# Effects of Tolbutamide and Insulin on Insulin Inhibitory Activity Associated with Plasma Albumin

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SUMMARY

**A reproducible pattern of change in insulin inhibitory activity associated with plasma albumin has been demonstrated. In six normal subjects, intravenous Na tolbutamide increased inhibitor activity to levels seen in many diabetics. Similar increases were seen in certain diabetic patients but not in all. Intravenous exogenous insulin also increased inhibitory activity. Since the latter could be demonstrated in the absence of hypoglycemia, the increase in inhibitor was probably related to the increase in insulin molecules. The time of inhibitor appearance suggests that it may be a product of insulin metabolism but other possibilities exist.** DIABETES *17*:746-51, December, 1968.

The physiopathologic significance of the insulin inhibitory activity associated with certain preparations of human albumin has been questioned as a result of (1) the inability of certain laboratories to demonstrate the inhibitor,[1-4] (2) suggestions that dialysis tubing used in the preparation of albumin may introduce an artifactual insulin inhibitor,[4-5] and (3) failure to demonstrate that preparations active in vitro have any insulin inhibitory activity in vivo.[6] Our laboratory and others[7-12] have reported confirmation of the basic observations of Vallance-Owen et al.[13] that albumin preparations obtained from diabetic subjects have quantitatively more insulin inhibitory activity than those obtained from normals, when tested at concentrations of 1.25 to 2.0 per cent in vitro. Furthermore, we have not been able to implicate a dialysis tubing artifact in any of our inhibitory preparations.[14] Similar failure to relate inhibitory activity to dialysis tubing has been reported by Dulin,[15] although he reports other insulin antagonistic artifacts which may be involved in the re-extractions of albumin preparations.

It seemed reasonable, therefore, to continue our studies of this inhibitor in an attempt to learn something of the "dynamics" of its behavior in patients. If indeed evidence could be obtained for a consistent pattern of response to a variety of physiologic stimuli, the possibility of ascribing a significant role for this inhibitor would be considerably enhanced.

Most reported studies concerning the insulin inhibitory activity of albumin describe the behavior of preparations obtained from fasting subjects. Jervell et al.[16] studied the response of insulin inhibitory activity to oral glucose loads in twenty normal subjects. They demonstrated a decreased activity thirty to sixty minutes after glucose. Our laboratory also reported a fall in insulin inhibitory activity three to five hours after an oral glucose load. In contrast, however, we noted suggestive increases in insulin inhibitory activity at the one to one-and-one-half-hour points.[17]

The purpose of this paper is to report studies of the response of insulin inhibitor activity associated with albumin to intravenous tolbutamide in normal and diabetic subjects. Significant *increases* in insulin inhibitory activity occurred concomitant with tolbutamide injection. A role for the insulin molecule per se in production of the inhibitor is supported.

## MATERIAL AND METHODS

*Experimental subjects:* Normal subjects, mildly diabetic subjects and one prediabetic subject were studied. Table 1 provides data on the age, sex, family history and body weight of the subjects. No patient was under treatment at the time of the study. All were receiving essentially normal diets with adequate carbohydrate

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TABLE 1

Data pertaining to subjects studied

| Diabetic subjects | Age | Sex | Family history of diabetes | Per cent DBW* | Per cent insulin inhibition by 2 per cent fasting albumin† | |
|---|---|---|---|---|---|---|
| LE | 40 | M | + | 80 | 37 | Tolbutamide studies |
| RC | 48 | M | − | 91 | 33 | |
| MC | 60 | F | − | 91 | 28 | |
| JB | 31 | F | + | 88 | 0 | |
| RM | 44 | M | − | 128 | 37‡ | |
| OE | 62 | F | + | 145 | 69‡ | |
| GZ | 59 | M | − | 134 | 15‡ | |
| PD | 44 | F | − | 98 | 17 | |
| RM§ | 62 | M | + | 118 | 49‡ | |
| RE | 62 | F | + | 80 | 45 | Glucagon studies |
| Normal subjects | | | | | | |
| RR | 32 | M | + | 107 | 23 | Tolbutamide studies |
| WW | 39 | M | + | 89 | 28 | |
| ON | 50 | M | − | 131 | 0 | |
| ET | 58 | M | − | 120 | 16 | |
| SM | 39 | F | + | 112 | 30 | |
| DW | 46 | F | − | 114 | 14 | |
| NV | 23 | F | + | 130 | 3 | Insulin studies |
| WK | 29 | M | − | 95 | 0 | |
| AS | 29 | F | − | 105 | 0 | |
| DO | 48 | M | − | 98 | 5 | |
| EL | 50 | M | − | 120 | 8 | |
| AS | 37 | F | + | 102 | 15 | Glucagon studies |
| JB | 45 | M | − | 100 | 5 | |

*Desirable body weight.

†P = .009 for the difference between the mean inhibitor activity in the ten diabetic subjects vs the thirteen normal subjects studied. (Determined by the standard error of the difference between means and calculation of k values.)

‡Failed to increase inhibitory activity following tolbutamide.

§Prediabetic—Patient's two children are diabetics.

intake prior to testing.

*Experimental design:* Oral glucose tolerance tests were performed (100 gm. glucose) and samples were obtained for blood glucose and plasma insulin levels at 0, ½, 1, 2 and 3 hrs. Following this study, but on a separate day, 1 gm. of sodium tolbutamide was given intravenously. Samples for glucose and insulin were obtained at 0, 20, 30, 60 and 90 min. Plasma for insulin inhibitor preparations was taken in the fasting state and 30, 60 or 90 and 120 min. following tolbutamide In addition, six normal subjects received 2 U. of glucagon-free exogenous insulin intravenously. Samples for glucose, insulin and insulin inhibitor activity were obtained at 0, 15 and 60 min. One normal subject received 50 gm. of glucose per os, followed ten minutes later by 2 U. insulin. The point just prior to insulin administration was considered the zero time.

Glucagon effects upon insulin, glucose and inhibitor were also studied in two normal and one diabetic subject. One milligram of glucagon was given intravenously with samples obtained at 0 and 30 min.

*Analytical procedures.* Blood glucose was measured by the Somogyi-Nelson procedure[18] and plasma insulin by the double antibody immunoassay of Morgan and Lazarow.[19] Albumin was prepared by the Debro trichloracetic acid-alcohol method previously described in detail.[20] Albumin was subsequently assayed for insulin inhibitory activity. The degree of inhibition of 1,000 μU. per ml. insulin produced by 2 per cent albumin in vitro with cut rat hemidiaphragms was expressed as the per cent of the insulin effect on glucose uptake noted in the paired buffer controls. The insulin effect in albumin was the difference between the effect of albumin in buffer vs albumin plus insulin. Samples of albumin were tested in triplicate and inhibition of greater than 30 per cent was considered significant.[7]

## RESULTS

*Response to oral glucose.* Oral glucose tolerance and insulin responses were measured in the two groups of subjects who subsequently received tolbutamide (table 1, figure 1). The diabetic subjects showed only mildly disordered blood sugar levels. The fasting insulin levels were the same in the two groups, but the insulin response of the diabetic subjects was significantly greater than that of the normal group and was characterized by a delayed insulin peak seen two hours after glucose. Calculation of the insulin/glucose ratios revealed that the diabetic subjects had consistently lower ratios than the normals. Table 1 indicates that the over-all body weights were comparable in the normal and diabetic subjects.

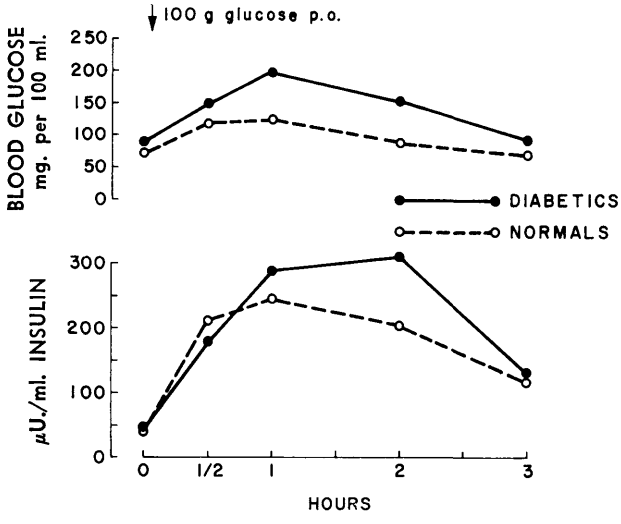


FIG. 1. Oral glucose tolerance performed in six normal subjects and nine diabetic subjects.

*Response to intravenous tolbutamide.* Figure 2 demonstrates the mean glucose and insulin response to tolbutamide in the subjects studied above. The diabetic responses were less than the normal in both parameters. Since the insulin response to tolbutamide is maximal within minutes of injection, the twenty-minute point permits comparison but does not represent the insulin peak. By sixty minutes, in both groups, the insulin level appears to have returned to fasting values. The blood sugar levels in the diabetic subject remained below the fasting levels for the two hours of observation, while in the normal subjects the levels tended to return more rapidly towards the fasting level. Insulin inhibitory activity measurements in the fasting state revealed differences between the two groups (table 1). The mean

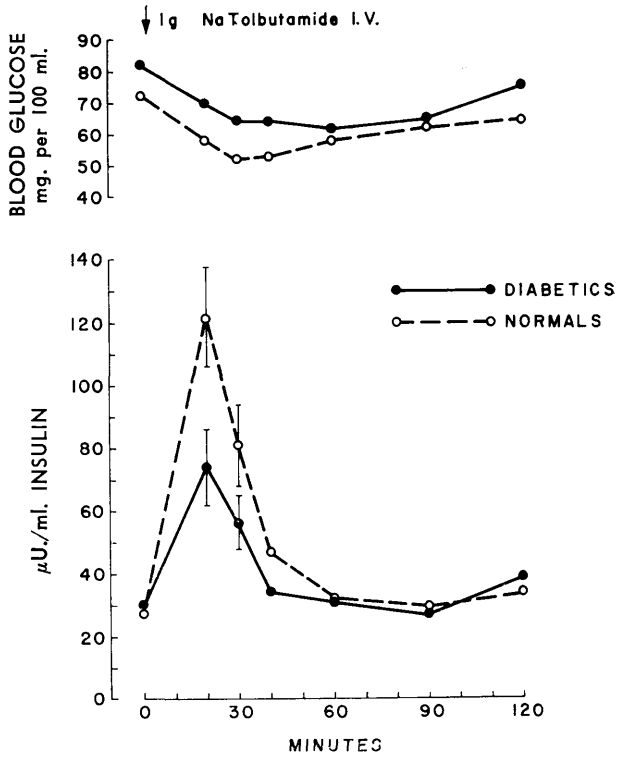


FIG. 2. The glucose and insulin responses to 1.0 gm. of sodium tolbutamide given intravenously in six normal and nine diabetic subjects. The standard error of the mean is shown in the insulin curves.

(± S.E.M.) inhibitory activity was 34 per cent ± 6 in the diabetics and 18 per cent ± 6 in the normals ($p = .06$). Serial determinations of insulin inhibitory activity of albumin obtained from normal subjects 30, 60 to 90 and 120 min. following tolbutamide revealed increased levels of insulin inhibitory activity (figure 3) compared with the pretolbutamide samples. However,

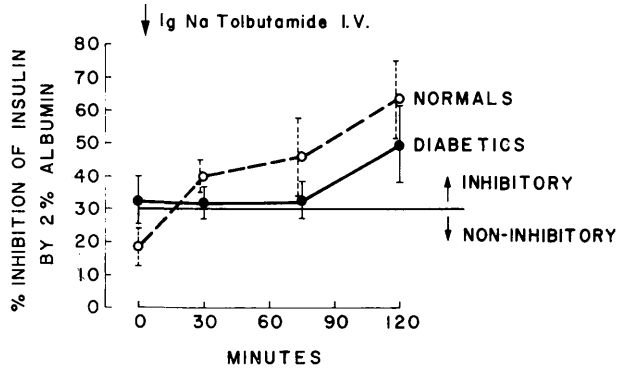


FIG. 3. The response of the insulin inhibitory activity associated with plasma albumin to tolbutamide. Note the increase in inhibitor in the normals. The standard error of the mean is shown.

the time of the peak increase varied amongst these subjects. All normal subjects had prompt increases at thirty or sixty minutes following tolbutamide ($p < .01$ by paired $t$ test). Although half of the diabetic subjects had similar increases in inhibitory activity, the others showed no increase following tolbutamide. Comparison of those diabetics who did not respond with those who did revealed that the nonresponders had a smaller insulin peak (56 uU./ml. vs 75 uU./ml. at 20 min.), a slightly smaller blood sugar response (lowest sugar levels of 57 mg. per 100 ml. vs 52 mg. per 100 ml.) and a higher fasting level of insulin inhibitory activity (table 1). These differences, however, were not statistically significant (S.E.M. and differences).

*Response to exogenous insulin.* Two units of Crystalline insulin were injected intravenously in six normal subjects. As expected, plasma insulin levels rose rapidly but returned to fasting levels by thirty minutes.[21] Blood glucose levels were lowest at twenty to thirty minutes and were normal by sixty minutes. Albumin inhibitory

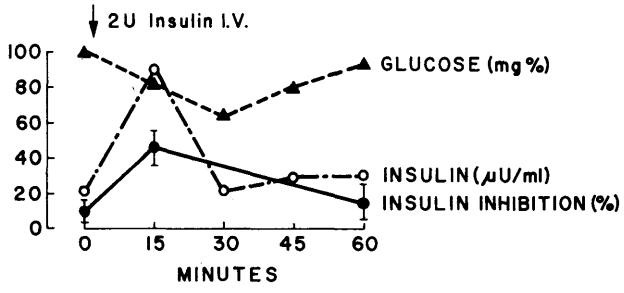


FIG. 4. The response to intravenous insulin observed in six normal subjects is shown. The increase in insulin inhibitory activity is demonstrated at the fifteen-minute point.

activity measured before, fifteen and sixteen minutes after insulin injection, showed the pattern seen in figure 4, namely a significant increase in inhibitory activity at fifteen mintues and a return towards normal by sixty minutes ($p < .01$ using paired data analysis).

*Response to insulin without hypoglycemia.* The preceding data indicated that increases in either endogenous or exogenous insulin resulted in increased levels of insulin inhibitory activity. Since hypoglycemia accompanied the increases in insulin levels, an attempt was made to produce hyperinsulinism without hypoglycemia by using either glucagon or insulin plus glucose.

Glucagon was administered to three subjects, two normals and one diabetic. One milligram intravenously produced short lived insulin rises within two minutes, followed by rises in blood sugar as previously reported.[22] Insulin inhibitory activity was measured before and thirty minutes after glucagon. There was no change in inhibitory activity in any of the three subjects (table 2).

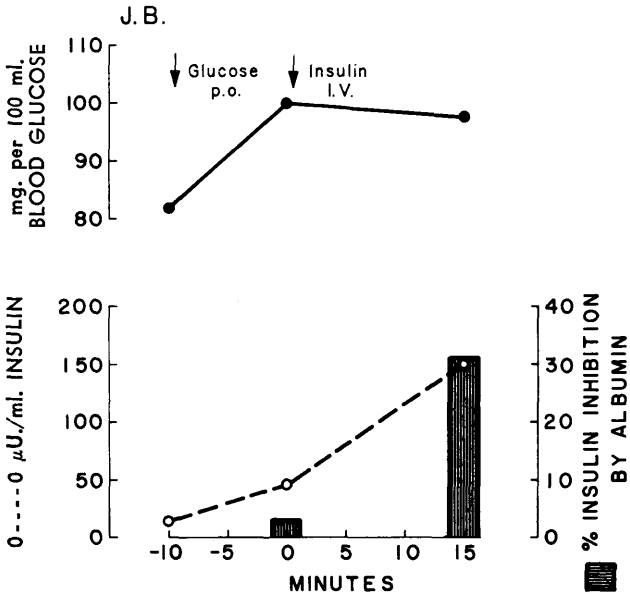


FIG. 5. The insulin inhibitor response of a single normal subject to 2 U. exogenous insulin. Fall in blood sugar was prevented by oral glucose. Note the increase in inhibitory activity.

TABLE 2

Response to glucagon

| Patient | Glucose mg. per 100 ml. Initial | Glucose mg. per 100 ml. Peak | Insulin (uU./ml.) Initial | Insulin (uU./ml.) 2-min. peak | Per cent insulin inhibition produced by albumin Initial | Per cent insulin inhibition produced by albumin 30-min. |
|---|---|---|---|---|---|---|
| R.E. | 140 | 200 | 25 | 70 | 45 | 44 |
| A.S. | 75 | 90 | 18 | 72 | 15 | 10 |
| J.B. | 82 | 94 | 10 | 50 | 5 | 8 |

On the other hand, in one normal subject (J.B.), 50 gm. of glucose given per os ten minutes before 2 U. of exogenous insulin did not prevent a rise in insulin inhibitory activity fifteen minutes after insulin (fig. 5).

## DISCUSSION

The data presented suggest that the insulin inhibitory activity associated with albumin is responsive to alterations in certain physiologic phenomena related to insulin. The administration of tolbutamide to normal subjects clearly resulted in an increase in insulin inhibitory activity, with levels quantatively greater than those obtained in many fasting diabetic subjects. Since tolbutamide increases *endogenous* insulin levels which presumptively are responsible for the depression in the blood sugar, it seems likely that these alterations might be responsible in some way for the increases in insulin inhibitory activity. This postulate appeared to be reinforced by the studies in which an increase in insulin inhibitory activity was also observed following administration of exogenous insulin to normal subjects. Plasma levels of insulin were still elevated fifteen minutes after exogenous insulin and thirty minutes after tolbutamide administration, the times when increased inhibitory activity was noted. However, elevations of inhibitory activity following tolbutamide persisted or increased despite return of plasma insulin to normal fasting levels. Often the maximal rise in inhibitory activity occurred 60 to 90 or 120 min. following tolbutamide. As a result, it seemed unlikely that the absolute level of plasma insulin per se was directly responsible for the level of inhibitory activity. Studies of fasting samples further support this point. In the fasting state, although plasma insulin levels were not different, a significant difference in insulin inhibitory activity was found when diabetics were compared with normals.

Further, no correlation between absolute level of insulin and inhibitor could be demonstrated in previous studies.[14]

In an effort to determine whether the increase in available insulin molecules or the hypoglycemia was responsible for the increment in insulin inhibitor, three subjects were injected with glucagon. Under these circumstances endogenous insulin levels rose and hyperglycemia rather than hypoglycemia obtained. Samples taken at thirty minutes revealed no increase in inhibitory activity in any subject. It should be pointed out that glucagon produced rises in insulin which were considerably smaller than those produced by tolbutamide or exogenous insulin. Samples of plasma insulin after 2 U. of insulin intravenously reveal levels at ten minutes which are essentially three to five times higher than those produced by glucagon. Further, with tolbutamide, the peak insulin response occurs within 1 to 2 min. and even at 20 min., the levels seen were higher than those with glucagon. Thus, the interpretation of the negative response of insulin inhibitor to glucagon is made difficult. The single observation, however, that exogenous insulin without hypoglycemia is accompanied at fifteen minutes by a rise in inhibitor activity *suggests* that the stimulus for inhibitor "production" is not hypoglycemia but insulin itself.

Clearly other factors must be involved in production of the inhibitor since endogenous hyperinsulinism as in obesity and hypoinsulinism as in juvenile diabetics do not respectively show positive and negative inhibitor levels.[4]

In essence then, it appears that increasing the circulating quantity of insulin molecules results in increases of insulin inhibitory activity persisting often an hour or more after the insulin level has returned to normal. A possible explanation for these findings is that the inhibitor may be a product of insulin degradation. Recent data presented by Roth[23] indicate that there are at least two molecular species of insulin in the plasma, namely, "big insulin" and "little insulin." These are immunoreactive insulins, but the former appears to have a larger molecular weight. It is of interest that this "big insulin" appears to be substantially increased at one to two hours following tolbutamide. The possibility exists that this "big insulin" is related in some way to the albumin associated insulin inhibitor.

The reason that four of nine diabetic subjects showed an essentially negative inhibitor response to tolbutamide may relate to their smaller insulin responses. However, the mechanism for their failure to respond is not entirely clear.

In summary, it appears that a reproducible pattern of insulin inhibitory activity can be produced in vivo. The mechanism may relate to the appearance of insulin degradation products following excessive levels of endogenous or exogenous insulin. At the present time there is no specific information to support or deny that the A or B chains of insulin or combinations of these are synonymous with this insulin inhibitory activity. On the other hand, it is possible that insulin may activate or alter the synthesis, degradation or release of the insulin inhibitor by some unknown mechanism which results in apparent increased levels of inhibitor. A role for growth hormone in this regard seems unlikely since

growth hormone levels are highest when inhibitor levels decrease three to five hours following oral glucose.[17]

ACKNOWLEDGMENT

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