity represented endogenous insulin. In the present case the source of ILA is uncertain, but it is possible that exogenous insulin still was present. The time of sampling was only six weeks after discontinuance of insulin treatment, and the levels of ILA obtained by the rat diaphragm and mouse hypoglycemia assays were above normal.

No conclusions can be drawn concerning the locus of resistance in the present case. Excessive plasma protein binding of insulin to as high as 4,700 mu./ml. has been reported.[20] Even if increased binding took place in the present case, there still was high ILA with in vitro and in vivo assay. If an antagonist was active, such as a degradation product of insulin,[21] it clearly was less effective in the mouse and rat than in the patient. In this regard, the case of Shipp et al.[16] is especially significant because plasma from their insulin-resistant patient caused hypoglycemia in a control patient, thus eliminating a species difference in antagonistic activity.

It is possible that loci of resistance existed beyond the plasma, either at the cell membrane or within the cell. Tissues generally held to be responsive to insulin are adipose tissue, muscle, and probably liver. The absence of ketonuria and normal blood levels of ketones, free fatty acids, and triglycerides later in her course suggest that there was glucose utilization, and thus insulin action, in adipose tissue. Whether interference with insulin action at muscle, liver, or other areas took place is beyond speculation now. The subject of insulin resistance remains complex and awaits studies at the tissue as well as the blood level.

SUMMARIO IN INTERLINGUA

Resistentia a Insulina e Acanthosis Nigricante

Es reportate un serie de studios de un femina de vinti-cinque annos de etate con resistentia a insulina. Le dosage diurnal de insulina variava ab zero a un maximo de 177.500 u. Le conditiones concomitante esseva acanthosis nigricante, multiple infectiones, infarcimento cerebellar, thrombosis de venas renal, e chronic nephritis. Plasma obtenite durante resistentia a alte dosage de insulina monstrava un alte contento de insulina per immunoessayo, alte activitate insulinoide per essayos a diaphragma de ratto, cossino grasse, e hypoglycemia de mus, e nulle excessive capacitate ligante per le methodo a trappamento in cellulosa.

Plasma obtenite quaranta-duo dies post le cessation de tractamento per insulina causava synthese de glycogeno in le diaphragma del ratto. Le causa del resistentia non es cognoscite.

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