

## Effect of protein dose on serum glucose and insulin response to sugars<sup>1-3</sup>

Gene A Spiller, PhD; Christopher D Jensen, RD; Thomas S Pattison, MS; Carol S Chuck, RD; James H Whittam, PhD; and James Scala, PhD

**ABSTRACT** To clarify the effects of protein on insulin and glucose response to sugars, 14 healthy normal-weight males and females were fed test meals containing 0, 15.8, 25.1, 33.6, and 49.9 g protein along with ~58 g carbohydrate. Serum samples were obtained at fasting time zero and 15, 30, 60, and 120 min postprandial. Mean areas of the glucose curves above fasting decreased with increasing protein dose. Protein-containing meals produced significantly lower ( $p < 0.01$ ) areas than the protein-free meal and the relationship between blood glucose area and protein dose was significant ( $p < 0.001$ ). Protein-containing meals produced significantly greater ( $p < 0.01$ ) insulin areas compared with the protein-free meal. However, no differences in insulin areas among the protein-containing meals were observed. These data support previous studies showing a blood glucose moderating and insulin-enhancing effect of protein ingestion. *Am J Clin Nutr* 1987;46:474-80.

**KEY WORDS** Protein, carbohydrate, glucose response, insulin response, glycemic index, diabetes

### Introduction

Protein ingestion and oral or intravenous administration of amino acids have been shown to stimulate insulin secretion and moderate postprandial hyperglycemia in normal and type II diabetic subjects (1-5). It is possible also that the beneficial effect that legumes have had in terms of their glycemic index (6) and long-term dietary treatment in diabetes (7) may relate to their higher-protein content by comparison with other starchy foods, in addition to their dietary fiber, type of starch, and content of other components.

To date, the few studies that have examined the serum insulin and glucose response to various protein doses have yielded mixed results. Day et al (8) fed a constant carbohydrate load from whole foods while varying the protein dose (3.6-75 g protein) and detected differences in glucose responses at 60 and 90 min between the high- and low-dose protein meals as well as differences in insulin levels at 60 min between the high-dose protein meal and the remaining meals. More recently, Nuttall (9) reported a protein-dose effect on serum insulin response when 0-50 g protein was fed with a 50 g glucose solution. However, no differences have been seen in the responses at the intermediate-protein doses. Jenkins et al (6) were unable to detect a significant change in the plasma glucose area above baseline in diabetic subjects fed a carbohydrate meal

of wholemeal bread and cottage cheese vs wholemeal bread alone where the protein difference was only 12 g.

The dose-response study reported here was undertaken to define the protein dose at which a significant effect on serum insulin and glucose response might be expected in healthy subjects.

### Subjects and methods

#### Subjects

Healthy individuals, eight females and six males aged 28-59 y (mean age 38 y), who were employees of the research center and free of diabetes or family history of diabetes participated after voluntary informed consent was obtained. Subjects had previously participated in similarly designed pilot studies that had examined the effects of various liquid test meals on glucose and insulin response to simple sugars. Consequently, subjects were familiar with the experimental regimen. Before participation in these studies, all subjects were given a standard oral glu-

<sup>1</sup> From the Shaklee Research Center, San Francisco, CA.

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<sup>3</sup> Address reprint requests to Christopher D Jensen, RD, Shaklee Research Center, 444 Market Street, San Francisco, CA 94111.

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TABLE 1  
Test meals\*

Ingredients	Pro0	Pro1	Pro2	Pro3	Pro4
<i>g</i>					
Maltodextrin	23.9	23.2	23.2	23.7	23.2
Fructose	18.3	19.0	19.1	19.0	19.1
Lactose	15.4	15.2	15.7	15.9	15.9
Total carbohydrate (g)	57.6	57.4	58.0	58.6	58.2
Milk proteins	—	5.2	8.1	10.8	16.0
Soy proteins	—	10.6	17.0	22.8	33.9
Total protein (g)	0.0	15.8	25.1	33.6	49.9

\* Ingredients for test meals came from the following sources: maltodextrin—Maltrin M100, Grain Processing Corporation, Muscatine, IA; fructose—D-Fructose 54016, Roche Chemical Division, Nutley, NJ; lactose—Edible Lactose, Land O'Lakes, Minneapolis, MN; milk proteins—Meloskim 500 (nonfat dry milk), Dairyland Products, Inc, Savage, MN, and TMP 1220 (total milk proteinate), New Zealand Milk Products, Inc, Petaluma, CA; soy proteins—Pro-Fam G-902 (soy protein isolate), Grain Processing Corporation, Muscatine, IA.

ucose tolerance test and found to be normal in this regard. Their mean percent of desirable body weight was  $104 \pm 8\%$  (range: 91–126%) using the 1959 Metropolitan Life Insurance Co tables for persons of medium frame. The design and procedures followed in the study were in accord with the Helsinki Declaration as updated in Tokyo, Japan in 1975.

*Design*

Subjects adhered to an alcohol-free, high-carbohydrate diet (> 200 g/d) for 72 h before each test-meal feeding and were monitored by dietary records. Exercise was discontinued 24 h before and fasting was instituted 12 h before each test meal was administered. On the mornings of the test meals, venous blood was drawn and then meals (Table 1) were consumed over a 2 min interval. Additional venous blood samples were drawn at 15, 30, 60, and 120 min after test meals were ingested. No exercise and additional food were allowed during the experimental period. The test meals were administered at least 6 d apart and on a given day subjects received the same test meal in the following order: 0 g, 25.1 g, 49.9 g, 15.8 g, and 33.6 g protein. The mean fasting blood insulin levels were similar on each test meal day. Mean fasting blood glucose levels were similar on the days the protein-containing meals were fed but lower ( $p < 0.01$ ) on

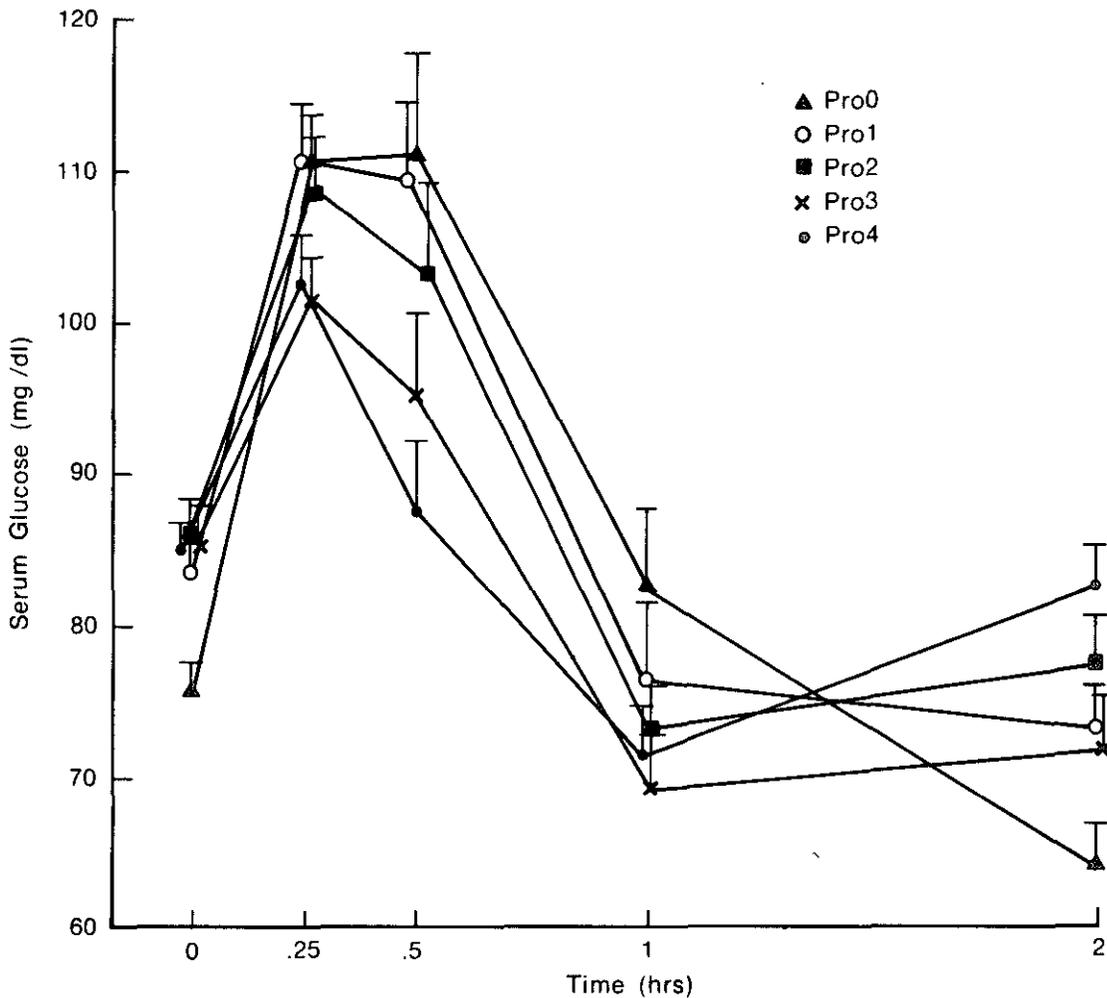


FIG 1. Serum glucose response to protein dose. Data are means  $\pm$  SEM.

the day of the protein-free test meal (Fig 1). One male subject was unavailable for the 25.1 g protein test meal and one female was unavailable for the 33.6 g protein test meal. Otherwise, all subjects ingested all test meals.

#### Test meals

Test meals (Table 1) were dissolved in 375 mL cold tap water. Each meal provided ~58 g carbohydrate, various amounts of protein, and trace amounts of fat. The levels of protein, fat, and carbohydrate of the various raw materials in the test meals were obtained from manufacturer specifications and confirmed by the standard methods of the Association of Official Analytical Chemists (10) for protein (#2.057), fat (#16.064), and fructose and lactose (#31.138). The degree of polymerization (DP) of the maltodextrin was characterized by high-pressure liquid chromatography (HPLC) (11) using a APX-42A column (Bio-Rad Laboratories, Richmond, CA). The approximate average glucose polymer molecular weight was 1800 and the average number of anhydrous glucose units per molecule was 11.1. The DP was distributed as follows: 0.5% DP1, 2.7% DP2, 4.3% DP3, 3.7% DP4, 3.1% DP5, 5.7% DP6, 7.1% DP7, 4.5% DP8, 3.1% DP9, 1.6% DP10, and 63.7% DP10 and above (Grain Processing Corporation, Muscatine, IA, personal communication).

#### Blood glucose and insulin analyses

Serum glucose was obtained by an automated glucose oxidase method (SMA24, Technicon, Terrytown, NJ) and serum insulin

was measured using a radioimmunoassay kit (Phadeseph Insulin RIA, Pharmacia Diagnostics AB, Uppsala, Sweden).

#### Statistical analyses

A one-way analysis of variance was used to test for significant differences in fasting serum glucose and insulin levels between the test meals and responses to the test meals. Differences were calculated from the actual values in the case of fasting levels and as increments from fasting values or incremental areas above fasting for the responses to the test meals. Incremental areas were calculated by computing and summing the individual areas described by the trapezoids above the fasting values between time zero and each separate sampling time. When the *F* statistic indicated significance, the method of least significant differences was used to compare means.

### Results

#### Compliance

Diet records indicated that subjects complied with the high-carbohydrate, alcohol-free regimen before the feeding of test meals.

#### Glucose response

The apparent maximum mean serum glucose rise from fasting for each protein-containing formula was seen at

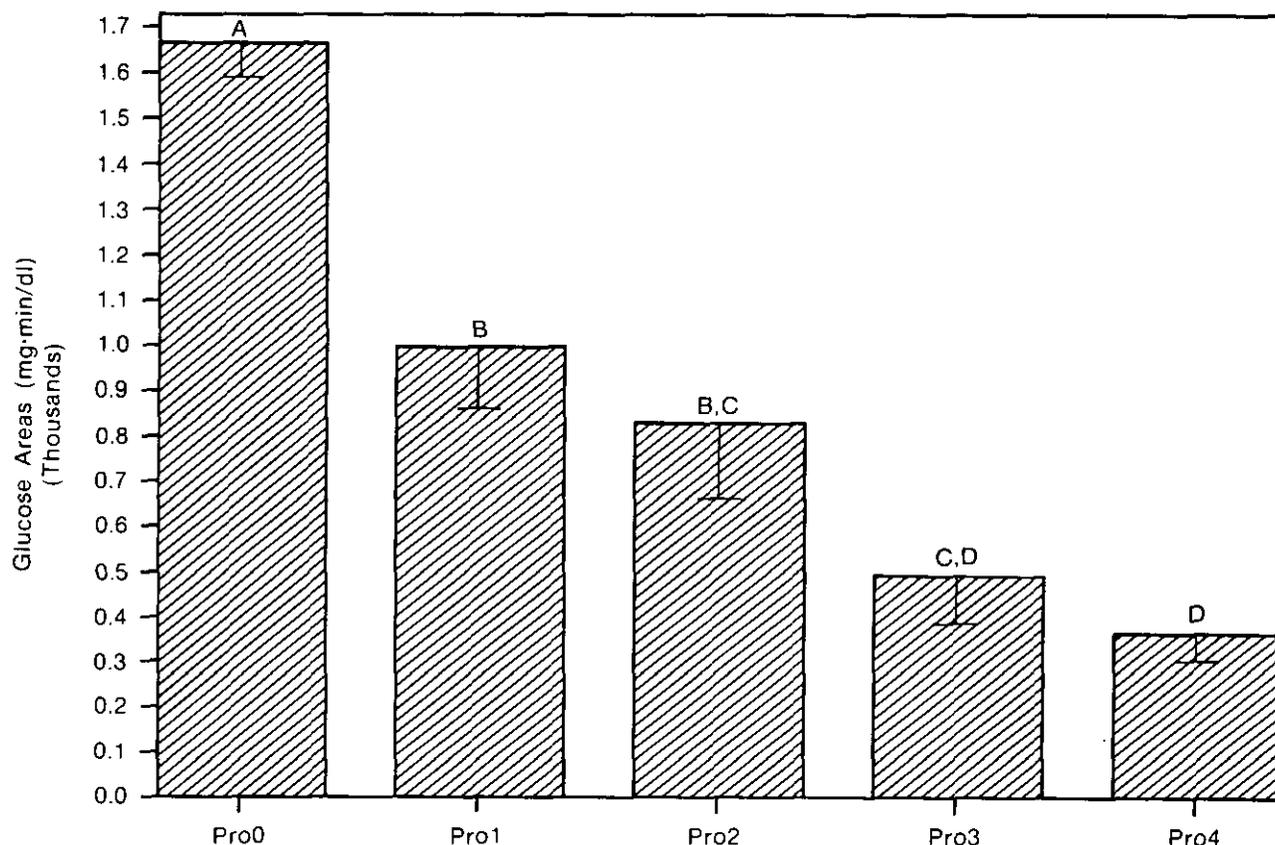


FIG 2. Areas above baseline under the glucose curves. Data are means  $\pm$  SEM. Areas are significantly different ( $p < 0.01$ ) if they do not share a common superscript letter.

15 min postconsumption, whereas, the test meal without protein (Pro0) appeared to reach its maximum increment at the 30 min mark (Fig 1). Each of the protein-containing test meals produced lesser maximum mean glucose increments as compared with the Pro0 test meal. Expressed as a percentage of the protein-free test meal, these differences were significant ( $p < 0.01$ ) except for Pro1 (15.8 g protein). In addition, Pro3 and Pro4 (33.6 g and 49.9 g protein, respectively) yielded significantly smaller ( $p < 0.01$ ) maximum glucose increments than did Pro1 and Pro2 (15.8 g and 25.1 g protein, respectively). The time estimated for the return to baseline of the mean glucose levels was found to decrease as the protein dose increased (Fig 1). The estimated mean times were 35, 43, 45, 58, and 76 min for Pro4, Pro3, Pro2, Pro1, and Pro0, respectively. The time differences between test meals were statistically significant ( $p < 0.01$ ) except for Pro3 and Pro2 ( $p > 0.05$ ). The maximum fall from baseline in mean serum glucose levels detected within 2 h after consumption did not differ significantly for any of the test meals ( $p > 0.05$ ).

The mean areas of the glucose curves above baseline for each test meal are shown in Figure 2. The glucose

areas produced by ingestion of the protein-containing test meals were significantly lower ( $p < 0.01$ ) than the area corresponding to Pro0. In addition, the mean areas for the protein-containing test meals decreased with increasing intakes of protein. The differences in areas between Pro3 and Pro4 versus Pro1 were significant ( $p < 0.01$ ). When the logarithm of these areas was plotted vs protein dose, a straight line resulted, suggesting a first-order relationship between moderation of the hyperglycemic response and dose of protein (Fig 3). The correlation coefficient was 0.986 ( $p < 0.001$ ).

#### Insulin response

For all of the test meals, the apparent maximum mean serum insulin increments from fasting were seen at 30 min (Fig 4). Pro0, the test meal containing the sugars alone, produced the lowest maximum rise from fasting, although none of the differences were significant ( $p > 0.05$ ). The only pattern related to protein dose was the insulin increment above baseline observed at the 2-h mark. The increment was progressively larger with increasing protein dose. The mean serum insulin increment above baseline for Pro4 was significantly larger ( $p < 0.01$ )

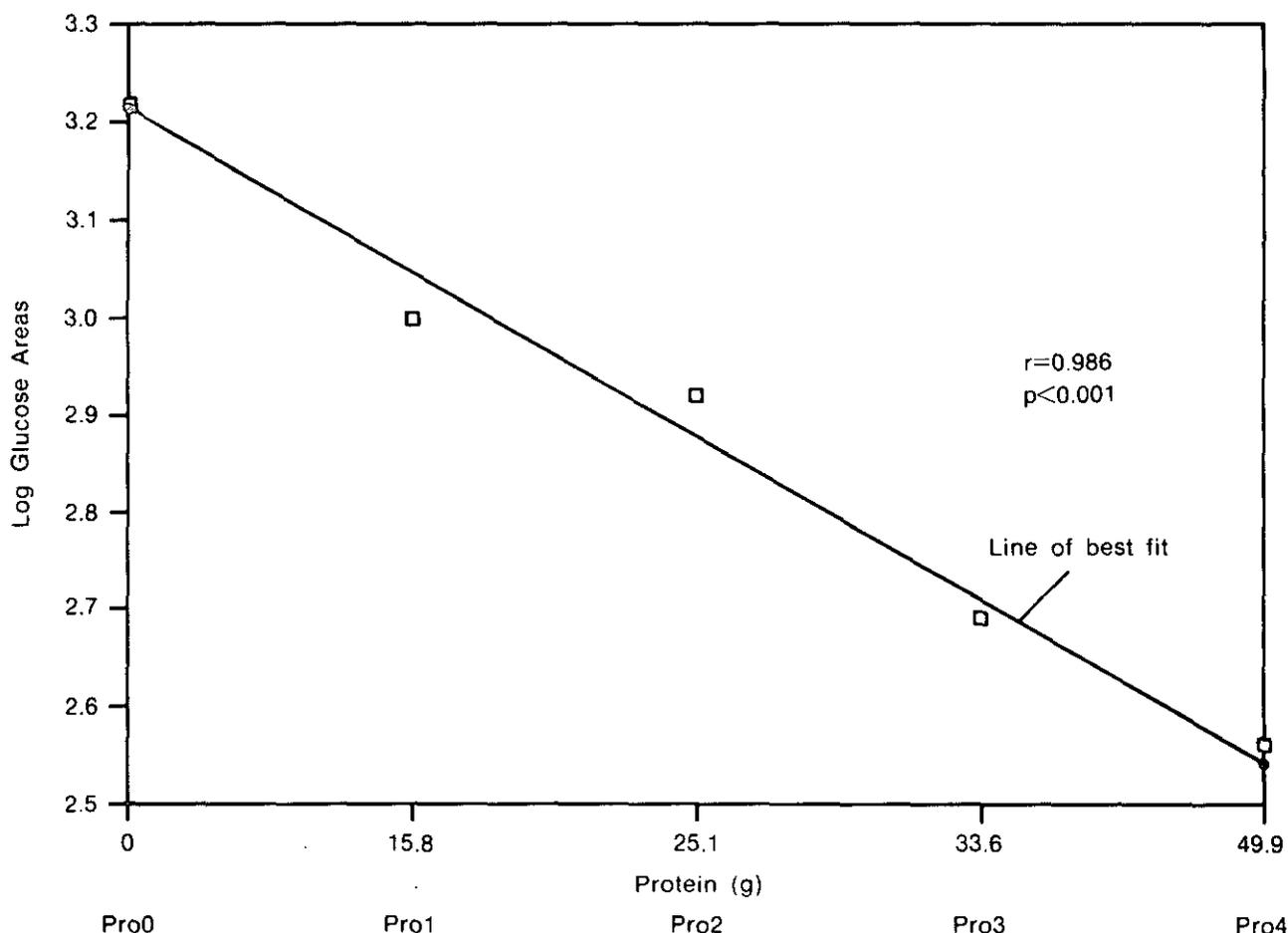


FIG 3. Logarithms of the areas above baseline under the glucose curves versus protein dose.

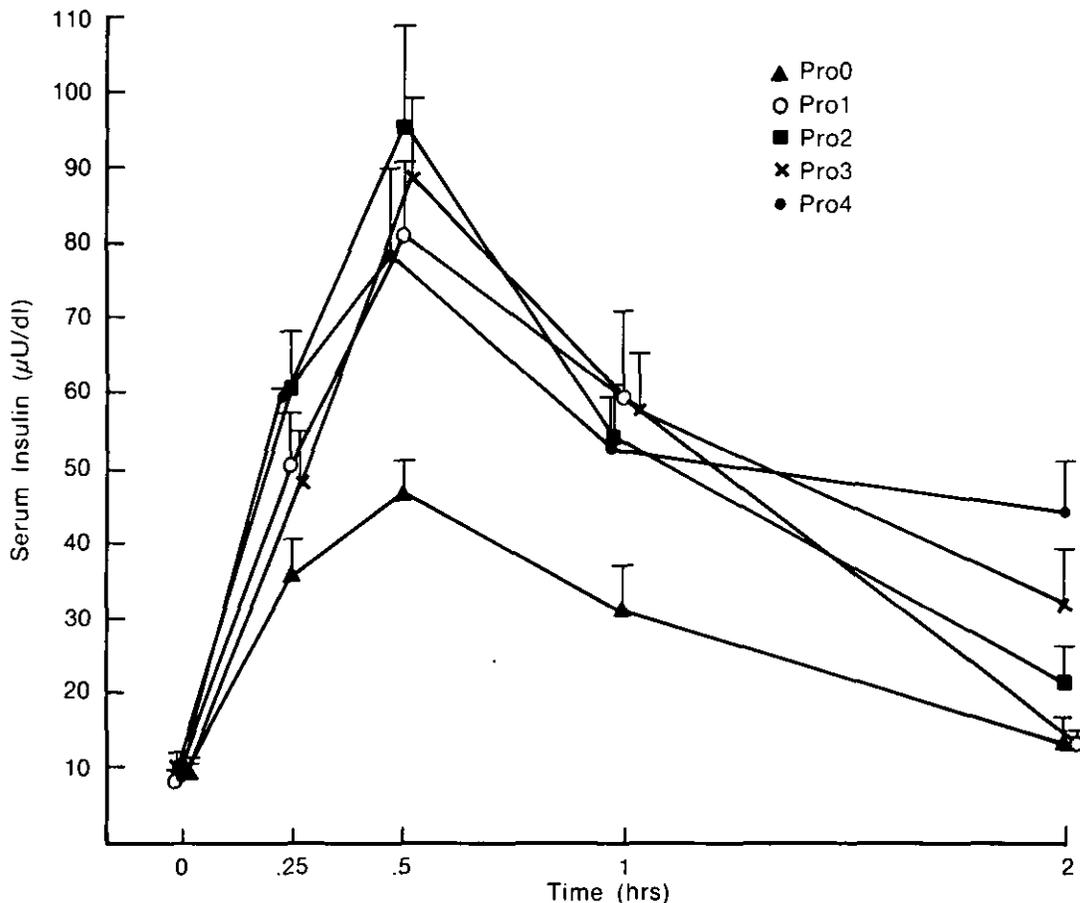


FIG 4. Serum insulin response to protein dose. Data are means  $\pm$  SEM.

than the increment produced by Pro3, which in turn was larger ( $p < 0.01$ ) than the responses to Pro1 and Pro0 (Fig 4). Mean insulin levels did not return to baseline within 2 h for any of the test meals.

The mean areas under the serum insulin curves above baseline for each of the protein-containing test meals were significantly greater ( $p < 0.01$ ) than the area corresponding to Pro0 (Fig 5). However, none of the differences in areas between protein doses achieved statistical significance at  $p < 0.05$  within the 2-h sampling period.

### Discussion

The lower maximum mean serum glucose increments and earlier times at which these increments were seen, along with the shorter periods of time required for glucose levels to return to baseline and the reduced areas under the glucose curves, confirm the results of previous studies in both normal (3) and type II diabetic (12) subjects. These studies found that after a carbohydrate load glucose elevations are clearly affected by concomitant protein ingestion if substantial differences exist in the protein levels in the meals. The finding that serum insulin response is enhanced by adding protein to a carbohydrate load is also in agreement with these studies. In addition, our results

can explain the failure of investigators in some situations to see an effect of protein. Jenkins et al (6) reported in diabetic subjects that a meal of wholemeal bread and cottage cheese (22.1 g protein, 50 g carbohydrate) did not yield a significantly lower plasma glucose area above baseline than did a meal of wholemeal bread alone (12.1 g protein, 50 g carbohydrate). A comparison of the effects of Pro1 (15.8 g protein) and Pro2 (25.1 g protein) in our study showed similar responses. However, when the protein doses were increased further, significant reductions in area did occur. In fact, a clear dose response effect was demonstrated from zero to  $\sim 50$  g protein.

Areas under the serum insulin curves did not support a protein dose-response effect. However, mean insulin levels did not return to baseline within 2 h (Fig 4). The fact that the insulin increment above baseline at the 2 h mark was increased with increasing protein dose suggests that, had the blood sampling period been extended, significant differences might have been observed.

Day et al (8) fed 3.6–75 g protein with a standard amount of carbohydrate from whole-food sources but detected little variation in the effects of protein dose on glucose and insulin response in normal subjects over 90 min. However, interpretation of their results is made difficult by the fact that meals were fed at noon and the fat content,

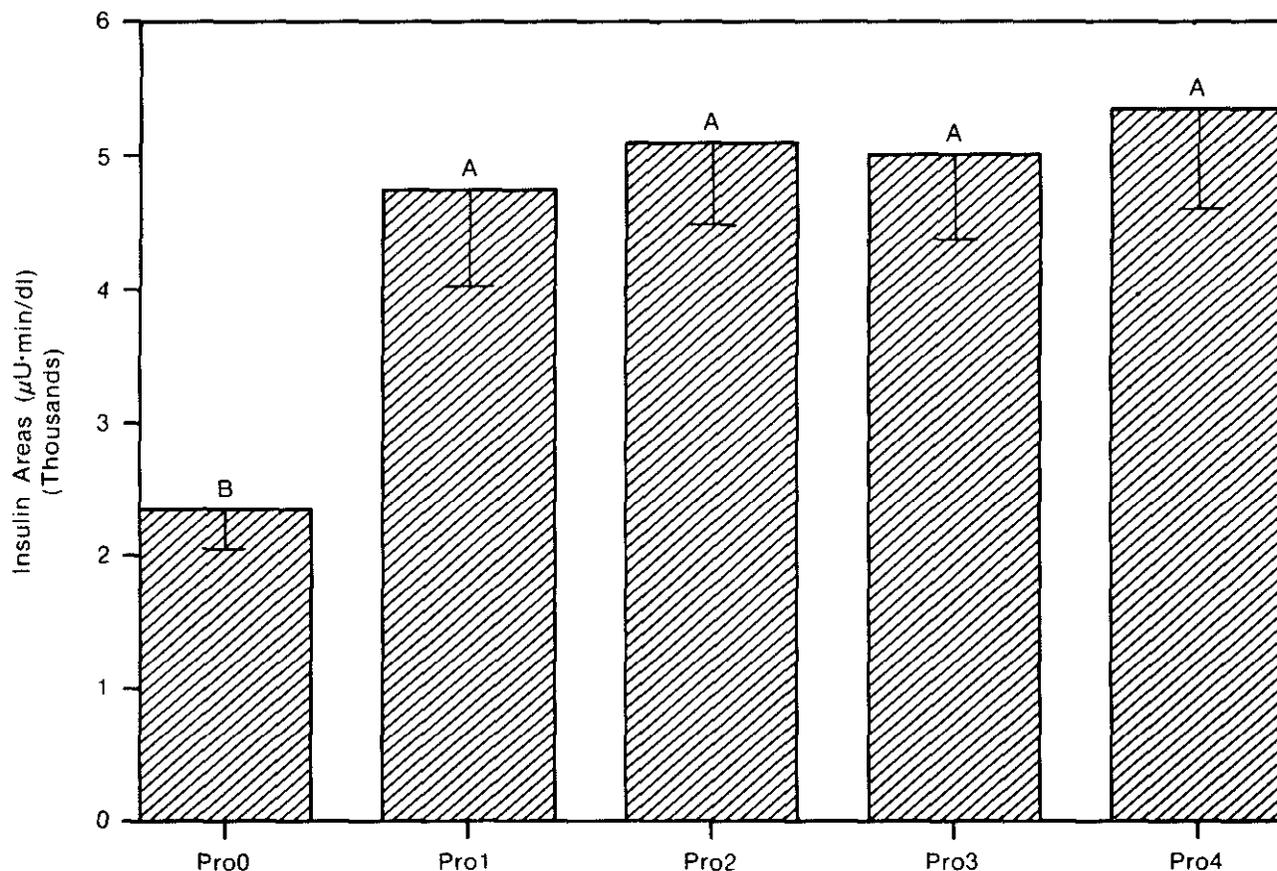


FIG 5. Areas above baseline under the insulin curves. Data are means  $\pm$  SEM. Areas are significantly different ( $p < 0.01$ ) if they do not share a common superscript letter.

known to alter gastric emptying time (13), varied between diets. The test meals used in our study were designed to contain negligible amounts of fat to avoid this difficulty.

Nuttall et al (9) recently fed 0, 10, 30, and 50 g protein with 50 g glucose to five mild untreated type II diabetic subjects whose percent of ideal body weight was  $123 \pm 23\%$ . Only the 50 g protein treatment yielded a significantly lower net area (net area equal to area below baseline subtracted from area above baseline) under the glucose curve as compared with the glucose treatment alone. Insulin areas were significantly greater for both the 30 and 50 g protein treatments as compared with the glucose treatment alone or in conjunction with 10 g protein. Fajans et al (14) have suggested that obesity in type II diabetics may lead to an exaggerated insulin response to protein dose. However, comparison of the insulin and glucose responses as indicated by areas above baseline or net areas (data not shown) under the respective curves from our mostly normal-weight healthy subjects to that of Nuttall's mildly overweight type II diabetic subjects suggests that the diabetic subjects were less sensitive to the lower doses of protein.

In summary, protein appears to exert a clear dose effect on glucose response as determined by mean areas above baseline under the glucose curve in normal fasting subjects

fed test meals consisting primarily of simple sugars and oligosaccharides. Due to the length of the blood sampling time in this study, similar conclusions regarding effects of protein dose on insulin cannot be made. Comparison of these results with those of mildly overweight type II diabetics fed similar protein levels point to a greater sensitivity to protein ingestion on the part of normal-weight healthy subjects. These findings are contrary to previous reports of greater insulin response observed with obese diabetics and indicate the need for additional research to clarify the effects of protein ingestion on serum insulin and glucose response in individuals with differing glucose tolerance status.

We are not suggesting at this point that the protein intake of the diabetic diet should be increased. Our results indicate that meal protein intakes must demonstrate differences of between  $\sim 10$ – $20$  g, when given in a liquid meal form, before significant differences in glucose response are observed in healthy subjects ingesting mostly simple sugars and oligosaccharides. Further studies comparing liquid test meals with traditional foods are needed before the relevance of these findings to the clinical setting is clear. It also should be noted that the carbohydrate distribution of these test meals is not typical of suggested diabetic meal plans. Advice to increase protein intake in

the diabetic diet is premature at a time when there is concern over high levels of dietary protein and renal damage (15, 16).

The assistance of Dr W Glenn Howells, Barbara Gifford, Mabel Rich, and Adib Nassar in various phases of the study is gratefully acknowledged.

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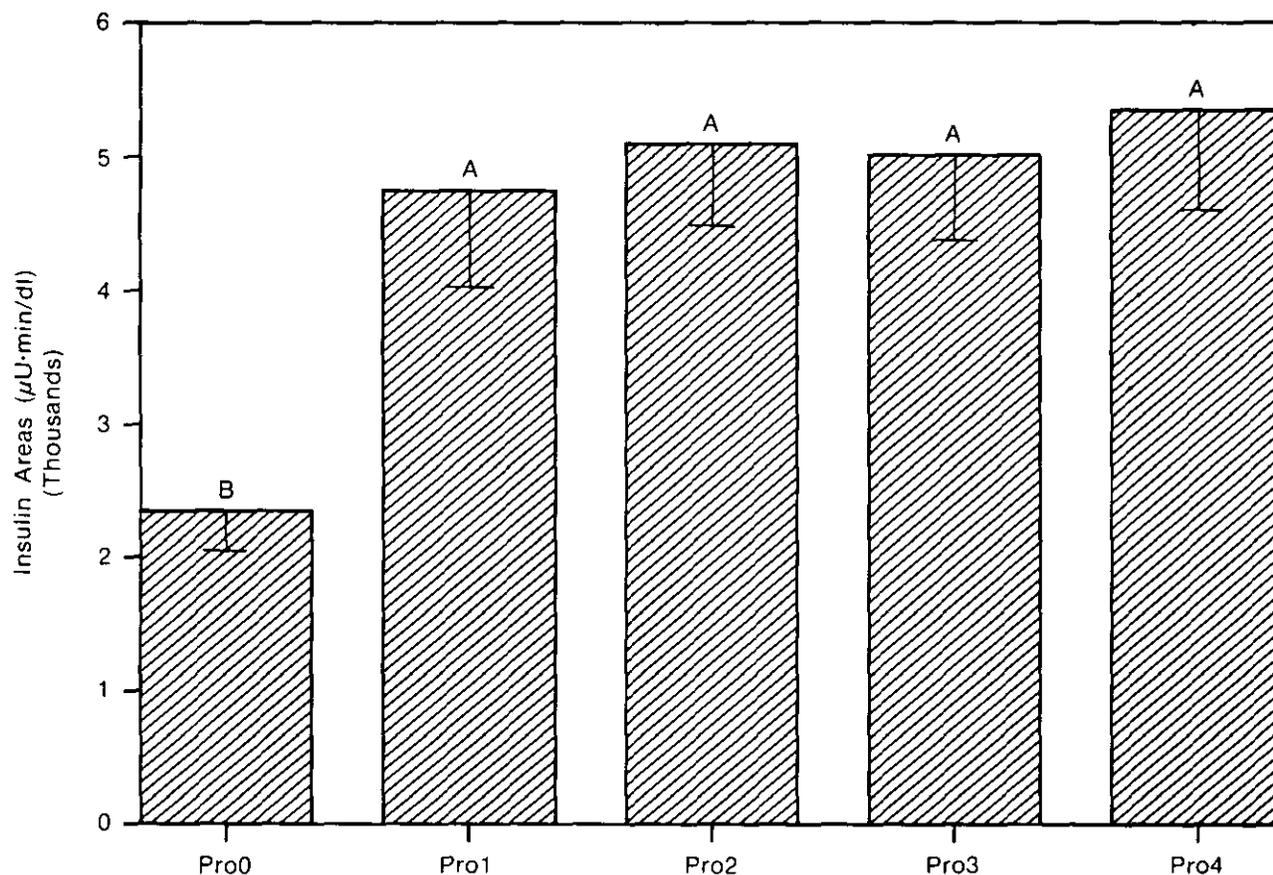


FIG 5. Areas above baseline under the insulin curves. Data are means  $\pm$  SEM. Areas are significantly different ( $p < 0.01$ ) if they do not share a common superscript letter.

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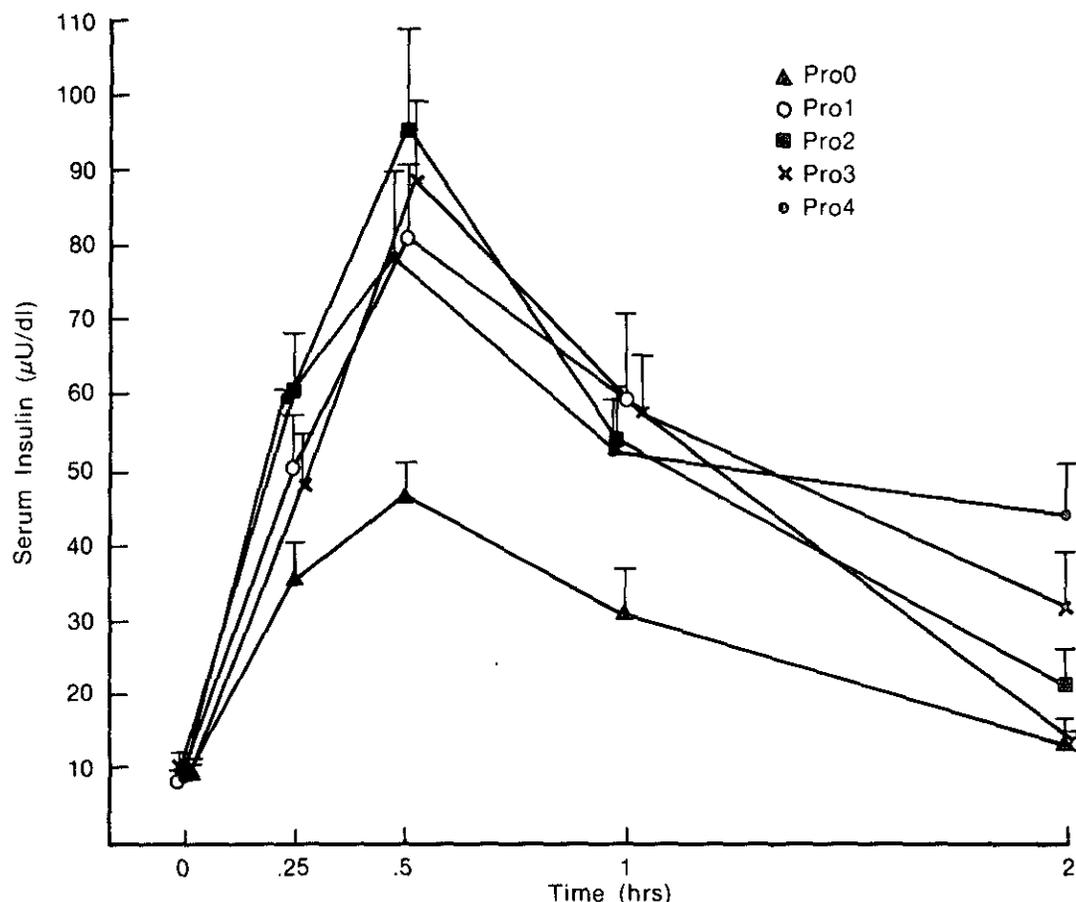


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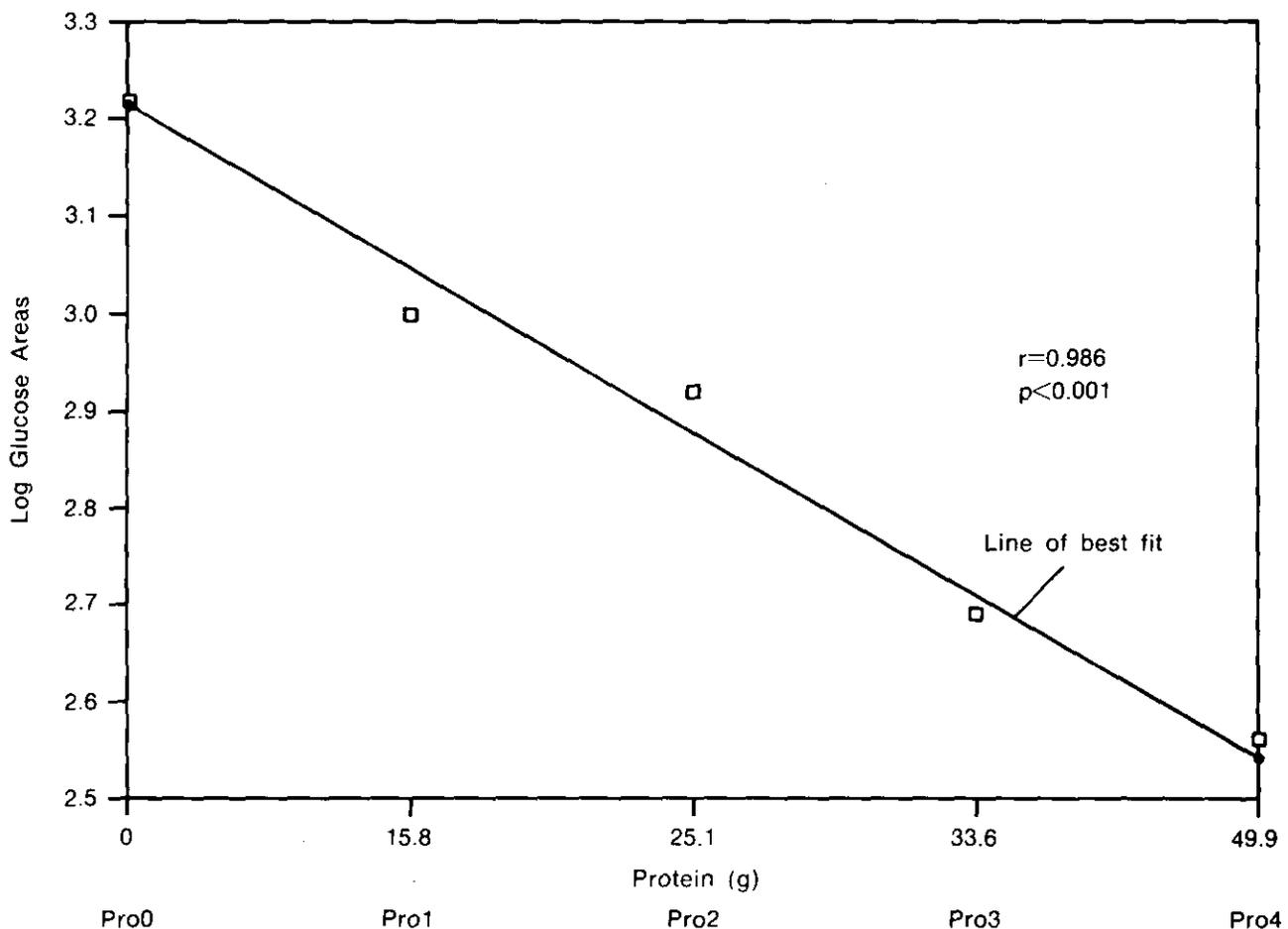


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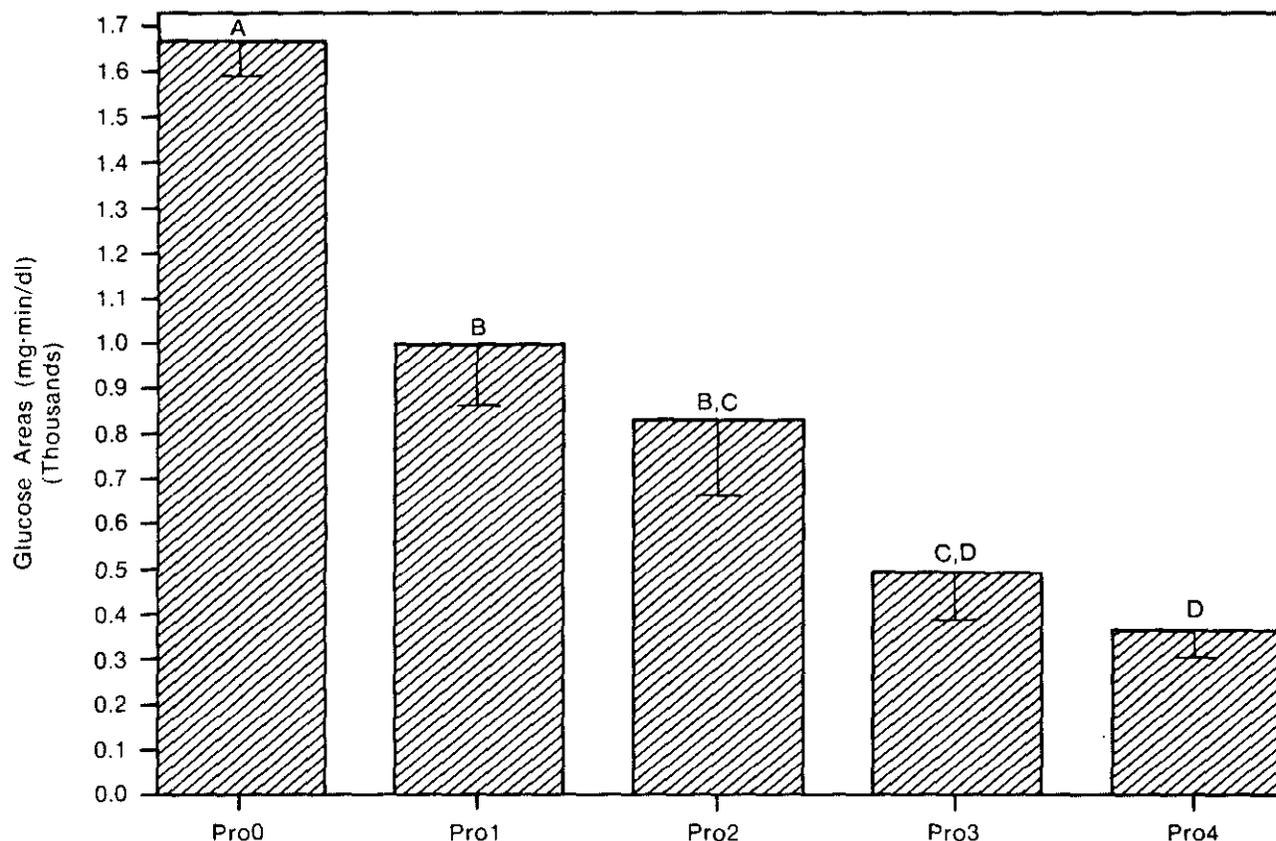


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Soy proteins	—	10.6	17.0	22.8	33.9
Total protein (g)	0.0	15.8	25.1	33.6	49.9

\* Ingredients for test meals came from the following sources: maltodextrin—Maltrin M100, Grain Processing Corporation, Muscatine, IA; fructose—D-Fructose 54016, Roche Chemical Division, Nutley, NJ; lactose—Edible Lactose, Land O'Lakes, Minneapolis, MN; milk proteins—Meloskim 500 (nonfat dry milk), Dairyland Products, Inc, Savage, MN, and TMP 1220 (total milk proteinate), New Zealand Milk Products, Inc, Petaluma, CA; soy proteins—Pro-Fam G-902 (soy protein isolate), Grain Processing Corporation, Muscatine, IA.

cose tolerance test and found to be normal in this regard. Their mean percent of desirable body weight was  $104 \pm 8\%$  (range: 91–126%) using the 1959 Metropolitan Life Insurance Co tables for persons of medium frame. The design and procedures followed in the study were in accord with the Helsinki Declaration as updated in Tokyo, Japan in 1975.

#### Design

Subjects adhered to an alcohol-free, high-carbohydrate diet ( $> 200$  g/d) for 72 h before each test-meal feeding and were monitored by dietary records. Exercise was discontinued 24 h before and fasting was instituted 12 h before each test meal was administered. On the mornings of the test meals, venous blood was drawn and then meals (Table 1) were consumed over a 2 min interval. Additional venous blood samples were drawn at 15, 30, 60, and 120 min after test meals were ingested. No exercise and additional food were allowed during the experimental period. The test meals were administered at least 6 d apart and on a given day subjects received the same test meal in the following order: 0 g, 25.1 g, 49.9 g, 15.8 g, and 33.6 g protein. The mean fasting blood insulin levels were similar on each test meal day. Mean fasting blood glucose levels were similar on the days the protein-containing meals were fed but lower ( $p < 0.01$ ) on

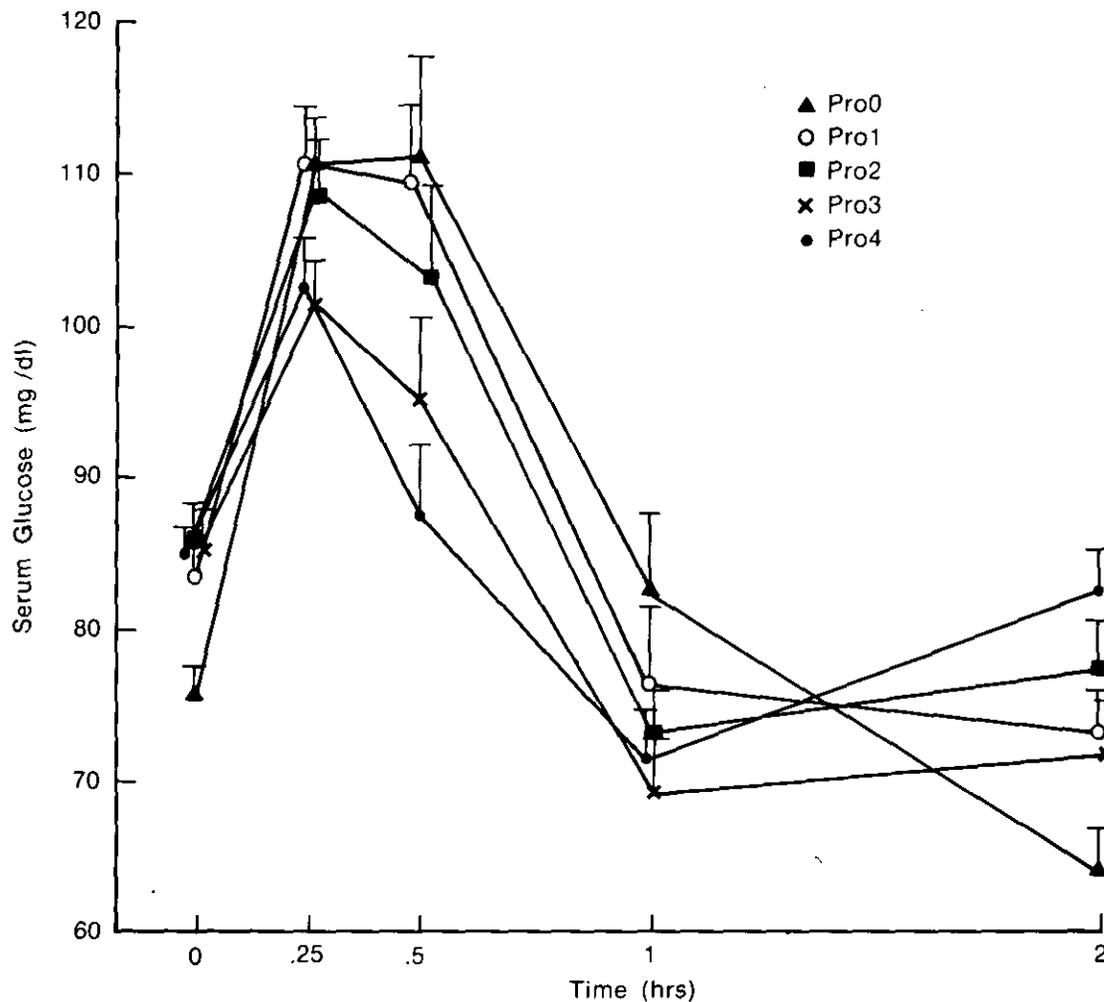


FIG 1. Serum glucose response to protein dose. Data are means  $\pm$  SEM.

# Effect of protein dose on serum glucose and insulin response to sugars<sup>1-3</sup>

Gene A Spiller, PhD; Christopher D Jensen, RD; Thomas S Pattison, MS; Carol S Chuck, RD; James H Whittam, PhD; and James Scala, PhD

**ABSTRACT** To clarify the effects of protein on insulin and glucose response to sugars, 14 healthy normal-weight males and females were fed test meals containing 0, 15.8, 25.1, 33.6, and 49.9 g protein along with ~58 g carbohydrate. Serum samples were obtained at fasting time zero and 15, 30, 60, and 120 min postprandial. Mean areas of the glucose curves above fasting decreased with increasing protein dose. Protein-containing meals produced significantly lower ( $p < 0.01$ ) areas than the protein-free meal and the relationship between blood glucose area and protein dose was significant ( $p < 0.001$ ). Protein-containing meals produced significantly greater ( $p < 0.01$ ) insulin areas compared with the protein-free meal. However, no differences in insulin areas among the protein-containing meals were observed. These data support previous studies showing a blood glucose moderating and insulin-enhancing effect of protein ingestion. *Am J Clin Nutr* 1987;46:474-80.

**KEY WORDS** Protein, carbohydrate, glucose response, insulin response, glycemic index, diabetes

## Introduction

Protein ingestion and oral or intravenous administration of amino acids have been shown to stimulate insulin secretion and moderate postprandial hyperglycemia in normal and type II diabetic subjects (1-5). It is possible also that the beneficial effect that legumes have had in terms of their glycemic index (6) and long-term dietary treatment in diabetes (7) may relate to their higher-protein content by comparison with other starchy foods, in addition to their dietary fiber, type of starch, and content of other components.

To date, the few studies that have examined the serum insulin and glucose response to various protein doses have yielded mixed results. Day et al (8) fed a constant carbohydrate load from whole foods while varying the protein dose (3.6-75 g protein) and detected differences in glucose responses at 60 and 90 min between the high- and low-dose protein meals as well as differences in insulin levels at 60 min between the high-dose protein meal and the remaining meals. More recently, Nuttall (9) reported a protein-dose effect on serum insulin response when 0-50 g protein was fed with a 50 g glucose solution. However, no differences have been seen in the responses at the intermediate-protein doses. Jenkins et al (6) were unable to detect a significant change in the plasma glucose area above baseline in diabetic subjects fed a carbohydrate meal

of wholemeal bread and cottage cheese vs wholemeal bread alone where the protein difference was only 12 g.

The dose-response study reported here was undertaken to define the protein dose at which a significant effect on serum insulin and glucose response might be expected in healthy subjects.

## Subjects and methods

### Subjects

Healthy individuals, eight females and six males aged 28-59 y (mean age 38 y), who were employees of the research center and free of diabetes or family history of diabetes participated after voluntary informed consent was obtained. Subjects had previously participated in similarly designed pilot studies that had examined the effects of various liquid test meals on glucose and insulin response to simple sugars. Consequently, subjects were familiar with the experimental regimen. Before participation in these studies, all subjects were given a standard oral glu-

<sup>1</sup> From the Shaklee Research Center, San Francisco, CA.

<sup>2</sup> Presented in part at the meeting of the American Society for Clinical Nutrition, Washington DC, May 1985.

<sup>3</sup> Address reprint requests to Christopher D Jensen, RD, Shaklee Research Center, 444 Market Street, San Francisco, CA 94111.

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