Effect of Protein Meals on Plasma Insulin in Mildly Diabetic Patients

Stefan S. Fajans, M.D., John C. Floyd, Jr., M.D., Sumer Pek, M.D., Ralph F. Knopf, M.D., Mitchell Jacobson, M.D., and Jerome W. Conn, M.D., Ann Arbor

SUMMARY

We have reported that in mildly diabetic patients increases in plasma insulin are subnormal in response to infused amino acids while others have reported excessive increases in response to ingested protein. Increases in plasma insulin exhibited by nonobese, mildly diabetic patients in response to the ingestion of protein meals and to the intravenous administration of a mixture of ten essential amino acids were compared to those of healthy nonobese control subjects. The plasma insulin responses to protein meals were also determined in a group of mildly obese, mildly diabetic patients. The results of these studies indicate that: (1) the mean insulin response of nonobese mildly diabetic patients to protein meals is subnormal and not excessive, (2) mildly obese, mildly diabetic patients respond excessively to protein meals as compared to control subjects of normal weight, and (3) the excessive insulin response to protein meals reported by others to occur in diabetics is probably the result of a greater degree of adiposity in their diabetics than in their control subjects, rather than of the presence of diabetes mellitus. Unless the degree of adiposity is similar in diabetic and healthy subjects, meaningful comparison of their insulin responses to protein meals or other stimuli cannot be made. DIABETES *18*:523-28, August, 1969.

We have reported that in healthy subjects the intravenous administration of some individual essential amino acids as well as of mixtures of essential amino acids[2-6] and the ingestion of protein meals[4,6,7] induce the release of insulin. We have also reported that in nonobese, mildly diabetic patients, increases in plasma insulin are subnormal in response to the intravenous administration of amino acids,[6,8] as well as in response to the oral administration of glucose.[8] On the other hand, Berger and Vongaraya[9] have reported that in response to ingested protein mildly diabetic patients exhibit increases in plasma insulin which are higher than normal. Although their diabetic patients were matched for weight with nondiabetic control subjects their respective heights were not reported. The mean fasting plasma insulin of their diabetics (40 μU./ml.) was almost twice that of their control subjects (22 μU./ml.). This suggests that their diabetic patients had a greater degree of adiposity than their control subjects and that the hyper-responses of plasma insulin to protein meals of these diabetic subjects may have been related to that factor. Several investigators have reported that fasting levels of plasma insulin are elevated in obese subjects whether nondiabetic or mildly diabetic.[10-13] Increases in plasma insulin in response to a variety of stimuli to insulin release have also been shown to be exaggerated in obese nondiabetic as well as in obese diabetic subjects when compared to their respective nonobese groups. This has been demonstrated for glucose,[10-16] amino acids,[17] proteins,[17] tolbutamide,[12] and glucagon.[18,19]

In the present study, the increases in plasma insulin exhibited by nonobese mildly diabetic patients in response to the ingestion of protein meals and to the intravenous administration of a mixture of ten essential amino acids (10 AA) were compared to those of healthy nonobese control subjects. The plasma insulin responses to protein meals were also determined in a group of mildly obese, mildly diabetic subjects. The results of these studies indicate that: (1) the mean insulin response of nonobese, mildly diabetic patients

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From the Department of Internal Medicine (Division of Endocrinology and Metabolism and the Metabolism Research Unit), the University of Michigan, Ann Arbor, Michigan.

to protein meals is subnormal and not excessive, (2) mildly obese, mildly diabetic subjects respond excessively to protein meals as compared to control subjects of normal weight, and (3) excessive insulin responses to protein meals in diabetics reported by others are probably related to a greater degree of adiposity as compared to their control subjects, rather than to the presence of diabetes mellitus.

MATERIAL AND METHOD

Healthy individuals, eighteen to thirty-five years of age (mean 22.2), of normal body weight (80-112 per cent of desirable body weight*) served as control subjects. Forty-three of these subjects (forty-two males and one female) received intravenously a mixture of ten essential amino acids and twenty-four subjects (twenty males and four females) ingested a protein meal of ground beef. Nineteen of the subjects (eighteen males, one female) received both tests. Fourteen patients (ten males, four females) with mild maturity onset-type of diabetes, aged twenty to forty-five (mean 32.2) years and of normal body weight (90-102 per cent, mean 98 per cent of desirable body weight), received the infusion of amino acids and ten of these patients (nine males and one female) aged twenty to thirty-six (mean 30.2) years also ingested the protein meals. Two of the patients with diabetes had definite fasting hyperglycemia (149 and 177 mg./100 ml.). Three others had fasting blood sugar levels of 102, 111 and 114 mg./100 ml. Five of the fourteen patients with diabetes had been treated with diet alone and nine patients with diet and tolbutamide for periods of fifty-two to 120 months prior to testing. With follow-up of periods of up to six years we have demonstrated that the mean insulin response to glucose of mildly diabetic patients is unchanged by treatment with tolbutamide.[20] Tolbutamide had been discontinued for two to three days before studies, except in the case of two patients who received tolbutamide up to the day before infusion of the mixture of ten amino acids.

Ten obese, mildly diabetic patients, aged twenty to fifty-one (mean 38.1) years ingested the protein meals. Eight of the patients were males and two were females. They exceeded desirable body weight by 15-33 per cent (mean 22 per cent). Seven of these patients had been instructed in a reduction diet in the past. One patient had been treated with tolbutamide for a total of twenty-seven months.

*Metropolitan Life Insurance Tables.

Standard glucose tolerance tests (1.75 gm. glucose per kg. of ideal body weight), with determinations of serum insulin, were performed in thirty-one of the control subjects, on all of the nonobese and on nine of the obese mildly diabetic patients. Blood samples were obtained in the fasting state and at intervals of thirty minutes for a total of three hours.

The diabetic patients ingested diets containing 250-300 gm. of carbohydrate per day for at least three days prior to testing. The healthy young adults continued on their habitual ad libitum food intake which contained at least 250 gm. of carbohydrate per day. No food was ingested for at least nine hours prior to testing.

The mixture of ten essential amino acids was administered in amounts of 0.41 gm./kg. body weight with a maximum dosage of 30 gm. The composition of the ten amino acids mixture, as well as the method of preparation of this amino acid solution for intravenous administration, are described elsewhere.[5] Following a thirty-minute period of control measurements the solution was administered intravenously over the succeeding thirty minutes. Blood was obtained through a needle placed in a vein in the forearm. The needle was kept open by an occasional flush with a dilute solution of heparin. Blood was sampled during the control period (—30, —15, 0 minutes, as shown in the figures), every ten minutes during the first hour after the beginning of the infusion, and every fifteen minutes during the second hour.

Cooked, ground, lean beef, prepared as previously described,[7] was fed in amounts of 7 gm./kg. body weight but did not exceed a total of 500 gm. After a thirty-minute control period the subjects consumed their meals within fifteen to thirty minutes. Peripheral venous blood was sampled during the control period (—30, —15, 0 minutes) before ingestion of the meals. Thereafter samples were obtained at ten-minute intervals for the first hour and at fifteen-minute intervals for a total of four hours.

Levels of serum insulin were determined by the immunoassay technic of Morgan and Lazarow.[21] Increases in plasma insulin are expressed as maximal increases above control levels (the average of the —15 and 0 minute values). In addition, after the infusion of the amino acid mixture and after the ingestion of protein meals, increases in plasma insulin are also expressed in μU.•min./ml. for the entire period of the test. Increase in serum insulin (and blood glucose) during the glucose tolerance tests are also expressed as

the sum of increments over control levels for the first hour and for all six intervals of the test. Concentrations of blood glucose were determined by the Technicon AutoAnalyzer ferricyanide method and were frequently verified by the Somogyi-Nelson technic.[22]

RESULTS

The results of standard glucose tolerance tests performed in the three groups of subjects are given in figure 1. Blood glucose levels of the group of nonobese diabetic patients were significantly higher than for the control subjects in the fasting state and at all time intervals after the administration of glucose. From the magnitude of the standard errors obtained in the group of diabetic patients it can be seen that there was considerable range in the degree of their glucose intolerance. Mean increases in plasma insulin after the administration of glucose to the diabetic patients were significantly delayed and smaller than those obtained in the control subjects. Maximal increases in plasma insulin as well as the sum of increments were also significantly smaller for the nonobese diabetic patients than for the control subjects (table 1).

Blood glucose levels in the group of obese mildly diabetic patients were also significantly higher than for the control subjects at all time intervals during the glucose tolerance tests. Although blood glucose levels for the obese diabetics were somewhat lower than for the nonobese diabetics the differences between these two groups were not significant. Maximal increases in blood glucose and the sum of increments in blood glucose for the first hour or for the three-hour period of the tests were not significantly different for the two groups of diabetic subjects. However, for the obese diabetics fasting levels of serum insulin as well as increases in serum insulin after the administration of glucose were significantly greater than those both for the group of control subjects and for the nonobese diabetic subjects (figure 1, table 1).

Levels of plasma insulin reached during and after the infusion of the mixture of ten essential amino acids were considerably lower for the diabetic patients

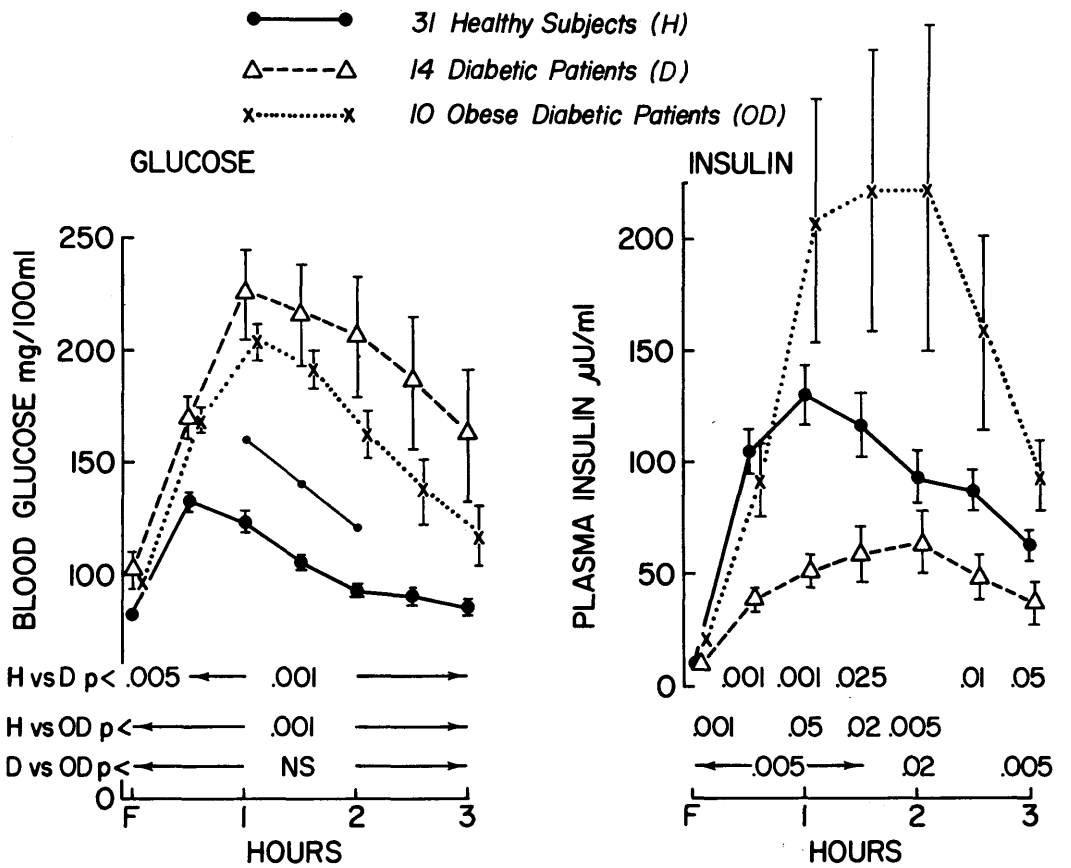

FIG. 1. Standard glucose tolerance tests on healthy subjects, nonobese diabetic patients and obese diabetic patients.

TABLE 1

Increases in serum insulin after oral glucose, intravenous amino acids and oral protein in healthy subjects, nonobese and obese diabetic patients

	Oral glucose			Intravenous amino acids		Oral protein	
Group	Maximum increases (μU./ml.)	Sum of increases first hour (μU./ml.)	Sum of increases 3 hours μU./ml.	Maximum increases (μU./ml.)	Increases μU.•min./ml. (0-120 min.)	Maximum increases (μU./ml.)	Increases μU.•min./ml. (0-210 min.)
Nonobese diabetic patients	62.9±12.5	70.9±11.7	239.4± 47.8	47.6±8.5	1,647±274	15.3±2.0	2,000± 349
p	<.005	<.001	<.001	<.001	<.001	<.005	<.05
Healthy subjects	143.7±15.4	213.7±20.3	537.5± 57.6	109.4±8.7	3,228±270	28.8±2.7	3,240± 315
p	<.05		<.05			<.001	<.001
Obese diabetic patients	244.8±68.0	260.8±64.8	895.3±236.6			59.2±8.8	8,204±1,360

than for the healthy control subjects (figure 2). Differences were statistically significant between five and forty minutes after the beginning of the infusion. When the increases in plasma insulin are expressed as either maximal increases or as increments μU.•min./ml. over a period of 120 minutes after the beginning of the infusion, the responses exhibited by the diabetic patients were significantly smaller than those of the healthy subjects (table 1). Both patients who were treated with diet alone as well as patients treated with diet and tolbutamide had lower responses than did the healthy subjects.

The effect upon plasma insulin of the ingestion of cooked ground beef for the nonobese diabetic patients and for the healthy control subjects is shown in figure 3. Mean increases in serum insulin were smaller for the diabetic patients than for the healthy subjects, the differences being significant at 20, 40, 50, 60 and 75 min. When the increases of plasma insulin after the ingestion of cooked ground beef are expressed as either

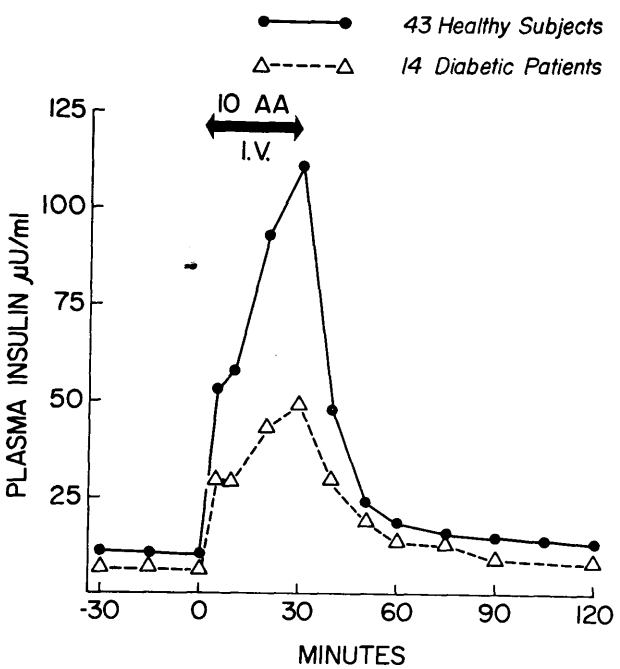

FIG. 2. Effect upon plasma insulin of the infusion of a mixture of ten essential amino acids. Increments of plasma insulin were significantly smaller for the nonobese diabetic patients than for the healthy subjects from five to forty minutes after the beginning of the infusions.

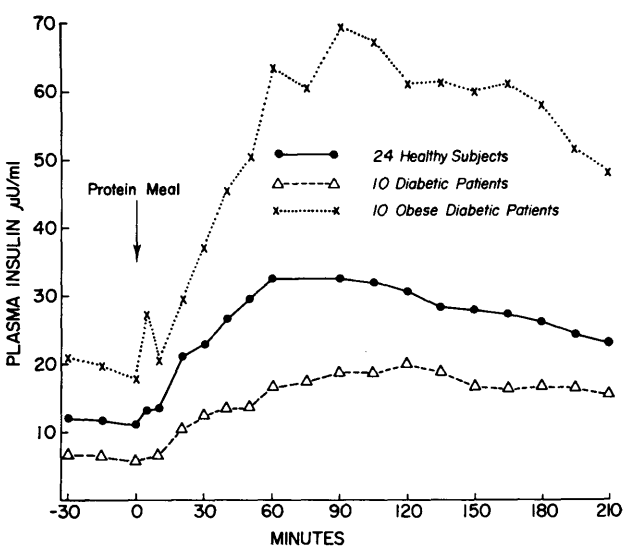

FIG. 3. Effect upon plasma insulin of the ingestion of cooked ground beef. Increments of plasma insulin were significantly smaller for the nonobese diabetic patients than for the healthy subjects at 20, 40, 50, 60 and 75 minutes after the beginning of the ingestion of the protein meals.

maximal increases or as increments μU.•min./ml. over a period of 210 minutes, the mean responses for the diabetics are again significantly lower than for the control subjects (table 1). The mean of maximal increases was 28.8 μU./ml. for the healthy subjects and 15.3 μU./ml. for the diabetics. Mean increments μU.•min./ml. were 3,240 and 2,000 respectively. Not a single patient exhibited a response which was above the range exhibited by the normal subjects.

On the other hand when the mildly obese, mildly diabetic patients ingested the protein meals, increases in plasma insulin were excessive as compared to those of healthy nonobese subjects (figure 3). Both maximal increases (59.2 μU./ml.) as well as increments over a period of 210 minutes (8,204 μU.•min./ml.) were significantly greater ($p < 0.001$) than for the control subjects (table 1).

DISCUSSION

As we and other investigators have reported before, the mean plasma insulin response of nonobese, mildly diabetic patients to infused and to ingested glucose[8,11,12,13] and to infused amino acids[6,8] is subnormal as compared to that of normal control subjects. Thus, the report by others[9] that mildly diabetic patients exhibit an excessive insulin response to ingested protein seems difficult to reconcile with these data. The results of the present studies indicate that the mean plasma insulin response of nonobese, mildly diabetic patients is also subnormal with respect to ingested protein when compared to that of healthy control subjects. Maximal increases in plasma insulin for the diabetic patients were approximately one half of those exhibited by control subjects whether the mixture of amino acids was infused (48 and 109 μU./ml., respectively) or the protein meals ingested (15.3 and 28.8 μU./ml., respectively) (table 1). The mean of increments in plasma insulin expressed as μU.•min./ml. for the diabetic subjects was 50 per cent of that of control subjects for the infusion of amino acids, while increments were 61 per cent of normal for the tests involving ingestion of protein meals. When individual results are considered, not a single of these nonobese diabetic patients had increases in plasma insulin, after the ingestion of protein meals, which were in excess of the range obtained in the healthy nonobese control subjects.

On the other hand, the mildly obese, mildly diabetic patients showed mean increases in plasma insulin greatly in excess of those obtained in healthy control subjects of normal body weight (figure 3, table 1). On the basis of these findings it seems likely that the excessive insulin response to protein meals in diabetics reported by others[9] is related to a greater degree of adiposity in their diabetics as compared to their control subjects, rather than to the presence of diabetes mellitus. This conclusion is supported by the fact that the mean fasting level of plasma insulin of these diabetics was almost twice that of their control subjects.* It is generally accepted[10,12,13] that obesity, rather than diabetes, is responsible for elevation of fasting levels of plasma insulin in obese, diabetic subjects. Although it has been reported repeatedly that moderately or severely obese subjects exhibit an excessive insulin response to a variety of stimuli to insulin release, it needs to be emphasized that the same phenomenon is observed in mildly obese patients. Unless the degree of adiposity is similar in diabetic and healthy subjects, meaningful comparison of their insulin responses to protein meals or other stimuli cannot be made.

Others[23] have concluded that alimentary factors might be involved in the "exaggerated rise in plasma insulin in response to protein meals in subjects with mild maturity-onset type of diabetes mellitus,[9] which contrasts with the grossly impaired effect of intravenous infusions of amino acids on insulin secretion in this disease."[24] While alimentary factors might be involved in the insulin response to ingested protein the above conclusion of Dupré et al.[23] cannot be accepted either on the basis of the premise that mild diabetes is characterized by an excessive insulin response to protein meals, or on the basis of the comparisons which they have made. The two groups of diabetic patients which they compared differed greatly in regard to severity of carbohydrate abnormality. The five patients of Merrimee et al. who had a mean fasting blood sugar level of 276 mg./100 ml.[24] undoubtedly had considerably smaller pancreatic insulin reserves than the patients of Berger who had a mean fasting plasma sugar level of 115 mg./100 ml.[9] If these two groups of patients had been given glucose, amino acids or protein, each by the same route, great differences in their respective insulin responses would have been expected.

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*Recently Berger has stated (personal communication) that the weight of his diabetic patients exceeded ideal body weight and that of his control subjects by a mean of 21 per cent. Thus, Berger's diabetic patients exceeded ideal body weight to a degree similar to our mildly obese, mildly diabetic patients.

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