Serum Immunoreactive Insulin Levels During Glucose Tolerance and Intensive Islet Stimulation

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SUMMARY

Glucose tolerance and intensive islet stimulation tests were done on eighty-seven subjects who covered a broad range of age and glucose tolerance. The data suggest decreasing glucose tolerance with increasing age compatible with the observations of others. Over a broad range of glucose tolerance there was seen such a broad spread of serum immunoreactive insulin levels (IRI) that it was not possible to relate them directly to the degree of glucose tolerance seen. This suggested that multiple factors, including variations in peripheral resistance to insulin not attributable to known factors, are operative in determining glucose tolerance. Only when glucose tolerance was very abnormal or fasting blood sugar was elevated was there apparent decrease in serum IRI response to the stimuli used, making it difficult to attribute mild-to-moderate degrees of glucose intolerance to lack of capacity for IRI release. A close correlation between the two-hour IRI in a glucose tolerance test and the levels obtained after the stimulus of glucagon and tolbutamide suggested that the former may be an adequate reflection of islet secretory capacity. DIABETES *20*:404-09, June, 1971.

Since the introduction of the radioimmunoassay for insulin by Yalow and Berson in the late 1950's,[1] numerous studies have been performed on plasma or serum immunoreactive insulin (IRI) levels after various physiologic and pharmacologic stimuli and during various pathologic states. Yalow and Berson in their initial studies found that some noninsulin-dependent adult-onset diabetic subjects eventually attained plasma IRI levels during glucose tolerance which exceeded those of normal subjects, but juvenile diabetics had uniformly low IRI levels.

Since that time a number of studies have been done in an effort to assess further the IRI response of the early diabetic state, but it has become obvious that many factors other than blood sugar level must be taken into consideration when assessing IRI response to glucose administration. Factors including weight,[2] age,[3] disease states,[4] as well as hormone[5] and drug administration[6] have been found to influence plasma IRI levels after glucose loading.

Previous studies from this laboratory showed that oral glucose followed by intravenous pulsed glucagon and tolbutamide results in a rapid and marked rise in plasma IRI levels and probably provides an acute-near-maximal stimulus to IRI release from the β cell.[7] The present study was undertaken to see if this stimulus to IRI would be helpful in evaluating the IRI response of the early diabetic state, as defined by mild abnormalities of glucose tolerance. It was assumed that if islet dysfunction was primary in the genesis of diabetes, the additional stressful stimulus would disclose islet failure at an earlier stage of glucose intolerance.

MATERIALS AND METHODS

The subjects of this study were outpatients, inpatients and normal volunteers of Presbyterian-St. Luke's Hospital, arbitrarily selected to cover a wide range of age. Glucose tolerance among subjects varied widely from well-within-normal to frankly diabetic limits. Although some diabetics had very high fasting blood sugars, none were receiving insulin. A small number of the known diabetic subjects were taking oral hypoglycemic agents, but these were discontinued at least three days prior to testing. At the time of testing, a careful family and medical history was taken.

All tests were done after an overnight fast and in the resting state. Blood for IRI determination was allowed

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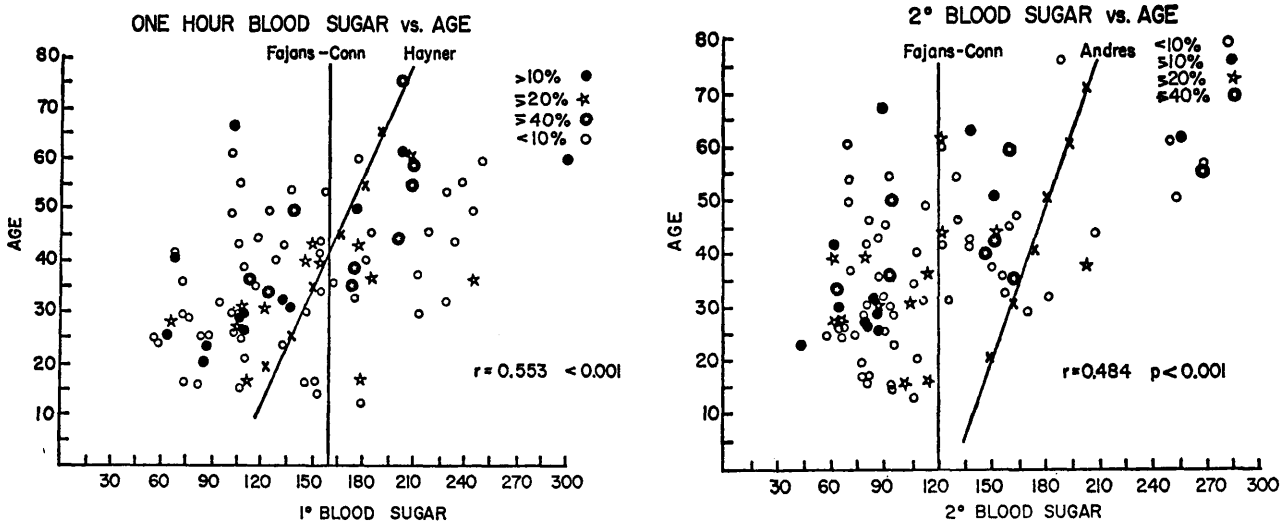

FIGS. 1. and 2. Relationships between one- and two-hour blood sugar and age of subjects tested. Symbols indicate per cent deviation from ideal body weight. See text for details.

to clot, chilled until the serum was separated, and the serum then stored frozen (—15° C.) until the time of analysis. Blood for sugar determination was collected in an oxalate fluoride tube and kept chilled until time of analysis. Whole blood determinations were done on a Technicon Analyzer by the ferricyanide method of Hoffman.[8] Serum IRI determinations were done by a dextran-coated charcoal modification[9] of the method of Goetz et al.[10]

Oral dextrose (100 gm.) was given as an orange-flavored solution (Dextrose 100) after the fasting blood sample was obtained. Bloods for the above determinations were obtained at one hour and two hours, at which latter time 1 mg. of glucagon (Lilly) and 0.5 gm. tolbutamide (Upjohn) were administered rapidly and sequentially intravenously. Blood samples were then taken exactly five minutes after injection. Mild nausea, flushing and tachycardia usually occurred after injection. Hypoglycemia frequently occurred thirty to sixty minutes after the injections if not prevented by further ingestion of carbohydrate.

RESULTS

Figures 1 and 2 show plots of one- and two-hour blood sugar levels obtained at the respective times after glucose ingestion versus the age of eighty-seven subjects tested. These subjects had normal fasting blood sugars (< 110 mg./100 ml.), were not taking any medications, and had no disorder known to affect carbohydrate metabolism. The per cent deviation from ideal weight is indicated by the symbols, and the dividing lines for normality and abnormality according to the criteria of Fajans and Conn[11] are indicated by the vertical line.

The diagonal line in the one-hour blood sugar plot indicates the mean of the one-hour blood sugar versus age in the study of Hayner et al.[12] of the populace of Tecumseh, Michigan. In this study of a randomly selected population, the mean blood sugar one hour after a glucose load increased significantly with age. It can be seen that the mean blood sugar of the subjects in the present study also was positively correlated with age. The plot also shows that in the present study obese subjects followed the same general distribution as nonobese.

As seen in figure 2, the same relationships were true for the two-hour blood sugar, but the correlation with age was not as strong as for the one-hour. The diagonal line in this figure indicates the division between normal and abnormal suggested by Andres[13] on the basis of his testing of glucose tolerance in a volunteer population spanning a large range of age. It can be seen that most of the subjects in the present study did not exceed his range of normal. However, as seen by the vertical line, if the criteria of Fajans and Conn are used, a considerable proportion of the subjects tested would be considered diabetic.

Figure 3 shows the relationships between the two-hour blood sugar versus two-hour IRI, and figure 4 the two-hour blood sugar and five-minute post-stimulation IRI levels. The former were fairly well correlated whereas the latter were poorly correlated. Inspection of the figures shows that the distribution of IRI levels for obese subjects was not different from those of normal

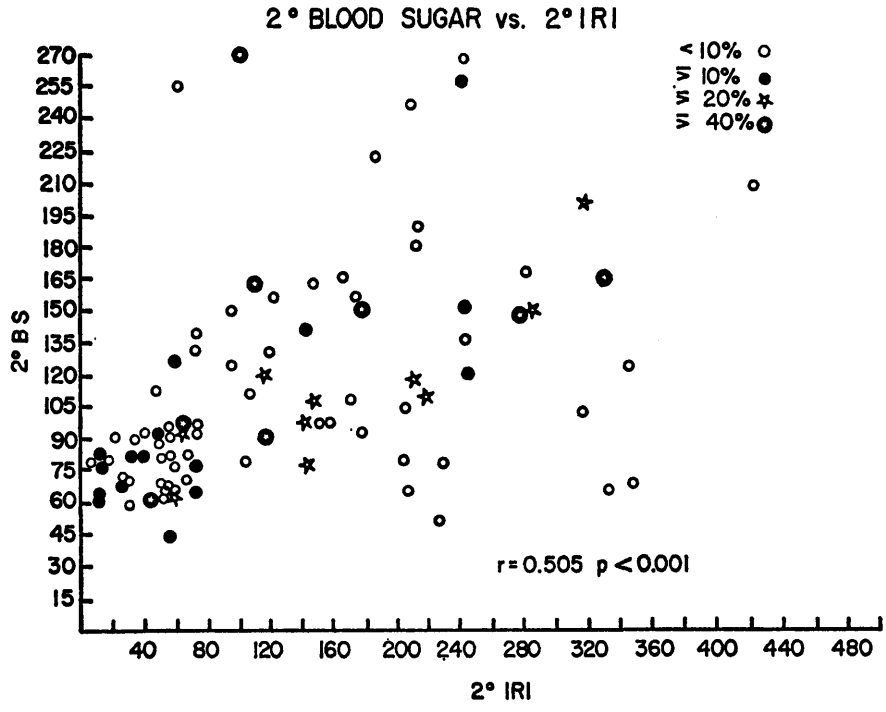

FIG. 3.

Relationships between blood sugar and serum IRI two hours after oral glucose. Symbols indicate per cent deviation from ideal body weight.

weight. The same type of plot (not shown) also revealed no influence of age on the IRI level of this group of subjects except as demonstrated previously that increasing age was associated with increasing blood sugar.

Figure 5 shows the relationship between the two-hour IRI and the five-minute post-stimulation IRI. These showed the highest degree of correlation of any of the observations made ($r = .726$). Obese and nonobese subjects were also distributed similarly in this relationship.

Table 1 shows the IRI responses of ten diabetic sub-

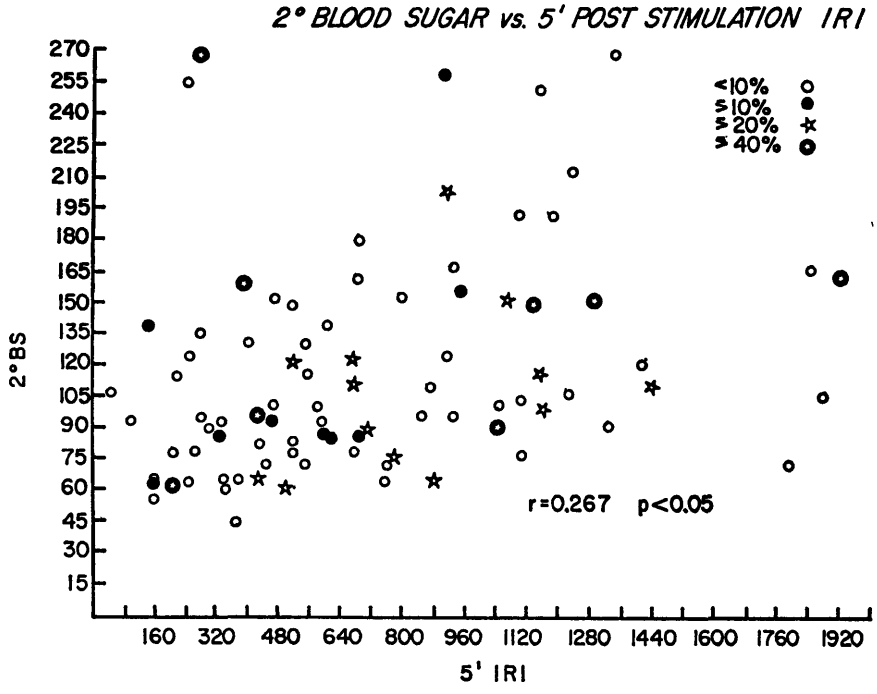

FIG. 4.

Relationship between blood sugar two hours after oral glucose and serum IRI five minutes after added stimulus of 1 mg. glucagon and ½ gm. tolbutamide intravenously. Symbols indicate per cent deviation from ideal body weight.

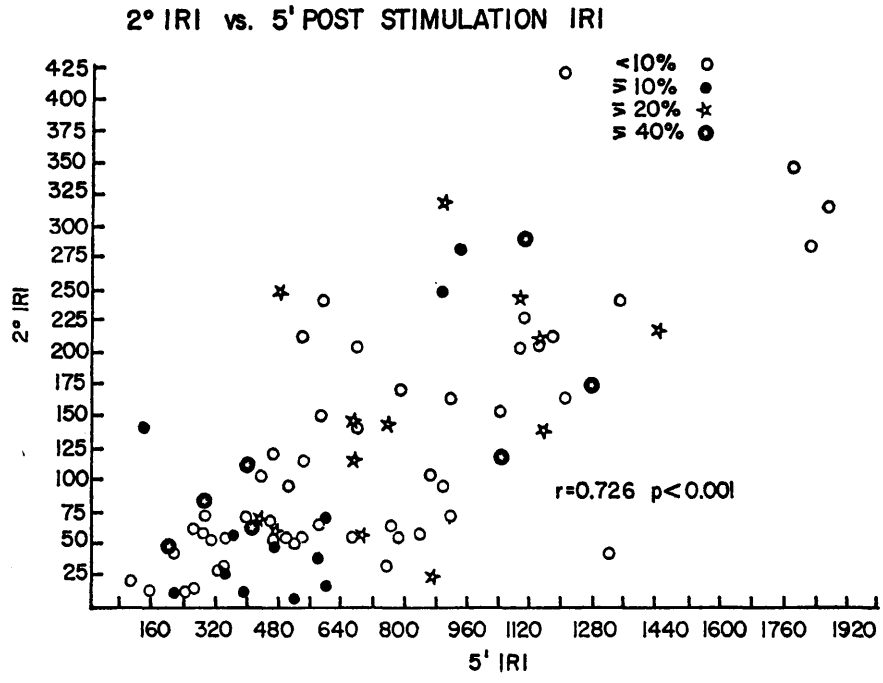

FIG. 5.

Relationship between serum IRI two hours after oral glucose and serum IRI after added stimulus of 1 mg. glucagon and ½ gm. tolbutamide intravenously. Symbols indicate per cent deviation from ideal body weight.

jects with elevated fasting blood sugar. The responses were generally low although frequently comparable to those of nondiabetic subjects. Only one subject had an elevated response and he was very obese.

DISCUSSION

As stated, the purpose of this study was to try to determine if a deficiency of IRI secretory capacity exists in the early diabetic state, as reflected by an acute and probably near maximal stimulus to the β cell. The early diabetic state was to be defined on the basis of abnormal glucose tolerance according to the criteria of Fajans and Conn.[11] As the study progressed it became apparent, however, that these criteria were probably inadequate for evaluation of glucose tolerance in our group of subjects whose ages ranged from the second to eighth decades. The studies of Hayner et al.[12] and Andres et al.[13] clearly showed the effect of age on glucose tolerance, and a similar effect was seen in our group of subjects.

It also became apparent that we were dealing with subjects who had a continuum of glucose tolerance.[14] Thus, a cutoff point for the labeling of a subject as "diabetic" was necessarily arbitrary, as discussed in detail by Andres.[13] Accordingly, we analyzed the results only on the basis of IRI response to blood sugar levels attained during the two-hour glucose tolerance test. As seen in figure 3, there was a broad range of IRI levels during glucose tolerance. This agrees with the data of Welborn et al.[15] who found a tenfold variation in IRI

TABLE 1

Immunoreactive insulin responses of subjects with elevated fasting blood sugar

Patient	Age	Per cent deviation from ideal body weight	Fasting blood sugar	Two-hour immunoreactive insulin	Five-minute immunoreactive insulin
H.N.	72	13	116	46	152
V.O.	52	97	132	400	1,580
I.N.	36	—3	137	25	64
A.S.	42	24	139	31	170
P.L.	38	29	155	82	330
H.K.	64	11	172	40	128
R.R.	36	34	230	27	228
T.D.	47	29	244	30	106
R.G.	43	25	246	17	152
O.J.	38	37	284	50	350

TABLE 2

Reproducibility of individual test responses

	W.R.			L.Y.			A.N.	
April 24, 1967	Blood sugar	Immuno-reactive insulin	Sept. 13, 1968	Blood sugar	Immuno-reactive insulin	April 5, 1967	Blood sugar	Immuno-reactive insulin
Fasting	86	18	Fasting	74	35	Fasting	77	12
One-hour	103	144	One-hour	107	147	One-hour	100	50
Two-hour	95	83	Two-hour	67	187	Two-hour	72	13
Five-minute	113	630	Five-minute	67	1,310	Five-minute	88	280
April 3, 1967	Blood sugar	Immuno-reactive insulin	Sept. 26, 1968	Blood sugar	Immuno-reactive insulin	April 24, 1967	Blood sugar	Immuno-reactive insulin
Fasting	75	23	Fasting	77	11	Fasting	68	10
One-hour	101	128	One-hour	118	375	One-hour	119	120
Two-hour	80	115	Two-hour	77	170	Two-hour	76	18
Five-minute	102	492	Five-minute	74	872	Five-minute	87	300

levels of a group of young normal subjects. Table 2 shows the reproducibility of the responses of a small number of subjects. The degree of reproducibility suggests that the wide ranges of response are not random but truly reflect differences among the individuals tested.

In addition, in the present study there was an increase in IRI response with increasing two-hour blood sugar (figure 3). The same was true for the IRI levels obtained after the glucagon-tolbutamide stimulus (figure 4). Surprisingly and perhaps importantly, there was a high degree of correlation between the post-stimulation IRI and the two-hour IRI level (figure 5). This suggests that the two-hour IRI level is proportional to the amount of IRI within the β cell available for immediate release. The glucagon and tolbutamide apparently trigger different release mechanisms resulting in a large outpouring of the IRI available. It thus shows that the IRI secretory capacity of the β cell is not exhausted under the stimulus of hyperglycemia alone.

The combination of a high blood sugar and elevated IRI level suggests that some form of insulin resistance is present which is not explained by the degree of obesity or other known factors. The presence of an ineffective insulin might be postulated and the recent discovery of proinsulin (which circulates but has little biologic activity) has generated considerable interest in its possible role here.[16] Evidence from others as well as investigations of our own suggest that this is not the case, but further work is needed in this area.

The combination of a high blood sugar level and low IRI level could be due to either insensitivity of the β cell to the signal of blood sugar elevation or an absolute inability to respond (β-cell failure). The close correlation of the two-hour IRI level and five-minute IRI level in this study suggests that the latter may be more likely when this combination prevails.

The low IRI responses exhibited throughout the test by subjects with elevated fasting blood sugars (table 1) suggests that they were in a state of relatively advanced β-cell failure. This was true for all these subjects except one who was very obese; thus it may be said that β-cell failure was characteristic of the subjects in this study who were definitely diabetic. Joffe et al.,[17] in using our procedure to study insulin secretory capacity, found that IRI response was markedly impaired in patients with chronic relapsing pancreatitis. This would also suggest β-cell failure or destruction. They also found that by carrying out the post-stimulation observations to thirty minutes, elderly subjects showed an initial delayed response, but it was elevated in the later periods tested. They interpreted this as indicating some degree of insulin resistance in the elderly in addition to their having a somewhat diminished early IRI response.[18]

Our present inability to ascertain the degree of glucose intolerance which precisely separates the diabetic from the nondiabetic does not allow us to define an IRI response characteristic of the diabetic in the earlier stages. The broad range of response seen suggests that multiple factors are operative in the genesis of glucose intolerance and that a stage of β-cell hyperfunction may even be present before β-cell failure supervenes. The long term follow-up of these subjects for the development of diabetes should help to answer some of these questions.

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REFERENCES

[1] Yalow, R. S., and Berson, S. A.: Plasma insulin concentrations in nondiabetic and early diabetic subjects. Diabetes *9*: 254-60, 1960.

[2] Perley, M., and Kipnis, D. M.: Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes *15*:867-74, 1966.

[3] Streeten, D. H. P., Gerstein, M. M., Marmor, B. M., and Doisy, R. J.: Reduced glucose tolerance in elderly human subjects. Diabetes *14*:579-83, 1965.

[4] Karam, H. J., Grodsky, G. M., Pavlatos, F., and Forsham, P. H.: Critical factors in excessive serum-insulin response to glucose. Lancet *1*:286-89, 1965.

[5] Perley, M., and Kipnis, D. M.: Effect of glucocorticoids on plasma insulin. New Eng. J. Med. *274*:1237-42, 1966.

[6] Graber, A. L., Parle, D. P., and Williams, R. H.: Clinical use of diazoxide and studies of the mechanism of its hyperglycemia effects in man. Ann. N.Y. Acad. Sci. *150*:303, 1968.

[7] Ryan, W. G., Nibbe, A. F., and Schwartz, T. B.: Beta-cytotrophic effects of glucose, glucagon, and tolbutamide in man. Lancet *1*:1255-56, 1967.

[8] Hoffman, W. S.: Technicon AutoAnalyzer methodology N-2a. J. Biol. Chem. *120*:51, 1937.

[9] Herbert, V., Lau, K., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocr. *25*:1375, 1965.

[10] Goetz, F. C., Greenberg, B. Z., Ells, J., and Meinert, C.: A simple immunoassay for insulin: Application to human and dog plasma. J. Clin. Endocr. *23*:1237, 1963.

[11] Fajans, S. S.: Diagnostic tests for diabetes mellitus. *In* Diabetes. R. H. Williams (Ed.). New York, Hoeber, 1960, p. 389.

[12] Hayner, N. S., Kjelsberg, M. O., Epstein, F. H., and Francis, T.: Carbohydrate tolerance and diabetes in a total community, Tecumseh, Michigan. Diabetes *14*:413, 1965.

[13] Andres, R.: Relation of physiologic changes in aging to medical changes of disease in the aged. Ann. Intern. Med. *63*: 988-1002, 1965.

[14] Schwartz, T. B.: Who is a diabetic? Ann. Intern. Med. *69*:181, 1968.

[15] Welborn, T. A., Rubenstein, A. H., Haslam, R., and Fraser, R.: Normal insulin response to glucose. Lancet *1*:280, 1966.

[16] Rubenstein, A. H., Sooja, C., and Steiner, D. F.: Evidence for proinsulin in human urine and serum. Lancet *1*:1353, 1968.

[17] Joffe, B. I., Bank, S., Jackson, W. P. U., Keller, P., O'Reilly, I. G., and Vinik, A. I.: Insulin reserve in patients with chronic pancreatitis. Lancet *2*:890, 1968.

[18] Joffe, B. I., Vinik, A. I., and Jackson, W. P. U.: Insulin reserve in elderly subjects. Lancet *1*:1292, 1969.