Insulin Resistance

Differentiation into Two Types by Measurement of Serum Insulin-like Activity in Vitro

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SUMMARY

In eleven insulin sensitive and in six insulin resistant diabetic patients, insulin-like activity was measured in unmodified serum and in the corresponding acid ethanol extract shown to be free of insulin antibodies. Whereas most of the insulin responsive and four insulin resistant diabetic patients exhibited a similar ratio of insulin-like activity of serum to the respective serum extract, two patients with insulin resistance exhibited a more than one-hundredfold increase in this ratio.

Of the factors analyzed, i.e., length of insulin treatment, daily insulin dose, maximum serum insulin binding capacity, serum ILA and serum extract ILA, only insulin binding capacity and serum extract ILA exhibited a positive correlation in both the insulin responsive and insulin resistant patients. DIABETES *14*:432-35, July 1965.

A diabetic patient is classified as insulin resistant when he requires more than 200 units of insulin per day for adequate control.[1,2] Insulin resistance is seldom encountered in the absence of ketoacidosis, acute infection or obvious endocrine disorders. The pathogenic mechanism is still poorly understood and treatment is empiric. It has been well established that insulin resistance is associated with circulating antibodies to insulin; however, the causal relationship has been repeatedly challenged, as antibodies to insulin are also found in the majority of insulin treated diabetic patients who do not have insulin resistance.[3,4]

It seemed of interest, therefore, to measure serum insulin-like activity (ILA) in such patients, not only in the unmodified serum, but also in serum extracts from which the insulin antibodies had been removed. The following brief report presents results obtained in insulin sensitive and insulin resistant diabetic patients, as well as in nondiabetic subjects serving as controls.

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MATERIALS AND METHODS

All diabetic subjects were treated as hospital inpatients and we are indebted to Dr. George W. Thorn of the Peter Bent Brigham Hospital, Dr. Alexander Marble and Dr. Leo Krall of the Joslin Clinic, Dr. Samuel Beaser of the Beth Israel Hospital, and Dr. Russell Randall of the Boston Veterans Administration Hospital for permission to use their patients. Seventeen diabetic patients were studied who ranged in age from eighteen to seventy-two years. Insulin treatment had been initiated six months to thirty years previously. Of these seventeen patients, eleven were insulin responsive diabetics; their daily insulin dose ranged from 40 to 70 U. Six patients were resistant to insulin without presence of ketoacidosis, infection or obesity. Their daily insulin requirement ranged from 250 to 2,000 U. The nondiabetic subjects were in apparent good health, had never received any insulin and were ambulatory. Blood was collected after an overnight fast from the diabetic patients prior to their daily insulin administration. Therefore, all diabetic patients were studied twenty-four hours after their last insulin injection. Blood was allowed to clot at room temperature; serum was separated by centrifugation and stored at minus 20° C. The acid ethanol extract of serum was prepared as previously described, the final material was dialyzed, lyophilized and taken up in buffer.[5] If crystalline insulin was processed this way, approximately 80 per cent of activity was recovered. Insulin-like activity was determined by the rat adipose tissue technic where oxidation of glucose-I-C-14 to C-14-O_2 is the index of activity.[6] Each sample of serum and of the extract was assayed at least twice on two different assay days, with care taken to assay serum and corresponding extract within

the same assay, as the intra-assay error is smaller than the inter-assay error. Due to the often elevated level of ILA, dilution with buffer was performed in all instances, ranging from one part serum or extract to three to 999 parts buffer. As the results of the resistant patients RM and YW were significantly different from the other subjects studied, serum and extract of patient RM was assayed in four, and that of YW, in three different assays. Results were expressed as microunits per ml. in equivalents of crystalline pork insulin standards and were corrected for dilution. Maximum insulin binding capacity of serum was assessed by the method of Berson and Yalow.[7]

RESULTS

Table 1 indicates the age, serum insulin binding capacity, serum insulin-like activity and insulin-like activity of the corresponding acid ethanol extract for the ten nondiabetic subjects. In addition it shows for the diabetic patients, the length of insulin treatment in years and their daily insulin requirements. The normal subjects exhibited a lower yield of insulin-like activity in the extract than in the serum except for subject GC. His serum has been extensively investigated and found

TABLE 1

Clinical data and values for insulin-like activity (ILA) of serum and corresponding acid ethanol extract (AEE) from nondiabetic subjects, insulin sensitive and insulin resistant diabetic patients

Patient	Age	Length of insulin Rx (years)	Daily insulin dose in units	Maximum serum insulin binding capacity (U./L.)		Serum ILA (μU./ml.)	AEE-ILA (μU./ml.)	Ratio ILA (AEE/Serum)
A. Nondiabetic subjects (n=10)								
G.C.	36	—	—	0		50	270	5.4
M.G.	43	—	—	0		200	70	0.4
J.L.	24	—	—	0		210	200	0.9
M.K.	47	—	—	0		260	110	0.4
M.G.	36	—	—	0		300	140	0.5
J.S.	24	—	—	0		400	130	0.3
A.R.	37	—	—	0		400	150	0.4
G.H.	30	—	—	0		450	330	0.7
D.C.	43	—	—	0		740	190	0.3
G.U.	31	—	—	0		800	160	0.2
					Mean	381	175	0.95
					± S.E.M.	±67	±24	±0.50
B. Insulin sensitive diabetic patients (n=11)								
M.G.	38	4	40	22		200	1,200	6
B.E.	68	30	40	16		320	320	1
D.J.	22	7	44	40		400	1,040	2.6
B.R.	37	24	46	15		240	520	2.2
D.W.	40	10	60	10		400	600	1.5
A.M.	25	23	62	25		480	1,400	2.9
M.P.	38	11	66	7		920	400	0.4
P.D.	49	12	68	6		240	200	0.8
L.C.	18	3	70	1		360	160	0.4
W.A.	58	14	70	17		680	1,400	2.1
T.J.	21	9	70	25		840	2,920	3.5
					Mean	462	924	2.04
					± S.E.M.	±75	±243	±0.54
C. Insulin resistant diabetic patients (n=6)								
Y.W.	67	4	250	300		570*	70,000‡	123
R.M.	66	6/12	500	650		300†	45,000§	150
C.R.	53	10	750	600		13,800	90,000	6.5
H.R.	59	20	800	800		114,000	147,000	1.3
B.C.	63	3	1,200	600		37,000	86,000	2.3
P.E.	72	1	2,000	1,200		125,000	175,000	1.4
					Mean	48,445	102,167	

*Standard error of the mean ± 180 (n=8).
†Standard error of the mean ± 80 (n=4).
‡Standard error of the mean ± 3,100 (n=6).
§Standard error of the mean ± 4,800 (n=4).

to have persistently low insulin-like activity on rat adipose tissue, however, normal activity on a muscle preparation.[8] For the insulin sensitive diabetic patients there was no relationship between duration of insulin treatment and daily insulin dose nor was there a particular relationship between the dose of insulin administered subcutaneously twenty-four hours previously and the level of circulating ILA. However, there was a definite relationship between insulin binding capacity and insulin-like activity of the acid ethanol extract. Contrary to the nondiabetic subjects, the mean ratio of insulin-like activity of acid ethanol extract to serum was greater than one. The insulin resistant diabetic patients all showed a marked elevation of their insulin binding capacity which in turn was closely related to their daily insulin requirement. The ratio of insulin-like activity of acid ethanol extract to serum was similar to those of the insulin responsive diabetics in four patients, although of much higher magnitude. Two insulin resistant patients exhibited a strikingly different behavior in that the acid ethanol extract yielded a more than hundredfold increase of insulin-like activity. In no instance could any insulin binding capacity be demonstrated in any acid ethanol extract.

DISCUSSION

The acid ethanol procedure not only extracts nonantibody bound circulating insulin but also extracts insulin from antibody. This has been demonstrated previously by Grodsky and Forsham[9] in the case of one insulin resistant patient. In most insulin responsive diabetic patients, the ILA of the extract was higher than the ILA of the serum, which corresponded to their increased insulin binding capacity. On the other hand the three patients with the lowest insulin binding capacity exhibited in each instance lower values for ILA of the extract than for ILA of serum, quite similar to the nondiabetic subjects. This positive relationship between insulin binding capacity and ILA of serum extract was also present in the insulin resistant diabetic patients. The highly significant increase in ILA following extraction of serum in two of the six insulin resistant patients suggests the presence of a different insulin antibody. However, using insulin binding capacity as a parameter, we were unable to distinguish this antibody from the one encountered in the insulin responsive and in the other four insulin resistant diabetics.

Our data lend support to the findings of other workers[10,11] that in *some* instances of insulin resistance the factor responsible for, or at least associated with such resistance, behaves differently from the insulin antibody commonly encountered. The existence of two different insulin antibodies was first reported in 1942 by Lowell,[12] and more recently confirmed in immunized guinea pigs and rabbits.[13,14] At any rate, neutralization of insulin could explain failure of the patient to respond to exogenous insulin. To explain insulin resistance in those other patients where abundant ILA can be detected in serum in vitro, one has to assume a weaker binding of the circulating insulin to antibody[7] or a failure of the target organs to respond to insulin.[15,16,17]

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EDITORIAL

INFANTS OF DIABETIC MOTHERS

Management of the newborn infant of the diabetic mother is still one of the most challenging unsolved problems in clinical medicine. In spite of extensive investigations of the physiologic and biochemical status of the infant,[1-4] approximately 35 per cent die either in utero or in the neonatal period. Prematurity and anomaly are increased in prevalence, albeit to what degree has not been established. The infants behave in many respects like premature infants even though they often are large and appear postmature. The most common cause of death in the neonatal period is hyaline membrane disease.

It is now realized that increase in fetal survival has been achieved by reduction of stillbirth rather than of neonatal death.[1] The most successful results have been reported from centers where therapy is characterized by collaboration between diabetician, obstetrician, and pediatrician. Factors related to unsatisfactory outcome have been: long duration of known diabetes, complicating vascular disease, and uncontrolled diabetes. Although it is generally held that early interruption of pregnancy will reduce the occurrence of stillbirth, conclusive evidence of this is not yet available. Good diabetic control is very important, a view which we base on long-term observation of a large group of juvenile diabetic pregnancies at the University of Iowa.

Fetal loss is also increased in patients destined to develop chemical diabetes in later years.[5] Unfortunately, neither prediabetes or mild gestational diabetes may be suspected, and consequent lack of appropriate management may account in part for the fetal loss. In both these patients and those with established diabetes there has been no significant reduction in mortality beyond that made possible by improved care of the mother.

Very little is known about the physiology of the placenta in diabetic pregnancy. There is evidence that the organ remains immature. Of particular interest are the recent reports of Joron et al.[6] and Kyle.[7] These investigators studied maternal urinary estriol excretion during pregnancy and found it below that of nondiabetic pregnancies. Moreover, rapidly decreasing urinary estriol values indicated fetal distress. In fact, when levels fell to 4 mg./24 hrs. for forty-eight hours, fetal death had occurred.[7] Another significant study recently is that from Daughaday's laboratory.[8] Herein a growth hormone-like substance, probably placental in origin, was found in elevated concentration in diabetic pregnancy. The physiology of this substance remains to be determined, however.

At present much attention is being directed to the side effects of drugs on pregnancy. Of particular interest in the management of diabetic pregnancy are the chlorthiazides and the sulfonylureas. The former have been used liberally in the pregnant diabetic without deleterious effect reported so far. Though they create antagonism to insulin action, it has been difficult to separate a rise in insulin requirement brought on by chlorthiazides from that usually occurring as pregnancy progresses.[9] The latter compounds, the sulfonylureas, have not been recommended in gestational diabetes because of scattered reports of fetal abnormalities in pregnancies wherein these drugs were employed. No proof of a cause