Plasma Insulin Responses to Glucose and Tolbutamide of Normal Weight and Obese Diabetic and Nondiabetic Subjects

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SUMMARY

Plasma insulin responses to oral glucose (100 gm.) and intravenous tolbutamide (1.0 gm.) before and after dexamethasone were determined in normal, obese nondiabetic, normal-weight diabetic and obese diabetic subjects. Fasting plasma insulin levels were significantly higher in obese nondiabetic and obese diabetic subjects than in normal-weight nondiabetic and diabetic individuals. Total plasma insulin response to oral glucose in obese nondiabetics was threefold greater, in obese diabetics 2.2-fold greater, and in normal-weight diabetics 1.7-fold greater than that observed in normal individuals. When the plasma insulin responses of obese diabetic and nondiabetic subjects were measured at comparable blood sugar levels, insulin secretion was demonstrated to be significantly impaired in the diabetic group. The initial plasma insulin response (i.e., thirty-minute level) to glucose of diabetic subjects is markedly impaired when compared to normal-weight and obese nondiabetic individuals. Total plasma insulin response to intravenous tolbutamide was fourfold greater in obese nondiabetic and obese diabetic subjects than in normal-weight nondiabetic and diabetic individuals. Following dexamethasone (2 mg. q.i.d. x 2), plasma insulin response to intravenous tolbutamide was increased fivefold in normal subjects, threefold in obese nondiabetics, 1.6-fold in obese diabetics and twofold in nonobese diabetics.

The results of these studies indicate that obesity is associated with a hypersecretory insulin response to both tolbutamide and glucose. The presence of overt diabetes mellitus indicates an impairment in insulin response in both obese and nonobese individuals when compared to their appropriate nondiabetic controls. Dexamethasone provokes a marked compensatory insulin response in nondiabetics and makes more apparent the impaired secretory capacity of diabetic subjects. Maturity-onset diabetes in nonobese individuals appears to be a consequence primarily of insulin deficiency. In obese diabetic individuals, however, even though secretory capacity may be significantly greater than observed in nonobese diabetics, impaired carbohydrate tolerance develops because of insulin antagonism associated with obesity per se. DIABETES *15*:867-74, December, 1966.

Greater than normal plasma insulin levels in response to oral glucose have been reported in maturity-onset diabetes mellitus,[1,2] pregnancy,[3,4] hyperadrenocorticism,[5] hypersomatotropism,[6,8] carbohydrate induced hyperlipemia,[9,10] and obesity.[11,12] It has been suggested that the high levels of hormone observed in response to oral glucose in these conditions may reflect either a compensatory response of the pancreas to a state of insulin antagonism or a continuing stimulus to insulin secretion by hyperglycemia or a combination of these two factors.[13,15] If insulin antagonism does indeed lead to the development of diabetes mellitus, it is reasonable to expect that a hypersecretory insulin response would be observed early in the development of the disease either before it becomes clinically manifest or when it is still mild in form. Karam et al.[11] recently reported that nonobese adult diabetic individuals in contrast to obese maturity-onset diabetics do not exhibit an excessive insulin response to an oral glucose load. Furthermore, Kalkhoff et al.[4] observed that gestational diabetics did not demonstrate excessive plasma insulin responses to oral glucose when their carbohydrate tolerance was normal postpartum.

In the present study, the influence of obesity per se and the diabetic state on pancreatic insulin secreting capacity has been examined by determining the plasma insulin responses of normal weight and obese nondiabetic and diabetic subjects to oral glucose and intravenous tol-

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butamide. Additional observations were made on the effect of a superimposed diabetogenic stress, in the form of a synthetic glucocorticoid (i.e., dexamethasone), on the insulin secreting responses of these various groups of subjects.

MATERIALS AND METHODS

Subjects. The criteria used for classifying subjects on the basis of weight and carbohydrate tolerance are recorded in table 1. All subjects were in the postabsorptive state at the time tested and had been on diets estimated to contain in excess of 200 gm. of carbohydrate daily. None of the diabetic patients had ever received either insulin or oral hypoglycemic agents.

Procedures. Subjects in each group underwent two or more of the following studies: (1) a five-hour oral glucose tolerance test (100 gm.), (2) a 1.0-gm. intravenous tolbutamide tolerance test, and (3) a 1.0-gm. intravenous tolbutamide tolerance test following the ingestion of 2 mg. of dexamethasone four times a day x 2.

TABLE 1

Classification of patients on basis of weight and carbohydrate tolerance

Group	Number	Age	Per cent of ideal weight*	Blood sugar response to GTT: Fasting mg./100 ml.	One-hr. mg./100 ml.	Two-hr. mg./100 ml.
Normal	50	11-61	90-115	<90	<140	<110
Obese	12	14-62	138-412	<90	<140	<110
Obese diabetic	16	15-65	177-350	80-150	>170	>140
Nonobese diabetic	19	24-69	95-115	80-120	>170	>140

*Based on Metropolitan Life Insurance Tables, 1959.

Blood glucose was determined on the AutoAnalyzer by the potassium ferricyanide-potassium ferrocyanide method. Plasma insulin was assayed by the double antibody radioimmunoassay procedure of Morgan and Lazarow.[16] The immunoassay procedure used in this laboratory permits maximal precision between 10-100 μU. per milliliter and is sensitive to 1 μU. per milliliter plasma.

In order to examine the plasma insulin responses of diabetic and nondiabetic subjects at comparable blood glucose levels, 20 per cent glucose solutions were infused into an antecubital vein while monitoring the blood glucose level in a constant stream of blood introduced into an AutoAnalyzer from an indwelling venous catheter in the contralateral arm.[17-19] The lag between the time of aspiration into the indwelling venous catheter to completion of analysis was six minutes. The volume of blood aspirated was 0.18 ml. per minute. Glucose solutions were infused using a Harvard perfusion pump; rates of infusion were varied to reproduce a "standard diabetic glucose curve" which represented the mean oral glucose tolerance curve of our diabetic subjects.

To date methods have not been devised which permit direct measurement of pancreatic insulin secretion in man. In this study, the area circumscribed by the plasma insulin response curve has been used as an index for comparing relative quantitative changes of insulin secretion. The units used to express this function of insulin secretion are μU.-min. ml.$^{-1}$

RESULTS

Reproducibility of fasting plasma insulin levels and plasma insulin response to oral glucose. Experience in this laboratory has indicated that the fasting plasma insulin level of any given subject is relatively characteristic of that individual (table 2). Subjects of normal weight rarely show more than 8 μU. of insulin per milliliter, whereas obese subjects rarely show less than this level. Furthermore, the plasma insulin responses of thirteen normal individuals to oral glucose when repeated two or more times in a Clinical Research Center under controlled conditions (i.e., constant dietary intake, time of day) showed less than 20 per cent variation from the mean on two or more determinations. The responses of three such normal subjects are shown in figure 1.

Fasting plasma insulin levels in diabetic and nondiabetic subjects (table 3). The mean fasting plasma insulin level of obese nondiabetic and diabetic subjects is greater than three times that of normal individuals and twice that of normal-weight diabetics. In contrast, however, the fasting plasma insulin level of normal-weight diabetics was not significantly different from that of normal subjects.

Plasma insulin responses to oral glucose. The blood glucose and plasma insulin responses of fifty normal, twelve obese nondiabetic, nineteen normal-weight diabetic and sixteen obese diabetic subjects are shown in figure 2. Although the glucose tolerance curves of normal and obese nondiabetic subjects were not significantly different, the plasma insulin response of the obese group was threefold greater ($p < 0.001$) than that of normal individuals. Both diabetic groups in this study had com-

TABLE 2

Reproducibility of fasting plasma insulin levels drawn on different days in diabetic and nondiabetic subjects

Subject	Fasting plasma insulin levels (μU./ml.)			
Normal				
C.B.	<1	<1	1	
J.B.	3	5	2	<1
P.B.	2	2	1	3
R.B.	<1	<1	1	<1
M.F.	<1	<1	<1	3
P.S.	<1	<1	<1	
P.T.	7	8	<1	
W.Y.	<1	<1	<1	
Normal-weight diabetics				
D.G.	<1	<1	<1	
J.H.	2	2	<1	<1
H.W.	1	8	4	
Obese				
P.A.	10	8	8	7
M.S.	23	17	18	16
C.S.	31	9	16	26
Obese diabetic				
E.N.	13	15	11	

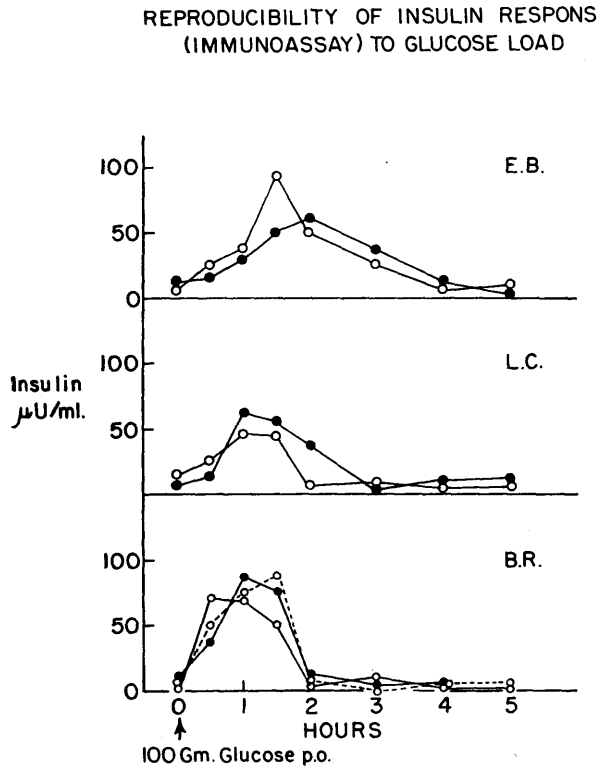

FIG. 1. Reproducibility in three subjects of plasma insulin responses during oral glucose tolerance tests repeated on different occasions.

TABLE 3

Fasting plasma insulin levels of normal-weight and obese nondiabetic and diabetic subjects

Group	Plasma insulin (μU./ml.)	Significance
Normal (50)*	4.4±0.8†	N vs O‡ $p<0.001$
Obese (12)	14.4±2.9	N vs ND n.s.§
Normal-weight diabetic (19)	7.3±1.5	N vs OD $p<0.01$
Obese diabetic (16)	15.2±4.2	O vs ND $p<0.01$
		O vs OD n.s.
		ND vs OD $p<0.05$

*Number of subjects
†Mean ± S.E.M.
‡N—Normal
O—Obese
ND—Normal-weight diabetic
OD—Obese diabetic
§n.s. = $p>0.1$

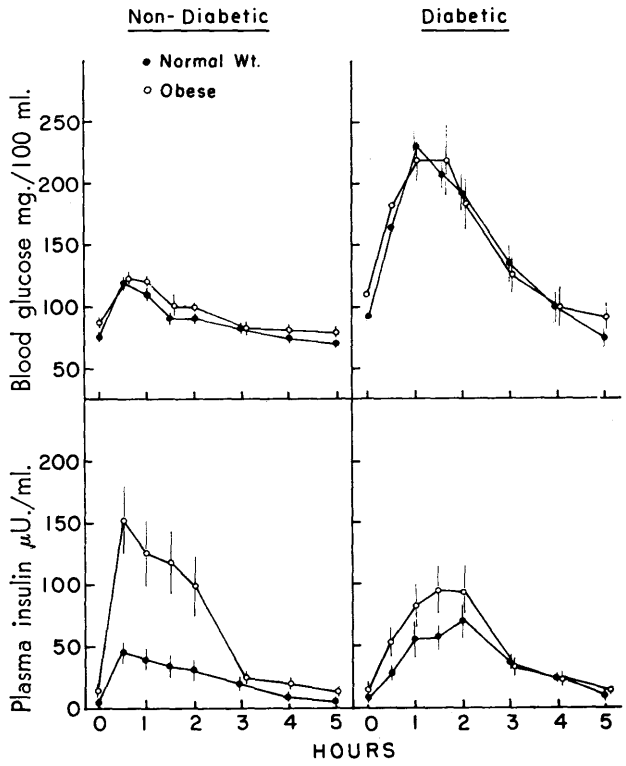

FIG. 2. Blood glucose and plasma insulin responses to oral glucose (100 gm.) in fifty normal, twelve obese, nineteen normal-weight diabetics and sixteen obese diabetic subjects. Values represent mean ± S.E.M.

parable degrees of carbohydrate intolerance, as measured by the oral glucose tolerance test, and secreted significantly greater amounts of insulin in response to oral glucose than normal individuals. Thus, the response of obese diabetics was 2.2-fold greater ($p < 0.01$) and that of normal-weight diabetics 1.7-fold greater ($p < 0.05$) than that seen in normal individuals. However,

both diabetic groups secreted significantly less than obese nondiabetic individuals. The plasma insulin responses of obese diabetic subjects were approximately 35 per cent greater than normal-weight diabetics.

The initial plasma insulin response of diabetic subjects was significantly delayed as compared with the brisk response typical of nondiabetic individuals of comparable weight. Thus, despite significantly greater blood glucose levels, the thirty-minute plasma insulin values of normal-weight and obese diabetics (27 ± 5 and 53 ± 13 μU./ml., respectively) were significantly less ($p < 0.01$) than those of normal and obese nondiabetic subjects (45 ± 9 and 152 ± 28 μU./ml., respectively).

Plasma insulin responses of obese diabetic and nondiabetic subjects at comparable blood sugar levels. Five obese nondiabetic subjects were infused with glucose in order to attain blood sugar levels comparable to those occurring in obese diabetic subjects during an oral glucose tolerance test (figure 3). The total plasma insulin response of obese nondiabetic individuals under these conditions was three times greater ($p < 0.001$) than that of the obese diabetic subjects.

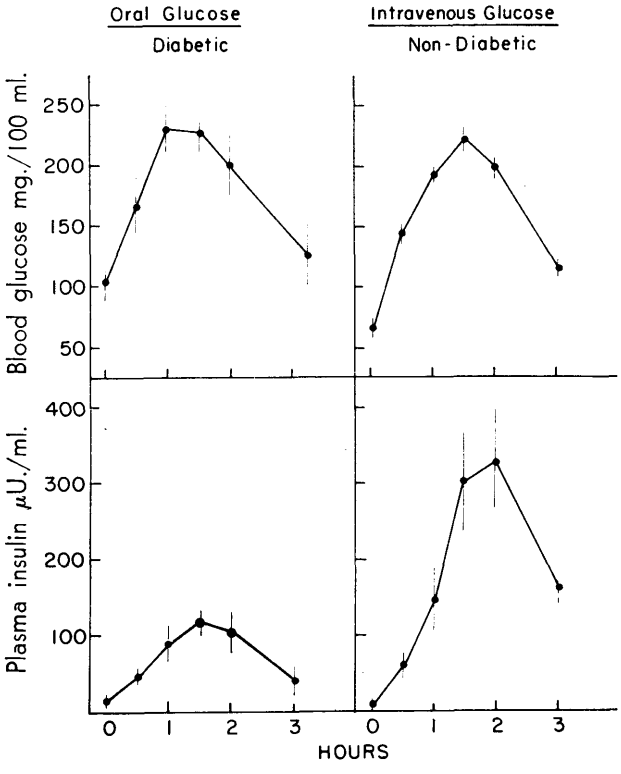

FIG. 3. Plasma insulin responses of twelve obese diabetic subjects to oral glucose and five obese nondiabetic subjects at comparable blood glucose levels maintained by glucose infusion. Values represent mean ± S.E.M.

Plasma insulin response to intravenous tolbutamide. The blood glucose and plasma insulin responses of thirty-two normal, fourteen normal-weight diabetic, eleven obese and eleven obese diabetic subjects to 1.0 gm. of tolbutamide intravenously are shown in figures 4 and 6. Obese nondiabetic subjects demonstrated a slightly impaired glucose response when compared to normal individuals despite a total plasma insulin response which was fourfold greater ($p < 0.001$) than that of the normal group.

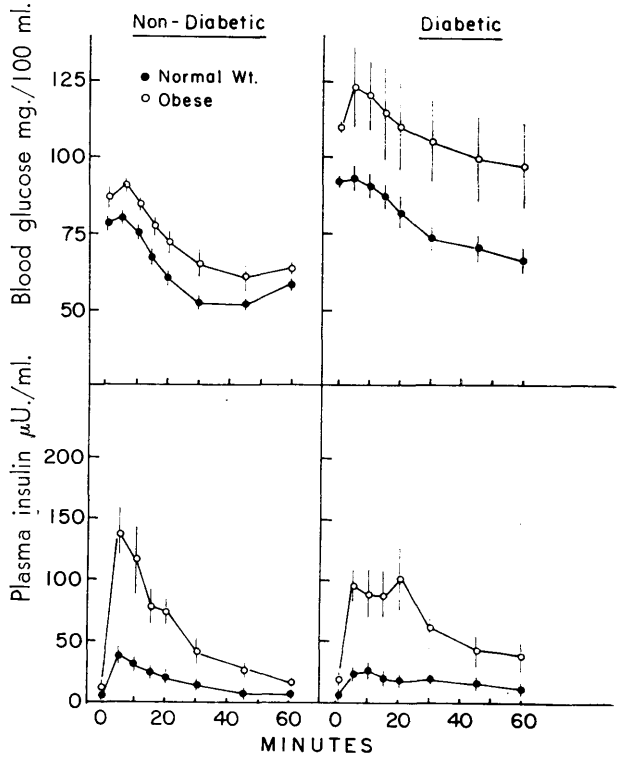

FIG. 4. Blood glucose and plasma insulin responses to intravenous tolbutamide in thirty-two normal, eleven obese, fourteen normal-weight diabetic and eleven obese diabetic subjects. Values represent mean ± S.E.M.

Tolbutamide-stimulated insulin secretion in obese diabetics did not differ significantly from that of obese nondiabetics but was fourfold greater ($p < 0.001$) than that observed in normal individuals. The initial plasma insulin response (five-minute) of the normal-weight diabetic group was significantly less ($p < 0.05$) than that of normal subjects although the total insulin secretory response over a sixty-minute period did not differ significantly. Correspondingly, the blood glucose response of normal-weight diabetics was only slightly abnormal. However, despite markedly elevated plasma insulin levels, the blood glucose response of obese

diabetic individuals to intravenous tolbutamide was severely impaired.

Effect of dexamethasone on the plasma insulin response to intravenous tolbutamide. The blood glucose and plasma insulin responses of twenty-seven normal, nine obese nondiabetic, fifteen normal-weight diabetic and ten obese diabetic subjects are shown in figures 5 and 6. Dexamethasone treatment resulted in a significant increase in the fasting blood glucose ($p < 0.001$) and fasting plasma insulin ($p < 0.001$) levels of normal and obese nondiabetic subjects. Steroid administration increased tolbutamide-stimulated insulin secretion fourfold in the normal group ($p < 0.001$) and threefold in obese nondiabetic subjects ($p < 0.001$). Obese individuals under these conditions still secreted insulin in excess of twice that seen in normal subjects ($p < 0.01$). In contrast to nondiabetic subjects, the fasting plasma insulin levels of both diabetic groups after steroid administration did not increase significantly ($p > 0.1$). Furthermore, the plasma insulin response to tolbutamide by diabetic subjects under these conditions did not exhibit the marked increase over baseline responses observed in the nondiabetic groups; the normal-weight diabetics attained less than a twofold increase ($p < 0.05$) and the obese diabetics a 1.6-fold ($p < 0.05$) increase. Furthermore, there was a marked increase in the fasting plasma glucose level and severe deterioration in carbohydrate tolerance in all diabetic subjects.

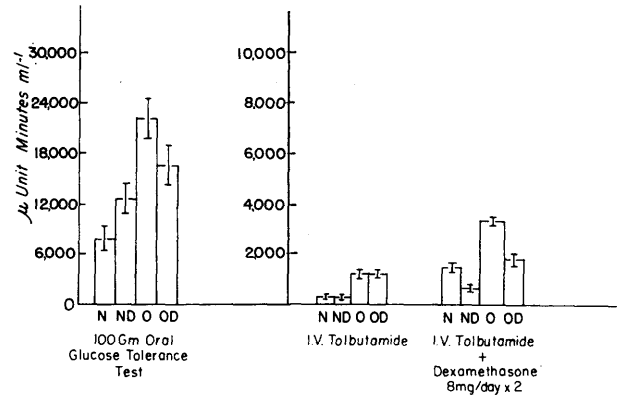

FIG. 6. Total plasma insulin responses to oral glucose and following intravenous tolbutamide before and after dexamethasone of normal-weight (N), obese nondiabetic (O), normal-weight diabetic (ND) and obese diabetic (OD) subjects. Values represent the integrated area circumscribed by the plasma insulin curve and are expressed as μU./min./ml. (mean ± S.E.M.).

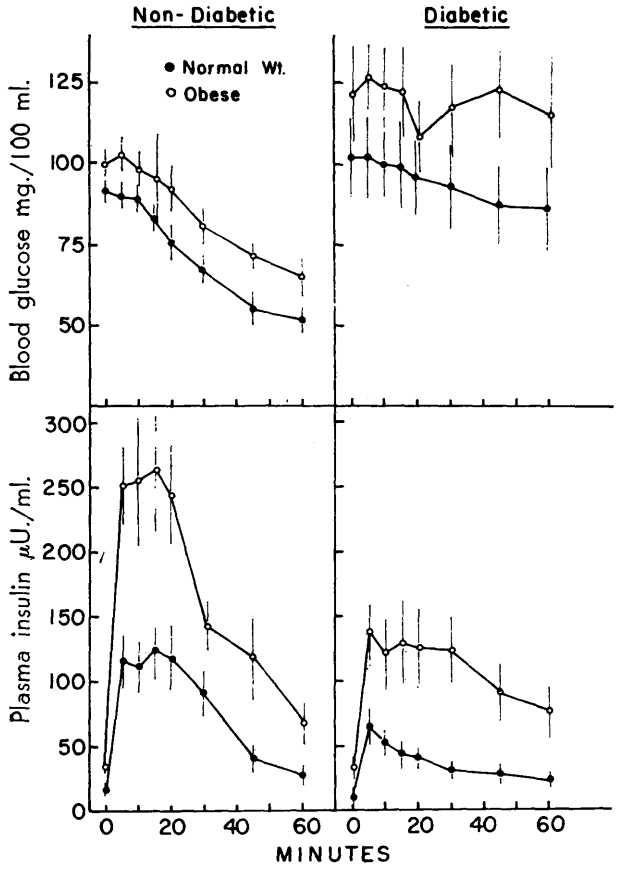

FIG. 5. Blood glucose and plasma insulin responses to intravenous tolbutamide of twenty-seven normal, nine obese, fifteen normal-weight diabetic and ten obese diabetic subjects following treatment with dexamethasone—2 mg. four times daily X 2. Values represent mean ± S.E.M.

DISCUSSION

A variety of conflicting results have been reported from several laboratories concerning the plasma insulin responses to both intravenous and oral glucose in diabetic patients. Berson and Yalow[1] had originally reported that diabetic subjects, as a group, have a greater total plasma insulin response to a standard oral glucose load (100 gm.) over a two-hour period than normal subjects. Karam et al.[11] later demonstrated that obese nondiabetic subjects responded to intravenous glucose with excessive plasma insulin levels and thereby raised the possibility that obesity per se can influence the plasma insulin response observed in the diabetic population which itself manifests a high incidence of obesity. Karam et al.[7] further reported that obese diabetics showed excessive insulin secretion in response to oral glucose, whereas nonobese diabetic individuals failed to hypersecrete. Recently, Yalow et al.[20] reported that "plasma insulin responses to glucose were somewhat higher than normal in obesity without diabetes" although

no statistical analysis of their results are given and the mean values for both groups were quite similar. Furthermore, in disagreement with the report of Karam, these authors claim that both obese and nonobese diabetic subjects exhibited a much greater than normal insulin response to oral glucose.

From the above studies, it is apparent that body weight influences the plasma insulin response to oral glucose. However, Karam et al.[7] failed to consider the effect of severity of the diabetic state in the selection of their patient groups. In their study, the degree of carbohydrate intolerance of the obese and nonobese populations was considerably different as measured by the standard oral glucose tolerance test. Thus, whereas the average fasting blood sugar of obese diabetic subjects was approximately 90 mg. per 100 ml., that of the nonobese diabetics was in excess of 150 mg. per 100 ml. Furthermore, during a glucose tolerance test only one of the eleven obese diabetic subjects had a blood glucose concentration in excess of 200 mg. per 100 ml., whereas only two of the fourteen nonobese diabetic subjects had blood glucose levels less than 200 mg. per 100 ml. In essence, therefore, the two groups of diabetics were not comparable in that one was far more diabetic than the other. One may question, therefore, the suggestion of these authors that the differential response of obese and nonobese diabetic subjects was correlated better with the associated obesity rather than differences in the severity of the diabetic state. Our results indicate, as did the recent report of Yalow et al.,[20] that the absolute plasma insulin levels seen in response to oral glucose of both normal-weight and obese diabetics is greater than that of normal subjects.

The elevated plasma insulin levels noted in mild maturity-onset diabetic subjects after oral glucose ingestion is subject to various interpretations. It may represent an excessive beta-cell response to a given stimulus reflecting a compensatory reaction to a state of insulin antagonism[11] or result from "continued stimulation by the persistent hyperglycemia" which in turn is secondary to impaired insulin sensitivity.[20] Clear-cut distinction between these alternative explanations can only be made when the added factor of different blood glucose levels in the various subjects studied has been excluded. As shown in figure 3, obese diabetic subjects, following oral glucose, secreted significantly less insulin than obese nondiabetic subjects maintained at comparable blood sugar levels by glucose infusion. Thus, by comparing the insulin secretory response of diabetic and nondiabetic subjects at similar blood glucose concentrations, it becomes evident that the response of diabetic subjects i impaired. The diminished secretory capacity of the dia betic group is even more evident when consideration i taken of the recent reports[21-24] that intravenously-ad ministered glucose is a less potent stimulus for pan creatic insulin release than orally administered glucose Additional studies in this laboratory further indi cate that nonobese diabetic subjects infused witl glucose to maintain a normal glucose tolerance curv also secrete significantly less insulin than norma control subjects.[23] Thus, although the total insuli secreted by subjects with mild maturity-onset diabete following oral glucose ingestion may be greater tha normal, the use of the terms "hypersecretory" or "ex cessive" to describe this phenomenon is misleading i that it implies a greater than normal response of th beta cell of the diabetic subject to a given insulinogeni stimulus. That such is not the case is also readily ap parent from the plasma insulin responses to intravenou tolbutamide noted in our subjects. Furthermore, whe the plasma insulin response is related to the correspond ing blood sugar level observed during the course of glucose tolerance test (figure 7), the diabetic respons is noted to be significantly delayed and not in exces of that seen in appropriate weight-control nondiabeti subjects.

An attempt was also made in this study not only t

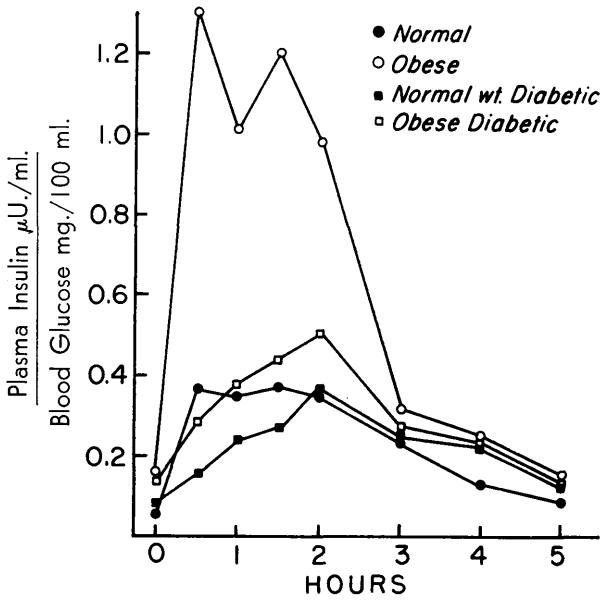

FIG. 7. Plasma insulin response expressed as the index—plasma insulin/blood sugar to oral glucose (100 gm.) of fifty normal, twelve obese, nineteen normal-weight diabetic and sixteen obese diabetic subjects.

clarify the influence of hyperglycemia on insulin secretion, but also to explore the interplay between the hypersecretory influence of obesity and the impaired insulin secretory capacity associated with diabetes. From the studies of Karam et al.[7,11] one may conclude that the biological effectiveness of endogenous insulin is impaired in the presence of obesity for greater concentrations of insulin are necessary to maintain normal blood sugar levels following a glucose load. Although the glucose tolerance curves of our obese and nonobese diabetic subjects indicated comparable degrees of carbohydrate intolerance, it should be noted that a 100-gm. glucose load is a significantly smaller test dose for the obese subjects when expressed in terms of grams of glucose per kilogram of metabolic mass. This would indicate that our obese diabetics had a greater degree of carbohydrate impairment than our nonobese diabetic subjects. Such proved to be the case with respect to the blood glucose response seen after intravenous tolbutamide. The obese diabetic subjects demonstrated a minimal blood glucose response (i.e., a maximum decrease of 13.5 per cent at 60 min.), whereas the nonobese diabetic subjects showed only a moderately impaired response (i.e., maximal decrease of 28 per cent at 60 min.).

There is now considerable evidence indicating that tolbutamide and glucose stimulate the pancreatic secretion of insulin via different mechanisms.[25-27] It is, therefore, not surprising that the plasma insulin responses to tolbutamide differed somewhat from those observed with glucose. For example, obese diabetic subjects secreted fourfold more insulin than normal-weight diabetic subjects in response to tolbutamide, but only 35 per cent more in response to glucose. Despite this excessive release of insulin, the impairment of the blood glucose response of the obese diabetic group in comparison to the normal-weight diabetic group suggests that the obese diabetic state is associated with a significant antagonism to the biological activity of insulin. Although the impaired blood glucose response to tolbutamide of nonobese diabetic subjects is consistent with some degree of hyporesponsiveness to endogenous hormone, it could also be completely attributed to the delayed pattern of insulin release observed in these individuals.

Tolbutamide tolerance tests performed after the administration of dexamethasone made more apparent the impairment of pancreatic insulin secretion characteristic of diabetic subjects. Following dexamethasone, normal subjects had a fourfold increase and obese nondiabetics a threefold increase in insulin secretion. Dexamethasone must have produced a severe degree of insulin antagonism in these subjects for despite these elevated levels of plasma insulin, the blood sugar response was slightly impaired. The compensatory response of both diabetic groups, however, was less than 30 per cent of that noted in either normal or obese nondiabetic individuals. Correspondingly, the blood sugar response to tolbutamide in diabetic subjects was more severely impaired by steroid treatment than in nondiabetic individuals. The demonstration of impaired pancreatic-islet function following a diabetogenic stimulus is consistent with the observations of Seltzer[28] who demonstrated that prolonged glucose infusions in normal and diabetic subjects eventually resulted in marked impairment of plasma insulin response in the diabetic group.

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Blood Lipids and Various Dietary Carbohydrates

Various aspects of the interrelationships among dietary fats and carbohydrates, blood lipids, and atherosclerotic cardiovascular disease have been discussed from time to time in *Nutrition Reviews* (*22*:301, 1964; *21*:228, 1963). Recently (*Nutrition Reviews* *23*:246, 1965), the reviewer noted that "work on the differential metabolic effects of various forms of carbohydrate becomes more intriguing with each additional research report." The appearance of two new papers in this area has not invalidated the observation.

R. E. Hodges and W. A. Krehl (*Amer. J. Clin. Nutrition* *17*:334, 1965) have presented a quite thorough review of the literature in the area of carbohydrate effects on lipid metabolism. Consideration of the complexities of this field suggests that more emphasis might have been given to the distinction between carbohydrate effects related to the calorie level of dietary carbohydrate as opposed to the exchange of various types of carbohydrate at a fixed calorie level. Adaptive changes to both kinds of manipulation have been reported. L. C. Fillios et al. (*Amer. J. Physiol.* *194*: 275, 1958) showed that the initial serum cholesterol response differential related to sucrose-starch substitutions in atherogenic diets in the rat had completely disappeared after twelve to seventeen weeks of feeding. Since A. Antonis and I. Bershon (*Lancet* *1*:3, 1961; *1*:998, 1960) have shown that the elevation in serum triglycerides associated with a change to a high carbohydrate-low fat type of diet gradually subsides to base levels after three to six months, a careful description of baseline as well as experimental diets and a consideration of the duration of experimental periods would seem to be necessary to meaningful interpretation of experimental results.

As an interesting addition to their contribution to the "Perspectives in Nutrition" pages, Hodges and Krehl have appended a section entitled "experimental studies." Data from a series of pilot studies, previously unpublished, are presented. Unfortunately the

(*Continued on page 888*)