Effect of Protein Ingestion on the Glucose and Insulin Response to a Standardized Oral Glucose Load

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Type II diabetic subjects were given 50 g protein, 50 g glucose, or 50 g glucose with 50 g protein as a single meal in random sequence. The plasma glucose and insulin response was determined over the subsequent 5 h. The plasma glucose area above the baseline following a glucose meal was reduced 34% when protein was given with the glucose. When protein was given alone, the glucose concentration remained stable for 2 h and then declined. The insulin area following glucose was only modestly greater than with a protein meal (97 ± 35, 83 ± 19 μU · h/ml, respectively). When glucose was given with protein, the mean insulin area was considerably greater than when glucose or protein was given alone (247 ± 33 μU · h/ml). When various amounts of protein were given with 50 g glucose, the insulin area response was essentially first order. Subsequently, subjects were given 50 g glucose or 50 g glucose with 50 g protein as two meals 4 h apart in random sequence. The insulin areas were not significantly different for each meal but were higher when protein + glucose was given. After the second glucose meal the plasma glucose area was 33% less than after the first meal. Following the second glucose + protein meal the plasma glucose area was markedly reduced, being only 7% as large as after the first meal. These data indicate that protein given with glucose will increase insulin secretion and reduce the plasma glucose rise in at least some type II diabetic persons. DIABETES CARE 1984; 7:465–70.

It is well known that protein ingestion or the administration of amino acids orally or intravenously will stimulate insulin secretion in normal or mildly diabetic subjects.[1–5] In normal subjects we have previously demonstrated that a diet composed of 40% of the food energy in the form of protein and 20% in the form of carbohydrate results in a clear increase in circulating insulin concentration after each meal. This occurred in the absence of a significant rise in glucose concentration after the second and third meals of the day.[6] This study suggested that a moderately high protein diet might be useful in the treatment of type II diabetic patients. To test this hypothesis, we have determined the plasma glucose and insulin response to a standard glucose meal in the absence and the presence of varying amounts of protein in mild type II diabetic subjects.

SUBJECTS AND METHODS

Nine male, untreated diabetic subjects were studied in a metabolic unit. All patients met the National Diabetes Data Group criteria[7] for the diagnosis of type II diabetes. The mean age was 61 ± 12 yr with a range of 38–74 yr. The mean percent of desirable body weight was 123 ± 23% using the 1959 Metropolitan Life Insurance Co. tables for persons of medium frame. All subjects signed an informed consent and the study was approved by the hospital committee on human subjects. All participants were on diets consisting of at least 200 g of carbohydrate/day with adequate food energy for 3 days before testing. None of the subjects had received treatment with either oral hypoglycemic agents or insulin previously. After an overnight fast of 8–10 h, an indwelling catheter was inserted into an antecubital vein and kept patent with small amounts of heparin.

The plasma glucose was determined by a glucose-oxidase method using a Beckman glucose analyzer (Beckman Instruments, Fullerton, California). Serum immunoreactive insulin (IRI) was measured in duplicate by a standard radioimmunoassay method[8] using a kit supplied by Pharmacia Laboratories (Piscatway, New Jersey). The glucose and insulin areas above the fasting baseline were determined by planimetry. Areas below the baseline were subtracted from areas above the baseline to give a net area. The fat content of the ham-

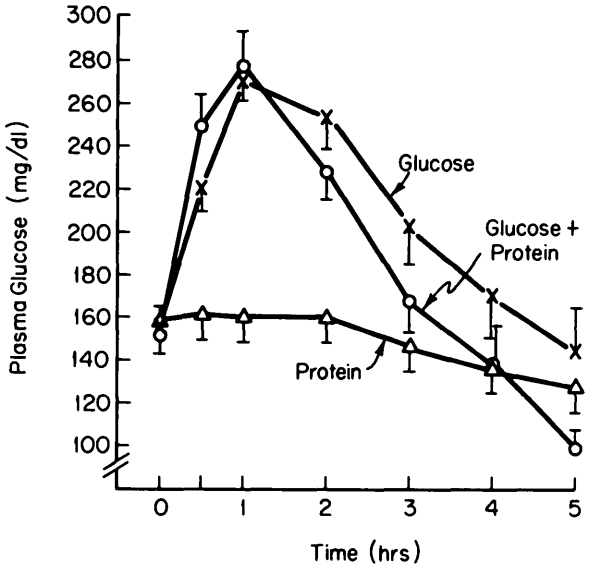

FIG. 1. Plasma glucose response to ingestion of 50 g glucose, 50 g protein, or a combination of 50 g glucose and 50 g protein. Seven male, untreated diabetic subjects were studied.

burger was determined by gravimetric analysis of several ether extractions of the meat. Student's *t*-test for paired variates was used for analysis of statistical significance. Data are presented as mean ± standard error of the mean.

The following three studies were done:

I. Seven type II diabetic subjects were given 50 g of glucose (Glutol, Paddock Laboratories, Minneapolis, Minnesota) or 50 g of protein or a combination of 50 g of glucose with 50 g of protein over 3 consecutive days in a random order. In all studies, protein was given as well-cooked very lean hamburger (236 g raw wt). Blood for glucose and insulin measurements was drawn at 0, ½, 1, 2, 3, 4, and 5 h after the ingestion of the test meal.

II. In five type II diabetic subjects the effect of adding 10, 30, and 50 g protein to a standard 50-g glucose dose was studied. Protein was given as well-cooked very lean (6.5% fat) hamburger (47, 142, and 236 g raw wt, respectively). Blood samples for glucose and insulin were collected at the time intervals indicated above.

III. Five type II diabetic subjects were given either two doses of 50 g of glucose 4 h apart or 50 g glucose with 50 g protein 4 h apart. Blood for glucose and insulin measurements was drawn before and ½, 1, 2, 3, 4, 5, 6, 7, and 8 h after the ingestion of the first test meal.

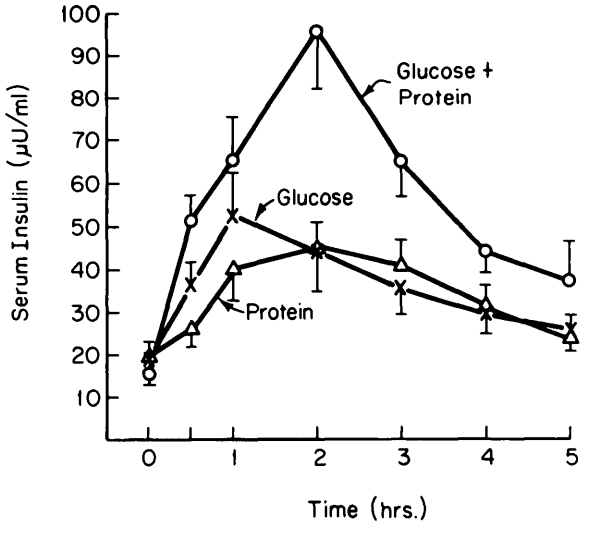

FIG. 2. Serum insulin response to ingestion of 50 g glucose, 50 g protein, or a combination of 50 g glucose and 50 g protein. The subjects are the same as indicated in Figure 1.

RESULTS

Following ingestion of 50 g glucose, the plasma glucose rapidly increased from the baseline of 156 ± 13 mg/dl to a peak of 271 ± 9.8 mg/dl at 1 h. It had returned to the baseline by about 4–5 h (Figure 1). When 50 g protein alone was given, there was no change in plasma glucose concentration for 2 h and then it began to decline gradually. By 5 h it was approaching a normal fasting level. When glucose and protein were given together, the plasma glucose concentration reached the same peak concentration as with glucose alone. However, the glucose concentration declined more rapidly. By 3.5 h it had returned to the baseline. It continued to

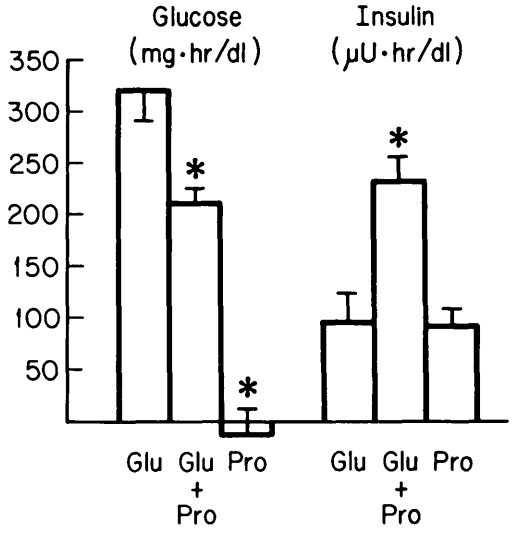

*FIG. 3. Areas above baseline for plasma glucose and serum insulin determined over the 5-h period following ingestion of glucose (glu), protein (pro), or a combination of glucose and protein. [*Indicates statistical difference from glucose administration alone for glucose area. For the insulin area glucose + protein is significantly greater than glucose or protein ingestion individually. The glucose and protein area is also significantly greater than the sum of glucose alone plus protein alone ($P < 0.01$). This indicates synergism between oral glucose + oral protein ingestion in the stimulation of insulin secretion.] The subjects are the same as indicated in Figure 1.*

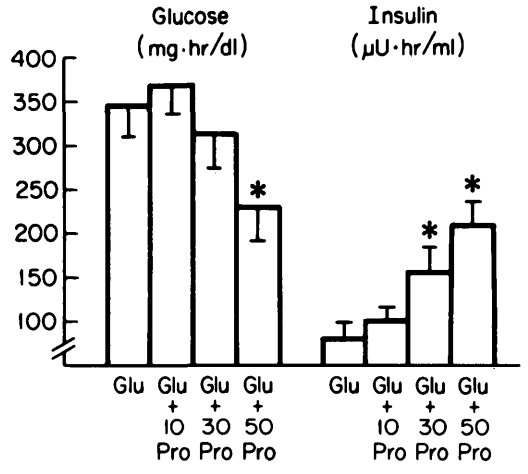

*FIG. 4. Areas above baseline for plasma glucose and serum insulin determined for 5 h following ingestion of 50 g glucose and increasing amounts of protein. (*Indicates results statistically different from those obtained after glucose ingestion alone.) Five male, untreated diabetic subjects were studied.*

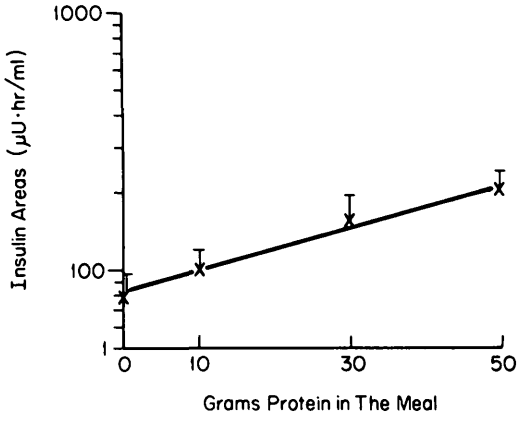

FIG. 5. Plot of insulin areas obtained with increasing amounts of protein ingested. The subjects are the same as indicated in Figures 1 and 4.

decline such that by 5 h it was well within the normal fasting glucose range (99.5 ± 7 mg/dl) (Figure 1).

Following glucose ingestion the mean serum insulin concentration rose to a maximum of 53 ± 13 μU/ml at 1 h and then gradually decreased but had not reached the baseline concentration by 5 h (Figure 2). When only protein was given, the peak in insulin concentration occurred later than when glucose was given (2 h and 1 h, respectively). The peak also was not quite as high (45 ± 6.9 μU/ml) as when glucose was given, although the difference did not reach statistical significance (Figure 2). By 5 h it also had not declined to the fasting level. When glucose was given with protein, the insulin peak (96 ± 18 μU/ml) was significantly greater than when glucose or protein was given individually. The peak occurred at 2 h, i.e., the same as when protein was given alone. By 5 h the insulin concentration was still considerably elevated. In fact, it was more than twice as high (38 ± 10 μU/ml) as the fasting value (16 ± 2 μU/ml).

The areas of the glucose and insulin curves above the baseline were determined and are shown in Figure 3. The glucose area after glucose plus protein ingestion was only 65% of the area observed with glucose alone (Figure 3) and the difference was statistically significant ($P < 0.05$). The glucose area following protein ingestion was slightly negative as expected. The insulin areas following glucose alone or protein alone were quite similar (93 ± 33 μU · h/ml and 87 ± 18 μU · h/ml, respectively). However, the area after the ingestion of glucose combined with protein was much greater (233 ± 39 μU · h/ml). In fact, it was approximately 2.5 times as high as with either glucose or protein alone. It was 30% greater than the sum of the areas of glucose and protein added together.

Addition of 10 g protein to a 50-g glucose load did not significantly affect the glucose curve (Figure 4). With addition of 30 g protein, the mean was modestly, but not significantly, reduced. With addition of 50 g protein, there was a further reduction that was statistically significant ($P < 0.05$).

The mean serum insulin area following 10 g protein with 50 g glucose was 125% of that obtained with glucose alone (Figure 4). However, this did not reach statistical significance. There was a further increase in insulin area when 30 and 50 g of protein were given with glucose, and these increases were statistically significant. When the dose response was plotted on semilog paper (Figure 5), the curve approximated a straight line, suggesting a first-order relationship between the response and dose of protein given. The calculated K (slope) was 2.8 μU · h/g protein/ml. However, there was a suggestion of a falloff in response at the 50-g dose.

The plasma glucose and insulin response to two sequential doses of glucose given 4 h apart is shown in Figure 6. The rise in plasma glucose concentration was modestly smaller after the second dose, although the difference was not statistically significant. The insulin curve after the second meal also was similar to the first. However, the rise started at a

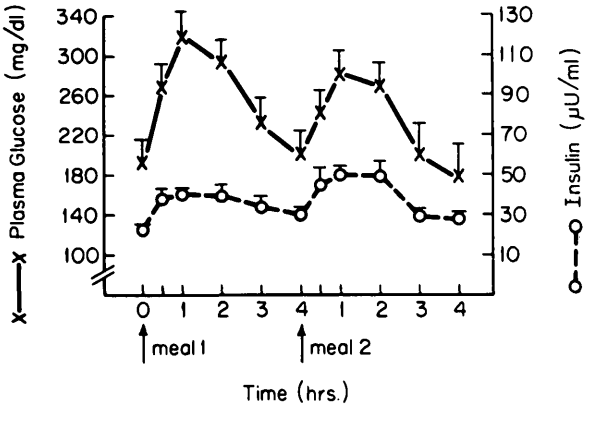

FIG. 6. Plasma glucose and serum insulin responses to 50 g glucose ingested twice, 4 h apart. Five male, untreated diabetic subjects were studied.

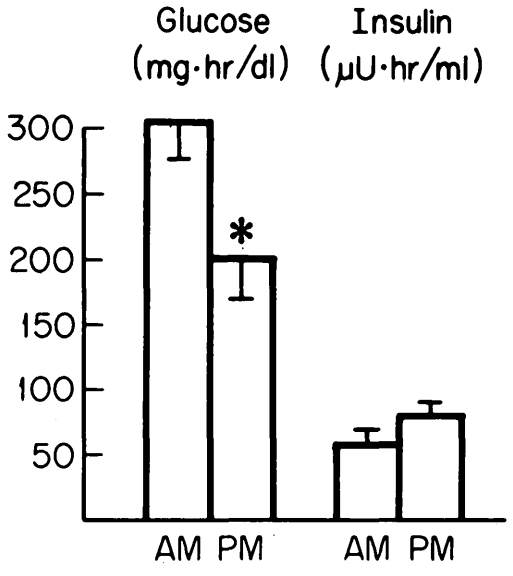

FIG. 7. Areas above baseline for plasma glucose and serum insulin determined over the 4-h period following consecutive 50-g glucose meals given 4 h apart (see Figure 6). The subjects are the same as indicated in Figure 6.

higher level than after the first meal. When the areas above the fasting baseline were determined, the glucose area after the second meal was significantly reduced compared with the first meal (307 ± 29 versus 202 ± 32 mg · h/dl). The insulin areas after each meal were not significantly different (58 ± 11 versus 82 ± 10 μU · h/ml, respectively) (Figure 7).

When glucose was given with protein 4 h apart (Figure 8), the peak glucose concentration after the second meal was considerably less than after the first meal. As expected, the rise in insulin concentration was greater than with the glucose administration alone. The rise after the second meal was less than after the first meal, but peak concentration after each meal was similar. The first glucose area (Figures 8 and 9) was less than when glucose was given alone (Figures 6 and 7) and the area was further reduced after the second meal compared with the first (17 ± 6 versus 230 ± 38 mg · h/dl). Actually, the second area was only 7% of the first. The insulin areas after the first (207 ± 26 μU · h/ml) and second (197 ± 37 μU · h/ml) meals were essentially identical (Figure 9). Compared with the response to glucose alone, the insulin area was 3.6-fold greater after the first meal and 2.4-fold greater after the second meal.

DISCUSSION

That protein stimulates insulin secretion in mild type II diabetic subjects is in agreement with previous studies.[4,5,9] In the present study, the stimulation of insulin secretion by protein in the form of hamburger was similar on a weight basis to that of glucose, although the peak response was delayed. In addition, the insulin response to co-ingestion of glucose and protein was greater than the sum of the responses to glucose and protein added together. This indicates a strong synergism between oral protein and glucose in the stimulation of insulin secretion. When increasing amounts of protein were given with a standard amount of glucose, the insulin response was first order in regard to the quantity of protein ingested. Thus, smaller amounts of protein were relatively more potent than larger amounts. A synergistic effect on insulin secretion also has been reported previously in four normal women who received beef steak with glucose. However, in this study there was little change in insulin concentration when steak containing approximately 60 g of protein was given alone.[10]

The hamburger given contained 6.5% fat. Dietary fat has been reported to delay gastric emptying.[11] Thus, the fat present could have affected the results obtained. A major effect on gastric emptying is not likely, however, since the peak glucose concentration following ingestion of the hamburger with glucose was similar to that observed with glucose ingestion alone (Figure 1). Also, the peak occurred at similar times, and the decrease in glucose concentration was more rapid when the combination was ingested. We would not have expected the increased food-energy load[12] following the ingestion of hamburger with glucose to have affected gastric emptying of glucose for the same reasons. Nevertheless, since the rate of gastric emptying was not measured, a small effect on the glucose response cannot be completely ruled out.

In a large group of normal subjects Floyd et al.[2] reported a rise in insulin concentration similar to that observed in the present study. The total amount of protein given was not stated, but calculations based on the usual protein concentration in lean beef and chicken livers, the foods used in the study, indicate that it was at least twice as much as was used in the present study. In a subsequent study[4] the same group reported the response to a protein meal in subjects with glucose intolerance and type II diabetes compared with normal subjects. In general, the insulin response to the protein meal correlated with the insulin response to a standard glucose meal. In mildly obese, glucose-intolerant subjects

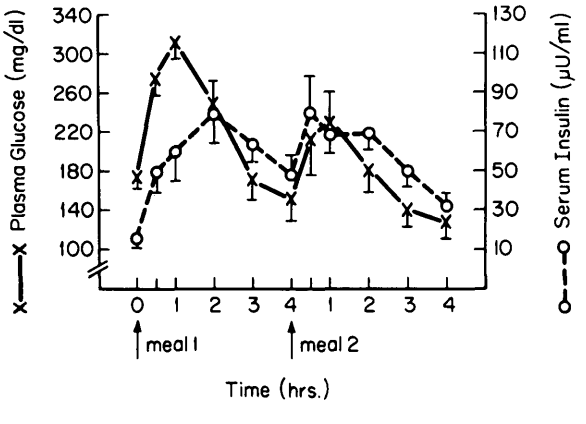

FIG. 8. Plasma glucose and serum insulin responses to 50 g glucose + 50 g protein ingested twice, 4 h apart. The subjects are the same as indicated in Figure 6.

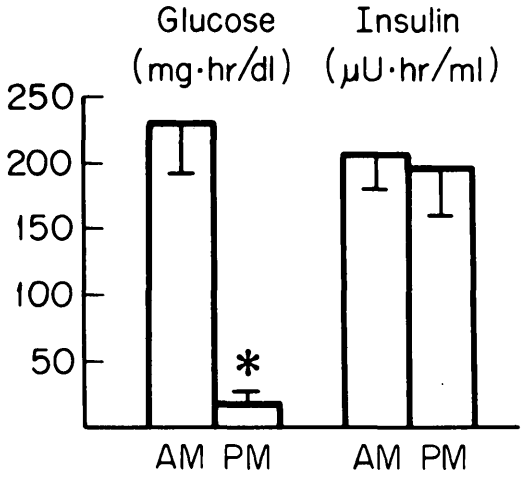

*FIG. 9. Areas above baseline for plasma glucose and serum insulin determined over the 4-h period following consecutive ingestion of 50 g glucose with 50 g protein given 4 h apart. (see Figure 8). (*Indicates response to second meal statistically significantly different from response to first meal.) The subjects are the same as indicated in Figure 6.*

insulin response to glucose was greater than in normal subjects. The response to a protein meal also was greater. In the normal-weight diabetic subjects the insulin response to the standard glucose meal was less than in the normal subjects. The response to the protein meal also was less. They suggested that the exaggerated insulin response to a protein meal in diabetic subjects compared with normal subjects reported by Berger and Vongaraya[5] was due to the use of obese, diabetic patients in their study, since the insulin response to a standard glucose meal also was greater in the diabetic subjects. However, review of the data indicates that the insulin response to glucose was only modestly greater, whereas the response to protein was considerably greater than in the normal subjects.

Thus, whether the insulin secretory response to protein ingestion in type II subjects is relatively better maintained than the secretory response to glucose ingestion is controversial. Our data would suggest that the response to protein is better maintained in at least some type II diabetic subjects. This is supported by data obtained in partially pancreatectomized rats.[13]

That a high protein content might be useful in the diet for diabetic persons was suggested as early as 1936.[14] It was based on the observation that ingestion of up to 140 g of protein as lean beef did not significantly increase the blood glucose concentration in either normal or mild type II diabetic subjects even though theoretically a large proportion of the amino acids was available for conversion to glucose.[14] Our data also indicate that protein ingestion does not cause an increase in plasma glucose and in fact may reduce the glucose concentration (Figure 1). In addition, when protein was given with glucose, the postmeal glucose area was reduced. Similar results have been reported in both normal[10] and type II diabetic subjects.[9] However, this has not been a universal observation. Jenkins et al.[15] have reported that addition of protein to a carbohydrate meal does not reduce the plasma glucose area above the baseline in normal subjects. Also, Day et al.[16] reported that addition of varying amounts of protein to a constant amount of carbohydrate in a meal did not significantly influence the plasma glucose rise. When there was less than 8 g of protein in the meal, the insulin response per unit increase in plasma glucose concentration was less than with a larger amount of protein; otherwise, they also noted little difference in insulin response as the protein content was increased up to as much as 25 g. When a greater amount of protein was given, both the glucose and insulin responses were increased. In the latter study the subjects were only studied for 90 min and the meal was given at noon.

It has been known for many years that giving normal individuals a second glucose meal approximately 4 h after a previous glucose meal results in an improved rate of glucose clearance.[17–19] This is the so-called Staub–Traugott effect. We were certainly interested in determining if this effect would be observed when protein was given with glucose to diabetic subjects for three reasons. First, we had demonstrated a much greater insulin rise when protein was given with glucose as a single meal than when glucose was given alone. Second, we had observed a greater plasma glucose area above baseline and smaller insulin area above baseline when mild type II diabetic subjects were given 50 g of glucose compared with a mixed breakfast meal containing approximately 70 g of carbohydrate and approximately 20 g of protein.[20] Lastly, in normal subjects receiving a 40% protein diet, we have previously demonstrated a reduced glucose response after a second and third meal were given 4 h apart.[6] In these subjects following each meal there was a distinct, sharp rise in insulin that could not be accounted for by a rise in glucose concentration or amino acids.[21]

In the present study, when glucose was given alone, a second meal effect (Staub–Traugott effect) was clearly observed even though the plasma insulin areas were similar after each meal (Figure 6). When protein was given with glucose, the second meal effect was much more striking. The second meal area was only 7% of that after the first meal. The insulin areas after each meal were essentially identical, although they were considerably higher than with glucose administration alone.

It is well known that protein ingestion stimulates a rise in circulating glucagon concentration; with glucose ingestion it is depressed.[22–26] It is also clear that the circulating glucagon concentration depends on the ratio of protein to carbohydrate in the meal. If the protein-to-carbohydrate ratio is high, it will increase, whereas if the ratio is low, it will decrease.[16,21] We had planned to determine the glucagon response in our studies; unfortunately, the tubes containing plasma samples for glucagon determination were either broken and/or the samples thawed when our laboratory was moved to another building. Thus, it was not possible to determine the glucagon response in these subjects. From previous experience, we would anticipate only a modest rise in glucagon when equal

amounts of protein and glucose are given.[21] In any regard, the insulin secreted in response to the mixture of protein and glucose was sufficient to reduce the postmeal glucose rise.

Whether a moderately high protein will be beneficial in type II diabetic patients remains to be determined. Nevertheless, the present studies suggest that protein ingestion is important in stimulating insulin secretion in these individuals. When carbohydrate is ingested, the simultaneous ingestion of protein may also prove useful in reducing the postmeal glucose rise. In addition, these data indicate the need to consider the insulin secretory response and a second and third meal effect on blood glucose concentration when determining the glycemic response to a food.

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