Insulin Resistance with High Levels of Circulating Insulin-like Activity Demonstrable in Vitro and in Vivo

J. C. Shipp, M.D., R. O. Russell, M.D., J. Steinke, M.D., M. L. Mitchell, M.D., and W. B. Hadley, M.D., Boston

The purpose of the present study was to examine some of the possible sites of interference with insulin action during insulin resistance. In theory, this interference can result from binding or inactivation of insulin in the vascular or interstitial compartments, from altered transfer of insulin across the capillary membrane to the reactive cell site or from a failure of the cell to respond normally to insulin (figure 1).

This report includes studies over a six-month period in a patient with insulin resistance and deals primarily with circulating factors which may alter the action of insulin. An increased serum binding capacity and precipitin antibodies were shown. High levels of circulating insulin-like activity were demonstrated by in vitro and in vivo methods. These abnormalities were no longer present when insulin sensitivity returned following prednisone therapy.

CASE REPORT

E.P., Joslin Clinic No. 54010, a seventy-three-year-old white female, was admitted to the New England Deaconess Hospital for the third time on Sept. 3, 1959, with diabetic acidosis.

The major clinical features are shown in table 1. Diabetes mellitus was discovered at age fifty-nine and treated with diet alone. A sister and paternal grandfather were diabetic. Thirteen years later, at age seventy-two, she was placed on 75 units of Protamine Zinc Insulin daily following a foot infection. She was first seen at the Joslin Clinic in May of 1959 when she was admitted to the New England Deaconess Hospital in diabetic coma precipitated by a foot infection. The keto-acidosis cleared with 1,200 units of insulin; subsequently 400 to 600 units of insulin daily were required to prevent ketosis. In July she was hospitalized because of bilateral foot ulcers. At this time her serum showed high levels of insulin-like activity and increased insulin binding capacity (see table 1). Tolbutamide (2.0 gm. daily for two days), tolbutamide (2.0 gm.) plus dexamethasone (3.0 mg.) daily for two days and dexamethasone (1.5 mg. for one day) alone had no effect on the insulin requirement. She was discharged on 650 units of U-500 insulin plus 100 units of Crystalline Insulin daily. Over the next four weeks the insulin need rose to 1,250 units twice daily. The detailed sequence of insulin requirements is shown in figure 2.

One day before the third hospital admission on Sept. 3, 1959, a necrotic area was noted on the right thigh at the site of the insulin injection. During the three hours before admission nausea and vomiting began. When first seen she was semi-stuporous with a blood pressure of 150/40 mm. of mercury, a pulse of 96 per minute and respirations of 36 per minute which were Kussmaul in type. There was an acetone odor to her breath. She had diabetic retinopathy, warm dry skin and a 0.5 cm. necrotic area on the right thigh. Heart and lungs were normal. Moderate hepatosplenomegaly was noted. Blood sugar was 726 mg. per 100 ml., serum acetone was positive in a 1:8 dilution; serum sodium, potassium and chloride were 126, 3.5, and 94 mEq./L., respectively. Arterial carbon dioxide content was 5 mM/L., arterial *p*H 7.22 with a calculated pCO_2 of 12 mm. of mercury. Hemoglobin was 13.0 gm. per cent. There were 8,400 leukocytes per cu. mm.; the differential smear showed 4 per cent eosinophils.

Over a period of twenty-four hours the patient received 6,000 units of insulin. Of this amount 2,000 units of U-5,000

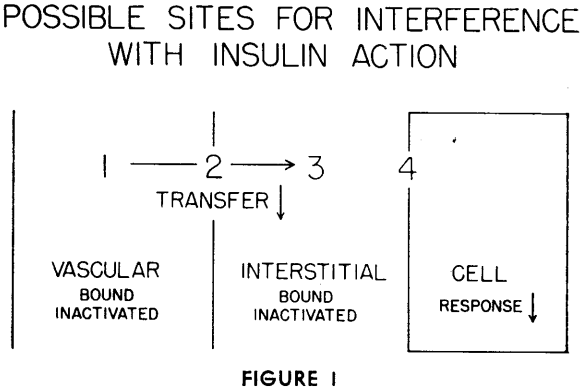

FIGURE 1

Presented at the Twentieth Annual Meeting of the American Diabetes Association in Miami Beach on June 12, 1960.

From the Joslin Clinic and New England Deaconess Hospital, Department of Medicine, Harvard Medical School and Lemuel Shattuck Hospital, Boston, Massachusetts. Dr. Shipp is a Fellow of Medical Foundation of Boston. Present address: University of Florida School of Medicine, Gainesville, Florida. Dr. Steinke is the recipient of a Research Fellowship of the American Diabetes Association.

TABLE 1
Insulin resistance (J.C. No. 54010)

Age	Date	Clinical	Insulin (U./day)	Anti-bodies	Binding	ILA* In vitro (mu/ml.)	In vivo
59	1945	Onset	0				
72	1958	Foot inf.	75				
73	1959						
	May	Coma (1,200 U.)	600				
	July	Foot inf.	600		10†	13	
	Aug.		2,500				
	Sept.	Coma (9,000 U.)	1,200	+	>20†	175	++++
	Oct.	Prednisone	1,200				
	Dec.		50		†	2.1	

* Insulin-like activity. † Level in insulin treated diabetic.

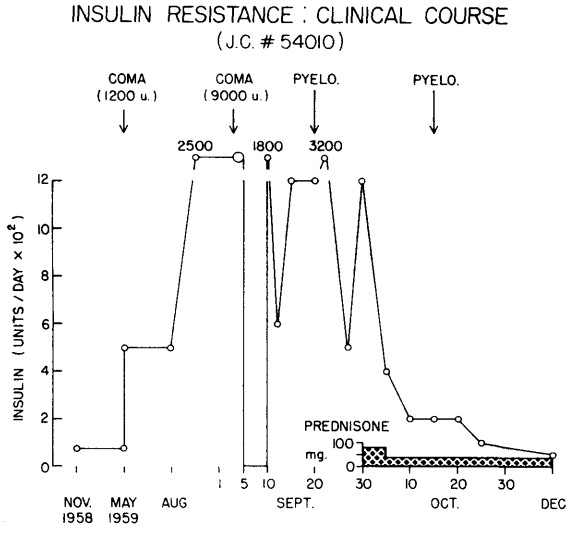

FIGURE 2

insulin‡ were given intravenously over about thirty seconds. Within five minutes she developed a rapid jerky motion of the jaw and became unresponsive. Blood pressure was unobtainable. Profuse sweating and an urticarial rash appeared. She responded to 100 mg. of hydrocortisone plus 40 mg. of metaraminol, given slowly intravenously, with a rise in blood pressure to 90/60. This was thought to be an anaphylactic reaction to intravenous insulin. Blood sugar thirty minutes after this episode was 330 mg. per 100 ml. Urine output, which had averaged 75 ml. per hour, fell to less than 15 ml. During the second day the blood sugar was 400 to 500 mg. per 100 ml. and she was given 3,000 units of U-5,000 insulin subcutaneously, and 100 mg. of hydrocortisone to help maintain blood pressure.

For the next five days urine output remained low at between 300 and 600 ml. daily. During this time, after the initial 9,000 units of insulin, she was given no insulin but required frequent intravenous and oral glucose to prevent symptomatic hypoglycemia. Blood glucose remained between 30 and 80 mg. per 100 ml. On the sixth day, urine output increased from 15 to 100 ml. per hour, blood sugar rose to 470 mg. per 100 ml. and she again required insulin.

‡ Courtesy of Dr. William Kirtley of Eli Lilly and Company, Indianapolis.

Insulin dosage rose steadily seeming to stabilize at around 1,200 units daily. With a urinary tract infection she required 3,200 units daily for three days. As the infection was controlled with chloramphenicol, insulin dose returned to around 1,200 units. Throughout this period insulin was given in amounts adequate to prevent ketosis. Blood sugars varied between 100 to 400 mg. per 100 ml.

Again, insulin requirement began increasing. On Oct. 1, 1959, when she received 1,600 units of insulin, prednisone (80 mg. daily) was started. There was a daily drop in insulin need which seemed to stabilize at 200 units daily on the sixth day; at this time prednisone was reduced to 40 mg. daily. During the second and third weeks of prednisone therapy from 150 to 250 units of insulin maintained the blood sugar around 100 to 200 mg. per 100 ml. In the fourth week between 50 to 100 units of insulin were given. Nine weeks after starting prednisone, while taking 50 units of insulin, a three-hour postprandial glucose was 68 mg. per 100 ml. While on prednisone a second episode of pyelonephritis did not produce an increase in insulin need.

On Sept. 15, 1959, serum, on paper electrophoresis, showed increased gamma globulin and decreased albumin. Total serum proteins were 5.6 gm. per cent with 2.0 gm. per cent albumin. There was 8 per cent bromsulphalein retention and a negative cephalin flocculation. Hematocrit was 32 per cent; leukocyte count was 5,000 to 8,400 with 33 per cent polymorphonuclears, 54 per cent band forms, 7 per cent lymphocytes, 3 per cent monocytes, 1 per cent atypical lymphocytes, 1 per cent metamyelocytes and 1 per cent plasma cells. Red blood indices were normal. Reticulocytes were 2.4 per cent. Platelet count was 68,000 to 75,000. No platelet or leukocyte agglutinins were demonstrated by Dr. James Tullis. A bone marrow aspirate showed moderate hypoplasia but no increased iron. Serum iron was 51 μg. per cent with an iron binding capacity of 243 μg. per cent. Hemochromatosis,[1] considered because of insulin resistant diabetes in a postmenopause female, was considered unlikely because of low serum iron in the absence of blood loss and with no increase of iron in the bone marrow. A liver biopsy to evaluate this possibility further was not advisable because of the thrombocytopenia. Stools were negative for occult blood. Lupus erythematosus cells were not demonstrated. Blood urea nitrogen was 14 to 24 mg. per cent, endogenous creatinine clearance 31 ml./min. and an intravenous urogram was normal.

She was discharged on Oct. 26, 1959, weighing 100 pounds on an 1,800 calorie diet. Her daily medications included 100

units of U-500 insulin, 40 mg. of prednisone, 2.0 gm. chloramphenicol, antacids and oral iron. On Dec. 7, 1959, while on 50 units of insulin the blood sugar was 68 mg. per 100 ml. three hours postprandially.

CLINICAL STUDIES

These clinical studies were performed over a six-month period during and following the insulin resistant phase. The results are shown in table 1.

1. *Antibodies to insulin.* Serum from the patient on Sept. 5, 1959, two days after the second episode of diabetic coma, showed faint precipitin bands with commercial beef-pork U-500 insulin (undiluted and diluted 1:10) with the Ouchterlony Technic[2] (courtesy of Dr. John Harter). A similar study on serum of Sept. 18, 1959, while she was taking 1,200 units of U-500 insulin daily, did not show evidence of precipitins.

2. *Insulin binding.* The insulin binding capacity of the serum was determined by means of the resin technic[3] during the months of July, September and December of 1959. The capacity of the serum to bind insulin was considerably elevated during the phase of insulin resistance as evidenced by more than a twentyfold increase in the insulin binding value over that seen during insulin sensitivity. However, during the phase of insulin responsiveness, the insulin binding capacity of the serum was no different from that of the insulin sensitive diabetic patients.

Studies had been done previously in insulin resistant and insulin sensitive diabetic patients to evaluate the possible differences in the uptake and release of I^{131}-labeled insulin by the liver, in vivo. The results clearly indicate that both the hepatic uptake and the release times of the insulin-I^{131} in the resistant patients were considerably prolonged when compared with insulin sensitive diabetic patients.[4] The patient described in this report also exhibited delayed hepatic uptake and slow release of insulin-I^{131}.

3. *Insulin-like activity (ILA) of serum. A. In vitro.* The rat epididymal adipose tissue method described by Martin, et al.[5] was used. In this procedure the amount of $C^{14}O_2$ produced from glucose-1-C_{14} is used as the index of insulin or ILA present in the incubation medium. With this technic undiluted fasting serum from apparently healthy adults[6,7] showed ILA ranging from 0.03 to 0.5 milliunits per ml., with a mean of 0.27. The ILA of insulin treated diabetics varies with the type of insulin, the total amount and the time of insulin administration before the blood sample was taken. For example, in a patient receiving 40 units of NPH insulin daily, serum taken twenty-four hours after the last insulin injection contained 2.0 milliunits per ml. of ILA.

In the present patient, serum was obtained for study of ILA during the period of moderate resistance, two months later at the peak of insulin resistance associated with keto-acidosis, and finally after the return of sensitivity to insulin. Detailed data are presented in table 2. During the resistant phase all sera studied showed markedly elevated levels of ILA. This was especially high during the episode of keto-acidosis (Sept. 3, 1959) and yet considerable additional insulin was needed for adequate treatment. After prednisone therapy the ILA was in the range usual in an insulin sensitive diabetic.

The following evidence suggests that the markedly elevated level of ILA was insulin. 1. At a serum dilution of 1:500 the ILA effect persisted. This makes nonspecific interference unlikely. 2. Anti-insulin serum, induced in guinea pigs with beef insulin, added to the incubation medium caused a marked reduction in ILA. However, the inhibition was not total, perhaps reflecting inhibition of only the beef component of the injected commercial mixture of beef and pork insulin. It is possible that incomplete inhibition at the time of the peak resistance (sample obtained on Sept. 3, 1959) represented saturation of the anti-insulin serum by the excessive amount of insulin present in the patient's serum at that time. Finally, there is the possibility that there was a small amount of endogenous human insulin circulating in the patient's serum.

TABLE 2

Insulin-like activity (ILA) of serum (J.C. No. 54010)

Date	Insulin (U./day)	Assay no.	Dilution	ILA (mu./ml.) Serum	Serum + AIS*
7/30/59	450	1	1:32	14.4	1.6
		2	1:32	11.8	
9/3/59	2,500	1	1:32	>16.0	14.7
		2	1:32	>32.0	
		3	1:128	>64.0	
		4	1:500	175.0	
9/24/59	3,200	1	1:128	>64.0†	
12/12/59	50	1	1:32	1.7	
		2	1:8	2.6	1.1

* AIS: Anti-insulin serum prepared in guinea pigs with beef insulin.
† Sample used for in vivo study.

B. *In vivo.* Plasma, 100 ml., which contained in excess of 6.4 units of ILA from the in vitro estimate, was infused into a patient with carcinoma of the lung over seven minutes. The glucose tolerance of the recipient, measured by the rate of disappearance of an infused load of glucose, was in the low normal range (K = 1.2).[8] The response of the blood sugar following infusion of the plasma from the insulin resistant donor is shown in figure 3. There is a definite hypoglycemic

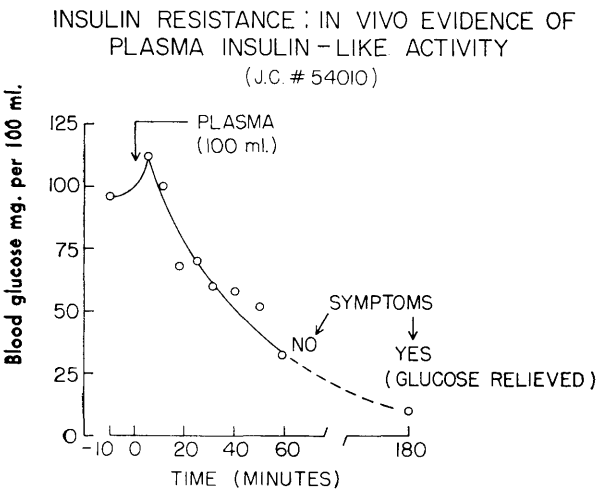

FIGURE 3

response. The peak depression of glucose in the recipient occurred later than one hour after the infusion suggesting a slow but continued release or activation of the insulin-like factor in the patient's plasma.

DISCUSSION

The patient demonstrated a number of clinical features known to be associated with insulin resistance.[28] The resistance per se was a significant factor in the cause of keto-acidosis and coma. Severe keto-acidosis is uncommon in patients with insulin resistance.[25,28] Among thirty-four patients seen at the Joslin Clinic during the last thirty years there are three in whom failure to respond to insulin resulted in coma.[9] Successful treatment required even larger amounts of intravenous insulin and was complicated by an anaphylactic reaction. The tendency of insulin-resistant subjects to develop hypoglycemia for several days after the administration of large amounts of insulin is consistent with the slow dissociation of insulin-protein complexes which have been observed in these subjects.[10,11] This complex of "bound insulin" serves as a depot of insulin which is released in significant quantities over a prolonged period of time.

It is established that antibodies to insulin occur.[29] Yet, these antibodies are not demonstrable by all of the technics for detection of antibodies. For example, immune rabbit serum, which has a high insulin antibody titer by a hemagglutination method, may not show precipitins.[27] The apparent discrepancy with the two studies in the present patient may be explained by antigen excess which can produce a soluble antigen-antibody complex.

Antibodies to insulin and an increased capacity of the serum to bind insulin were demonstrated. Neither of these features has been thought to be specific for insulin resistance.[12-16,30] However, the very high level of binding and the fall following the return of the insulin sensitive state suggest a possible role of the "binding process" in the perpetuation of the resistance. The difference between insulin resistance and nonresistance in insulin-treated patients may be a quantitative one. Berson and Yalow[11] have shown that serum from insulin resistant patients may bind up to 500 units of insulin per liter; they suggest that binding of administered insulin may be the important factor in certain patients with insulin resistance. Lowell,[18,19] Burrows[20] et al. and Vallance-Owen[21] have suggested that the need for large amounts of insulin is to overcome binding or to overcome a circulating insulin inhibitor. Field[22] found that ACTH abolished insulin resistance without altering the ability of the serum to bind large amounts of insulin. The complexity of the binding process, and the difference in technics used for its demonstration, have not permitted definitive evaluation of the role of this factor in insulin resistance.[11]

An increased ILA of serum has been demonstrated in the chronically resistant patient[32,34] and during diabetic acidosis poorly responsive to insulin.[33] In the present instance the high ILA is most likely a measure of the "bound insulin." This in vitro activity may reflect dissociation of insulin from the protein complex. Alternatively, the "binding" may not interfere with active sites of the insulin molecule if the insulin-protein complex can reach the cell. The failure of an in vivo response could be explained by an inability of this insulin-protein aggregate to cross the capillary membrane.

The hypoglycemia following infusion of plasma from the patient into a nondiabetic patient clearly indicates the biologic activity of the insulin. The magnitude of the response is consistent with an in vitro estimate of insulin-like activity made on an earlier serum sample (17.5 units per 100 ml.). The time required for the maximum hypoglycemia is consistent with a delayed release of "bound insulin" in the donor plasma. A slow release is also suggested by the prolonged time required for maximal hepatic uptake of I^{131} from I^{131}-insulin added to the donor plasma.[4] Unfortunately, the functional state of the pituitary, the adrenal and the liver in the recipient could not be evaluated. The low normal rate of glucose utilization observed may represent the impaired glucose tolerance which has been demonstrated in patients with neoplastic disease.[8]

The effect of prednisone, or any presumed specific therapy, is difficult to evaluate because of the variable course of insulin resistance.[28] ACTH[23-25] and prednisone[26] have been reported to decrease insulin requirements in insulin resistant patients. The mechanism for this ap-

parent effect is not clear. In the reported series there was usually a fall in insulin dosage in four to five days and within ten to fourteen days the insulin requirement was less than 100 units daily. This decrease in insulin requirement has been observed in patients with[23] and without hemochromatosis. An effect of adrenal steroids or ACTH on antibody formation has been suggested. In the six patients reported by Oakley et al.[26] the four who responded to prednisone had demonstrable antibodies as measured by the passive cutaneous anaphylaxis test in the guinea pig. The other two did not show antibodies and the insulin requirement increased during prednisone administration. It is difficult to explain the early fall in insulin dose by decreased antibody synthesis since gamma globulins have a half life of the order of fourteen days. This early decrease in insulin could be due to a direct effect on insulin-sensitive cells, an effect on capillary membrane permeability or an alteration of the protein binding. A recent report indicates that methyl prednisolone in a patient with insulin resistance lowered the serum binding globulin concomitantly with insulin.[31]

Figure 2 shows the insulin requirements after starting prednisone in the present patient. The drop to less than 100 units of insulin daily on the third day after prednisone may represent continued release of "bound insulin" following the 3,150 units during the preceding forty-eight-hour period. Over the next three weeks the insulin requirement remained at approximately 200 units per day, lower than at any period since resistance developed five months previously. In the fourth week after prednisone there was a decrease in insulin requirement to less than 100 units daily. By the eighth week, 50 units of insulin daily were adequate to maintain normoglycemia. The final sequence is consistent with an effect of prednisone on antibody formation. Further evidence on the role of prednisone might have been obtained by stopping the steroid. This was not felt advisable because of the severity of the clinical situation during the resistant period. The glucocorticoid property of prednisone did not produce any major problem.[24]

SUMMARY

An instance of insulin resistance of five months' duration is described in which the resistance per se was a major factor in producing diabetic coma. This was successfully treated with large amounts (9,000 units) of insulin. The intravenous insulin produced an anaphylactic reaction and was followed by a five-day period of hypoglycemia. The resistant state returned (1,200 units daily). Prednisone was associated with a return of insulin sensitivity.

Serum from the patient during the resistant period showed precipitin antibodies, increased insulin binding and high levels of insulin-like activity by the in vitro adipose tissue assay. Plasma, 100 ml., infused into a nondiabetic patient produced profound hypoglycemia. The time course of the induced hypoglycemia suggests a slow release of "bound insulin" in the patient's plasma. These observations suggest that the basis for insulin resistance in this instance is an inability of the insulin to reach the cell in a biologically active form at the necessary rate, or a failure of the cell to respond.

SUMMARIO IN INTERLINGUA

Resistentia a Insulina con Alte Nivellos de Activitate Insulinoide in le Circulation

Es describite un caso de resistentia a insulina de cinque menses de duration, in que le resistentia per se esseva un factor major in le production de coma diabetic. Isto esseva tractate a bon successo con grande quantitates (9.000 unitates) de insulina. Le administration intravenose del insulina produceva un reaction anaphylactic e esseva sequite per un periodo de cinque dies de hypoglycemia. Le stato de resistentia recurreva (con un dosage diurne de insulina de 1.200 unitates). Le uso de prednisona esseva associate con le retorno del sensibilitate pro insulina.

Le sero del patiente durante le periodo de resistentia monstrava anticorpore precipitinal, augmento del ligation de insulina, e alte nivellos de activitate insulinoide secundo le essayage in vitro a tissu adipose. Le infusion de 100 ml de plasma ab le patiente produceva profunde hypoglycemia in un subjecto non-diabetic. Le curso del hypoglycemia in le tempore pareva indicar un lente liberation de "insulina ligate" in le plasma del patiente. Iste observationes suggere que le base del resistentia a insulina in le presente caso esseva le incapacitate del insulina de attinger le cellula in un forma biologicamente active e in le quantitate requirite o le incapacitate del cellula de responder.

ACKNOWLEDGMENT

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ADDENDUM

A follow-up* on June 3, 1960, indicates that prednisone was stopped sometime in early 1960. Insulin requirement has progressively risen to the range of 600-800 units daily. This dose level has prevented ketosis;

* Courtesy of Dr. Art. B. Martin, Fort Smith, Arkansas.

fasting blood sugars range between 100 to 300 mg. per 100 ml.

Protein fractions of the patient's serum were separated by column electrophoresis and their effect on glucose uptake of the isolated rat diaphragm tested. Methods used have been reported by Randle, P. J. (*J. Endocrinol.* *14*:82, 1956) and Taylor, K. W., and Randle, P. J. (*J. Endocrinol.* *19*:221, 1959).

When these protein fractions were dissolved in a volume of buffered glucose-saline thirty times that of the original volume of serum a statistically significant increase in glucose uptake was observed with the gamma globulin fraction *only*. Since these fractions were highly diluted prior to assay the results suggest that a considerable quantity of insulin was associated with the gamma globulins and that this insulin possesses biologic activity in this in vitro system. These observations are reported through the courtesy of Dr. K. W. Taylor.

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