Insulin Response to Ingested Protein in Diabetes

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SUMMARY

The ingestion of protein by normal human subjects has been shown recently to represent a stimulus to the secretion of insulin. This response is probably related to the postprandial rise in plasma amino acid concentration.

Since disordered protein synthesis may contribute to the pathogenesis of diabetes and its complications, the insulin response to ingested protein (casein or gelatin, 50-100 gm.) was compared in twenty-eight normals and ten maturity-onset diabetics. Insulin response (microunits-minutes, μU.-min.) was considered to be the area included by that portion of the plasma insulin response curve above projected basal insulin secretion. Following protein ingestion the diabetics showed a mean insulin response (± S.E.M.) of 3,425 ± 367 uU.-min., compared with 1,005 ± 136 in the normals, a 3.4-fold difference ($p < 0.01$). In neither group were there significant or consistent changes in plasma glucose concentration. The fall in free fatty acids was comparable and no difference was seen in the plasma amino acid nitrogen curves.

These data indicate that the insulin response to ingested protein is excessive in diabetes. The mechanism of the hyperresponse would seem to lie within the beta cell since no abnormality of the presumed amino acid stimulus was demonstrated. DIABETES *15*:303-06, May, 1966.

Ingestion of various proteins by normal human subjects has been shown recently to represent a physiological stimulus to insulin secretion.[1,2] Since no significant changes in plasma glucose concentration were seen following these protein meals, the rise in plasma amino acid concentration was considered to be the insulin secretory stimulus. Because insulin acts within the cell to augment protein synthesis,[3] a relationship between aminoacidemia and insulin secretion is easily understood.

Since disordered protein synthesis may contribute to the pathophysiology of diabetes and its complications, the insulin response to ingested protein was compared in normal subjects and in diabetics with responsive islets.

Presented at the Twenty-fifth Annual Meeting of the American Diabetes Association in New York City, on June 20, 1965.

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SUBJECTS, RESEARCH PLAN, AND METHODS

Twenty-eight normal subjects and ten diabetics were studied. The diabetics were of the maturity-onset type, and they had received neither insulin nor the various oral hypoglycemic agents prior to the study. The groups were matched for weight in view of several recent reports of hyperinsulinism in obese but nondiabetic subjects.[4,5] Body weight (mean ± S.D.) was 170 ± 27 lbs. in the normal group and 163 ± 30 in the diabetics.

Prior to examining the effects of protein ingestion we chose to establish whether the groups responded characteristically to a well-known standard challenge, that is 100 gm. of glucose by mouth. Since plasma insulin[6] and free fatty acid responses[7] to ingested glucose have been defined in normal subjects and in maturity-onset diabetics, these parameters were assessed.

Protein meals consisted of casein or gelatin, in amounts of 50 to 100 gm. Since there was no significant difference between proteins, or between amounts, results have been pooled and hereafter considered "response to protein."

Three hundred grams or more of carbohydrate were ingested by each subject for three or more days prior to each study. Plasma glucose, insulin, free fatty acids (FFA), and amino acid nitrogen (AAN) were determined fasting and half-hourly for two hours. All analyses were performed on heparinized venous plasma. The plasma was separated immediately after venipuncture and stored at —20° C. until the time of analysis. Glucose concentration was determined by a standard glucose oxidase method,* insulin by the two-antibody immunoassay technic of Morgan and Lazarow,[8] free fatty acids by a modification of the method of Dole and Meinertz,[9] and amino acid nitrogen according to Hawk et al.[10]

Insulin response was considered to be the area included by that portion of the insulin response curve above projected basal insulin secretion. This was calculated as the product of the mean increment in insulin concentration above the fasting level and time. Plasma insulin content was derived in microunits, and time in minutes; insulin response, as recently used by Perley

*Glucostat, Worthington Biochemical Corporation, Freehold, New Jersey.

and Kipnis,[11] was therefore expressed as microunits-minutes (μU.-min.).

RESULTS

Response to glucose

Figure 1 compares the insulin response to glucose in the two groups, and several differences are seen. First, the fasting plasma insulin concentration (mean ± S.E.M.) was significantly higher in the diabetic group: 41.2 ± 2.7 versus 24.9 ± 1.3 μU./ml. ($p < 0.01$). Second, the insulin response shown by the diabetics was more sustained. These observations are quite characteristic of the maturity-onset diabetic.[6] The abnormally sustained plasma insulin response to glucose loading in diabetes generally parallels the plasma glucose response.[6] This relationship in our subjects is shown in figure 2 and it represents the basis for ascribing the abnormally sustained insulin response to glucose to abnormal persistence of the hyperglycemic stimulus.[6] The net insulin response (mean ± S.E.M.) for the two-hour period was not significantly different: 5,978 ± 1,164 uU.-min. in the diabetics compared with 6,104 ± 583 in the normals ($p = 0.9$). The plasma FFA responses are shown in figure 3 and the maximum fall of 46 per cent at 1.5 hrs. in the normal group and 53 per cent at 2 hrs. in the diabetics is similar to that reported by Kipnis and Stein.[7]

The groups, then, responded to glucose in characteristic fashion.

Response to protein

The insulin response to ingested protein is shown in figure 4. The diabetics' response (mean ± S.E.M.) was 3,425 ± 367 uU.-min. compared with 1,005 ± 137 in the normals, a 3.4-fold difference ($p < 0.01$). When these results are compared with the corresponding insulin responses to glucose the ratio of the protein:glucose responses (mean ± S.E.M.) was 0.21 ± 0.04 in the normals and 0.80 ± 0.17 in the diabetics ($p < 0.01$). Whereas only three of twenty-eight normals responded to protein with over 40 per cent of their insulin response to glucose, this was seen in eight of the ten diabetics. In spite of the greatly different insulin responses to protein, however, the fall in free fatty acids was surprisingly comparable (figure 5). A mean fall of approximately 30 per cent was seen in both groups at two hours. Although glucose levels were quite different, in neither group were there significant or consistent changes in plasma glucose concentration following the protein meals (figure 6). The amino acid nitrogen curves were virtually identical (figure 7).

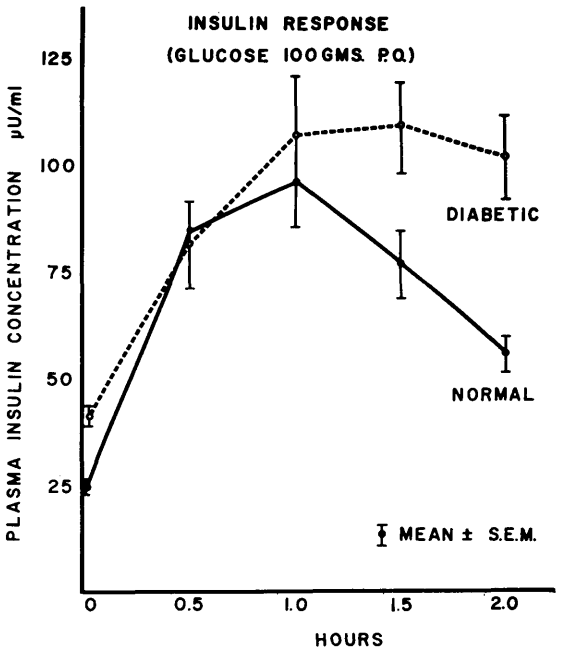

FIG. 1. Plasma insulin concentrations during standard oral glucose tolerance tests in twenty-eight normal subjects and ten maturity-onset diabetics.

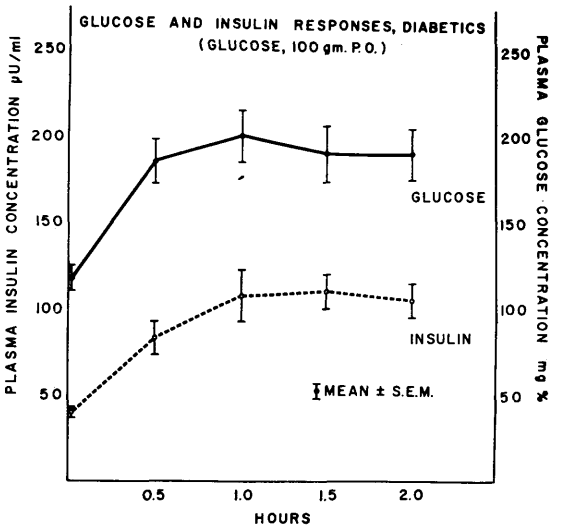

FIG. 2. Plasma glucose and insulin concentrations during standard oral glucose tolerance tests in ten maturity-onset diabetics.

DISCUSSION

These data indicate that the insulin response to ingested protein is excessive in diabetes. The difference in the protein:glucose insulin response ratios can be wholly explained in terms of the increased response to protein seen in the diabetic group.

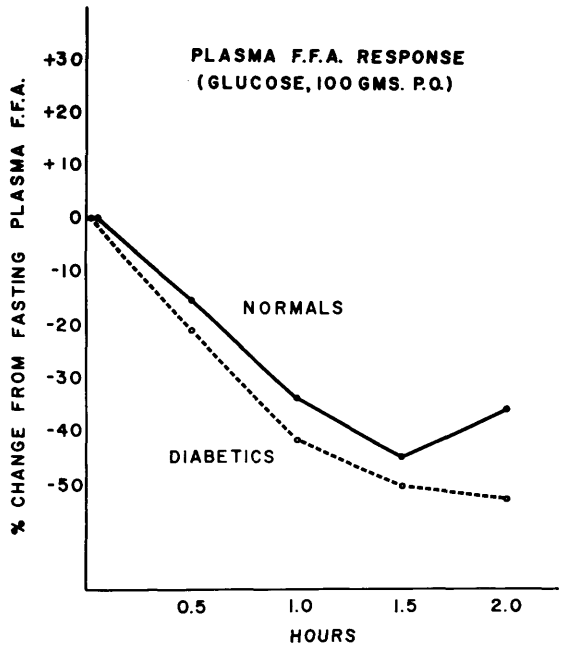

FIG. 3. Plasma free fatty acid changes during standard oral glucose tolerance tests in six normal subjects and ten maturity-onset diabetics.

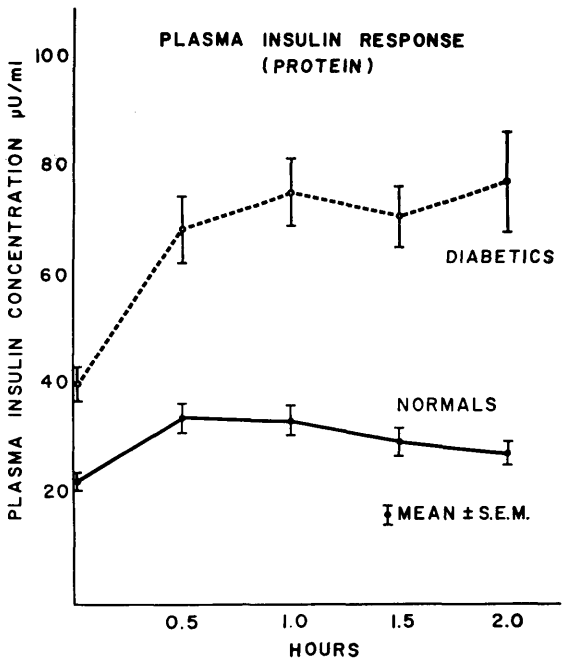

FIG. 4. Plasma insulin concentrations during protein meals in twenty-eight normal subjects and ten maturity-onset diabetics.

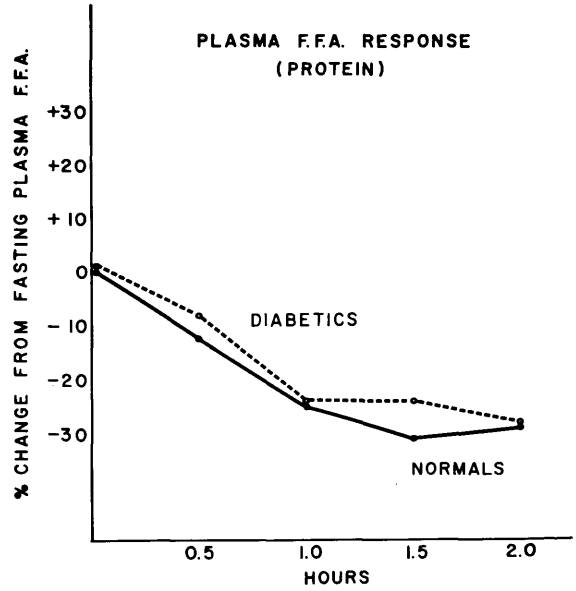

FIG. 5. Plasma free fatty acid changes during protein meals in eleven normal subjects and ten maturity-onset diabetics.

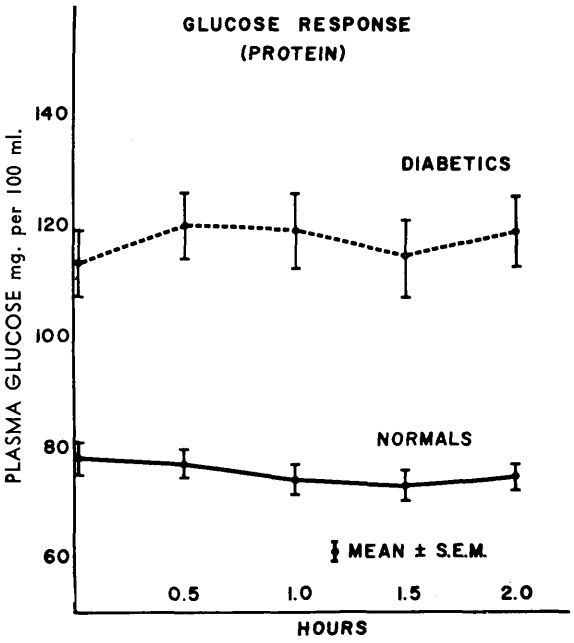

FIG. 6. Plasma glucose concentrations during protein meals in twenty-eight normal subjects and ten maturity-onset diabetics.

The mechanism underlying this observation requires some consideration. Although the insulin response to ingested protein is perhaps related in some way to the rise in plasma amino acid concentration which follows a protein meal, it is of interest that the AAN plot and insulin response are not parallel. Amino acids generally continue to rise for two hours whereas the peak insulin concentration occurs at thirty minutes. If, however, any portion of the amino acid nitrogen curve reflects the

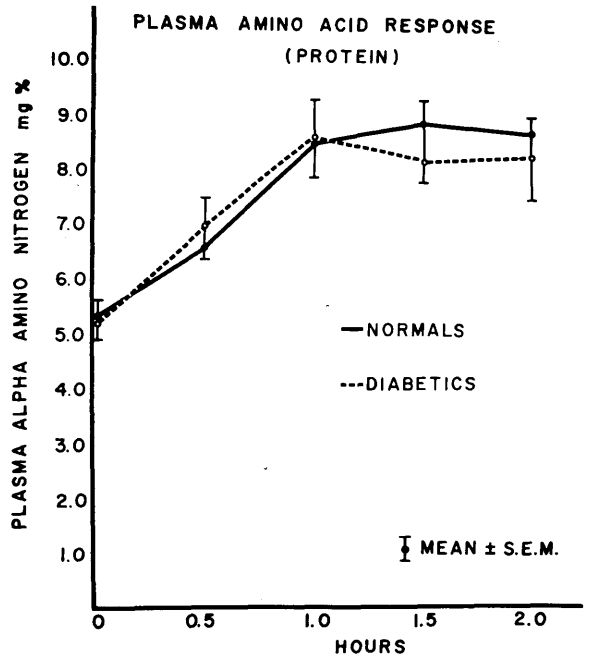

FIG. 7. Plasma amino acid nitrogen concentrations during protein meals in twenty-eight normal subjects and ten maturity-onset diabetics.

insulin secretory stimulus provided by protein ingestion, since the curves were virtually superimposable in the two groups studied, the mechanism of the hyperresponse seen in the diabetic group would seem to involve the beta cell primarily rather than the insulin secretory stimulus. Studies are in progress designed to test whether chronic "hyperglycemic priming" of normal islets may induce an excessive insulin response to ingested protein since the plasma glucose concentrations were significantly different in the two groups at the time that the protein meals were administered (figure 6).

A possible connection between the excessive insulin response to ingested protein in diabetics and excessive synthesis of protein by insulin-responsive tissues is evident but data bearing directly on the sensitivity of human diabetics to the protein-anabolic action of insulin is lacking.

The similar fall in plasma FFA following protein meals in the two groups, in spite of the very different insulin responses, requires comment. Since a much greater depression in plasma FFA levels was seen after glucose loading, the responses to protein cannot be considered maximal. The observed data would support the observations of Randle and co-workers[12] concer resistance to the free fatty acid-suppressing actio insulin in maturity-onset diabetics.

ACKNOWLEDGMENT

These studies were supported in part by rese grant AM-09370 from the National Institutes Arthritis and Metabolic Diseases, National Insti of Health, Bethesda, Maryland.

The authors are indebted to Mrs. Betty Rachm Mrs. Betty Rutschmann, Miss Kathy Hill, and Barbara Sabin for technical assistance in various pl of the work.

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