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Insulin Sensitivity Predicts Glycemia After a Protein Load

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Protein ingestion results in small but distinct changes in plasma glucose and insulin. We hypothesized that the glycemic and/or insulin response to protein might be related to the degree of insulin sensitivity. Our aim was to determine the relationships between insulin sensitivity (assessed by euglycemic-hyperinsulinemic clamp) and postprandial glucose, insulin, C-peptide, and glucagon responses to a 75-g protein meal and a 75-g glucose load. Sixteen lean healthy Caucasian subjects (mean ± SD age, 25 ± 6 years; body mass index [BMI], 23.1 ± 1.7 kg/m^2) participated in the study. After the protein meal, the mean plasma glucose declined gradually below fasting levels to a nadir of −0.36 ± 0.46 mmol/L from 60 to 120 minutes, showing wide intraindividual variation. Insulin sensitivity (M value) was 1.1 to 3.9 mmol/L/m^2 · min in the subjects and correlated inversely with the plasma glucose response to the protein meal ($r = -.58$, $P = .03$), ie, the most insulin-sensitive subjects showed the greatest decline in plasma glucose. In contrast, there was no correlation between insulin sensitivity and the insulin or glucagon response to the protein load, or between the M value and the metabolic responses (glucose, insulin, C-peptide, and glucagon) to the glucose load. Our study suggests that the net effect of insulin and glucagon secretion on postprandial glucose levels after a protein meal might depend on the individual's degree of insulin sensitivity. Gluconeogenesis in the liver may be less susceptible to inhibition by insulin in the more highly resistant subjects, thereby counteracting a decline in plasma glucose.

THE GLYCEMIC RESPONSE after protein consumption is not well described, perhaps because the changes are relatively small in comparison to the effect of a glucose load. Some studies report stable blood glucose levels within 0 to 6 hours of a protein load, while others report a decline.[1-4] One explanation for this variation may be the type of subject studied (normal *v* diabetic) and differences in ethnicity (Caucasian *v* Australian Aboriginal). Type 2 diabetic subjects and ethnic groups with a predisposition to diabetes are more resistant to the effects of insulin on glucose metabolism than healthy Caucasian subjects.[5-7] However, insulin also has profound effects on protein and amino acid metabolism that are less well studied,[8,9] especially in relation to insulin resistance.

Just as insulin-resistant subjects have been shown to have higher glucose and insulin responses after a glucose load,[9,10] we might also expect them to show differences after a protein load. In normal subjects, protein stimulates a significant increase in insulinemia, equivalent to about 30% of the increase found after an equivalent glucose load.[1,2] In combination with carbohydrate, protein reduces glycemia but increases insulinemia.[11] As the quantity of the protein increases, the insulin response increases in a dose-dependent manner.

Because protein stimulates insulin and this in turn stimulates the cellular uptake of glucose, we might expect blood glucose levels to decrease following a protein meal. However, protein also stimulates glucagon secretion,[8] which promotes glycogenolysis and gluconeogenesis, offsetting any insulin-induced decline in glucose levels. However, the net effect of insulin and glucagon secretion on the resulting plasma glucose levels might be different in insulin-resistant versus insulin-sensitive subjects.

We hypothesized that the degree of glycemia and/or insulinemia after a protein meal may therefore depend on the degree of insulin resistance. Within populations, insulin resistance has been shown to vary 4-fold even among those with normal glucose tolerance.[10] The defect of insulin action in 1 of 4 of these individuals does not differ substantially from that of patients with type 2 diabetes. In the present study, we postulated that a protein load may cause a distinct decline in plasma glucose in the most insulin-sensitive individuals, while more insulin-resistant subjects would "resist" this decline and maintain a more stable glucose profile. The aim of this study was therefore to determine the relationships between insulin sensitiv-

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ity (assessed by euglycemic-hyperinsulinemic clamp) and plasma glucose, insulin, C-peptide, and glucagon responses to a 75-g protein meal versus a 75-g glucose load.

SUBJECTS AND METHODS

Sixteen healthy Caucasian subjects (6 females and 10 males) participated in the study. They satisfied the following eligibility criteria designed to obtain a homogenous group: no family history of diabetes, no medication known to interfere with glucose tolerance, age range 20 to 40 years, body mass index (BMI) 20 to 25 kg/m^2, and female subjects not pregnant or using oral contraceptive agents. The mean age was 25 ± 6 years and mean BMI was 23.1 ± 1.7 kg/m^2 (mean ± SD).

The subjects attended the Metabolic Unit of the Department of Endocrinology, Diabetes and Metabolism, Prince of Wales Hospital, Randwick, on 3 separate mornings after an overnight fast. On 1 occasion, insulin sensitivity was assessed using the euglycemic insulin clamp. On the other 2 occasions, an oral glucose tolerance test (75 g glucose) and a protein meal (75 g protein) test were conducted in random order. Individual subjects were studied at least 48 hours apart and all 3 tests were completed over a 2- to 4-week period. In females, the tests were performed within the first 14 days (follicular phase) of the menstrual cycle.

Euglycemic Insulin Clamp

The clamp was maintained by the method of Pacini and Bergman.[12] Intravenous cannulas were inserted into both arms, one for periodic blood collection and the other for dextrose and insulin infusion. After 3 fasting glucose readings (−30, −15, and 0 minutes), insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused at a rate of 40 μU/m^2/min to achieve plasma insulin levels of approximately 100 μU/mL. The rate of glucose infusion (25% dextrose) was regulated by an IVAC 591 peristaltic pump (Phoenix Medical Division, West Ryde, Australia) and adjusted at 10-minute intervals to achieve blood glucose levels close to the fasting value. Stable plasma glucose levels were obtained after approximately 90 minutes, and the glucose infusion rate between 90 and 120 minutes was recorded. The M value was calculated as the rate of glucose infusion per minute per square meter of body surface area in the period between 90 and 120 minutes. During this period, plasma glucose was maintained at 4.3 ± 0.4 (mean ± SD) mmol/L and serum insulin at 109 ± 20 μU/mL.

The 75-g glucose load was given in the form of a 300-mL lime-flavored drink, Glucaid (Histo Laboratories, Sydney, Australia), after an overnight fast. The 75-g protein load was provided as cooked lean beef steak (350 g raw weight). The protein content (21.4 g/100 g raw beef) and fat content (3.5 g/100 g) were derived from Australian food composition tables.[13] The meat was cooked in a nonstick frying pan with 10 mL olive oil either on the morning of the experiment or on the night before and placed in a refrigerator overnight. The meal was reheated in a microwave oven for 2 minutes and served immediately.

After collection of 3 basal fasting blood samples (−30, −15, and 0 minutes), the test meals were consumed gradually over 10 minutes, with time 0 as the start of the meal. Blood samples (30 mL) were collected into heparinized tubes at 15-minute intervals for 180 minutes from a forearm vein via an indwelling cannula kept patent with normal saline (0.9%). After centrifugation, the plasma was removed and stored at −20°C for measurement of plasma glucose, insulin, C-peptide, and glucagon.

Plasma glucose was analyzed using the enzymatic glucose hexokinase method on a glucose multichannel analyzer (Olympus Au5031; Tokyo, Japan). The within-day assay variation for plasma glucose was 2.3% (at 1.3 mmol/L) to 3.5% (at 19.7 mmol/L). The between-day variation for plasma glucose was 3.3% (at 4.2 mmol/L) and 3.1% (at 17.3 mmol/L). Serum insulin and C-peptide concentrations were measured by the double-antibody radioimmunoassay method using protocols developed by the Department of Endocrinology at Prince of Wales Hospital. The interassay variation for the C-peptide assay was 16.6% (at 0.11 nmol/L), 7.3% (at 0.93 nmol/L), and 9.1% (at 1.1 nmol/L). The interassay variation for the insulin assay was 23.1% (at 6.8 μU/mL), 16.5% (at 33.1 μU/mL), 15.2% (at 80.2 μU/mL), and 30.2% (at 192.8 μU/mL). Plasma glucagon was determined using a glucagon double-antibody radioimmunoassay (DPC Diagnostic Products, Los Angeles, CA) at the Endocrinology Laboratory of Royal Prince Alfred Hospital. The intraassay variation was 15.7% (at 35 pg/mL), 4.4% (at 151 pg/mL), and 4.1% (at 564 pg/mL). The interassay variation was 30.3% (at 65 pg/mL) and 17.8% (at 367 pg/mL).

Glucose and hormonal responses to food ingestion were measured geometrically as both the incremental and decremental area under the curve (AUC) using the fasting level as baseline.[14] In all cases, the incremental or decremental areas were integrated over the entire 180 minutes of study and are expressed as the mean ± SD. Statistical differences were determined using Student's *t* test (Stat-View Software; Abacus Concepts, San Francisco, CA). Simple regression analysis was used to determine the relationship between the metabolic responses (AUC) and insulin sensitivity (M value). The significance level was set at .05.

The study was approved by the Medical Ethical Review Committee of Prince of Wales Hospital, and the subjects provided informed written consent.

RESULTS

Fasting glucose, insulin, C-peptide, and glucagon were within the normal range of values for healthy subjects (Table 1). Changes in plasma glucose after the glucose and protein challenges are shown in Fig 1. After the carbohydrate load, plasma glucose increased predictably to a mean peak of 2.4 ± 1.4 mmol/L above fasting levels, followed by relative "hypoglycemia." At 180 minutes after the glucose load, mean plasma glucose levels were still −1.6 ± 1.2 mmol/L below baseline.

After the protein meal, mean plasma glucose declined gradually below fasting levels to a nadir of −0.36 ± 0.46 mmol/L from 60 to 120 minutes. There was wide interindividual variation in the nadir, from as low as −1.7 mmol/L up to 0 mmol/L. By 180 minutes, the mean plasma glucose level returned to baseline, but in some subjects glucose remained 0.5 mmol/L below fasting levels at this time. The mean incremental glucose area after the protein meal (8 ± 16 mmol/L · 180 min) was significantly different versus the mean incremental area after the glucose load (122 ± 78 mmol/L · 180 min, $P < .0001$). The mean decremental areas were not significantly different (−48 ± 51 and −82 ± 77 mmol/L · 180 min, $P > .05$ for protein *v* glucose load, respectively).

The changes in serum insulin are shown in Fig 2. After the glucose load, the mean incremental insulin AUC was 8,020 ± 907 μU/mL · 180 min. In contrast, the subjects showed less insulinemia (incremental AUC, 2,461 ± 281 μU/mL · 180 min) after the protein meal ($P < .0001$). However, the mean insulin level after protein remained above the fasting level at 180

Table 1. Fasting Plasma Glucose and Serum Insulin, Glucagon, and C-Peptide at the Start of the Three Test Days in 16 Subjects (except glucagon, where n = 13)

Parameter	Mean ± SD	Range
Glucose (mmol/L)	4.6 ± 0.2	4.3-4.7
Insulin (μU/mL)	15.2 ± 3.9	7.5-24.0
Glucagon (pg/mL)	193 ± 26	156-253
C-peptide (nmol/L)	0.51 ± 0.16	0.20-0.84

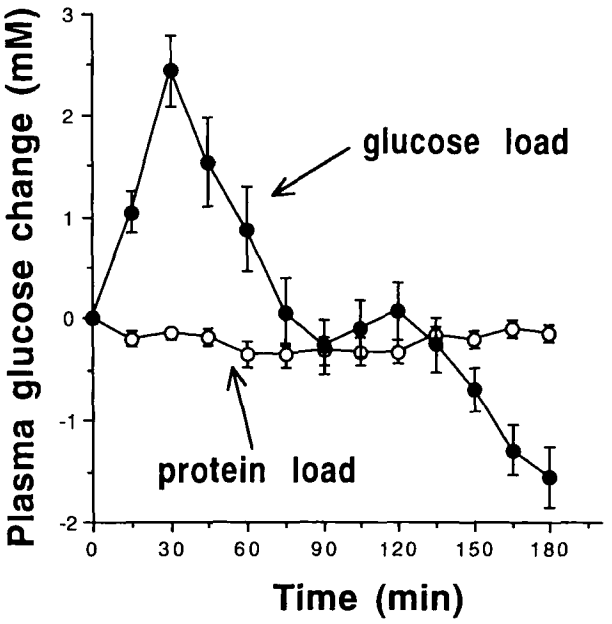

Fig 1. Change in mean ± SE plasma glucose response to 75 g glucose and 75 g protein (n = 16).

minutes (13 ± 20 μU/mL), while the values after the glucose load returned to baseline. Total insulinemia after protein intake may therefore be greater than indicated. As expected, the serum C-peptide response to the meals paralleled the mean serum insulin response (data not shown).

Changes in plasma glucagon are shown in Fig 3. After ingestion of the glucose load, the mean plasma glucagon concentration decreased modestly to a nadir at 15 minutes and remained below the fasting level for the rest of the 180 minutes. The mean decremental glucagon AUC after the glucose load was −3,978 ± 719 pg/mL · 180 min. In contrast, after the

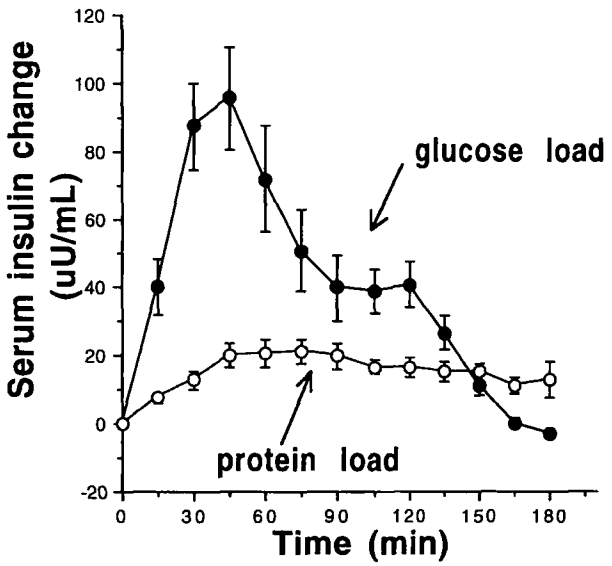

Fig 2. Change in mean ± SE serum insulin response to 75 g glucose and 75 g protein (N = 16).

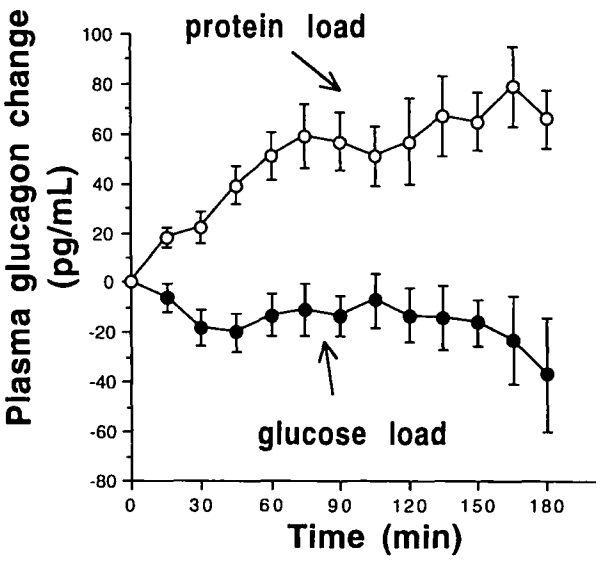

Fig 3. Change in mean ± SE plasma glucagon response to 75 g glucose and 75 g insulin (n = 13).

protein meal, there was an increase in plasma glucagon, which remained elevated for the whole study period (incremental AUC, 10,097 ± 1,377 pg/mL · 180 min, $P < .0001$). There was a nonsignificant trend for a higher plasma glucagon response to be associated with a lower plasma glucose level after the protein meal ($r = -.6$, $P = .06$).

The amount of glucose infused (M value) to maintain euglycemia during the euglycemic clamp was 6.8 to 25.9 mg/kg lean body weight/min (12.4 ± 4.7 mg/kg/min), or between 1.1 and 3.9 mmol/L/m^2 · min when expressed on a body-surface-area basis. The degree of insulin sensitivity (M value) correlated inversely with the decremental glucose AUC after the protein meal ($r = -.58$, $P = .03$; Fig 4), but there was no relationship to the insulin AUC ($r = .11$, NS). In other words, the most insulin-sensitive subjects showed the greatest decline in blood glucose, while the more resistant individuals showed little change (1 subject showed an increase). The slope of the line of best fit indicated that a 1-U increase in insulin sensitivity led to a 12% greater decline in plasma glucose.

There were no significant correlations (or trends) between the M value and the incremental C-peptide or glucagon response to protein ingestion, nor any relationship between insulin sensitivity and the metabolic response (glucose, insulin, C-peptide, or glucagon) to the 75-g glucose load (data not shown).

DISCUSSION

In this study, a significant relationship was found between insulin sensitivity and the extent of the decline in plasma glucose in healthy Caucasian subjects after a high-protein load ($r = -.58$, $P = .03$). The most insulin-sensitive subjects showed the greatest degree of hypoglycemia after protein, in contrast to the more resistant individuals who displayed a relatively stable plasma glucose profile. Surprisingly, there was no relationship between insulin sensitivity and protein-stimulated insulinemia ($r = .11$, NS). Thus, the more stable blood glucose levels after protein found in the more insulin-resistant subjects cannot be

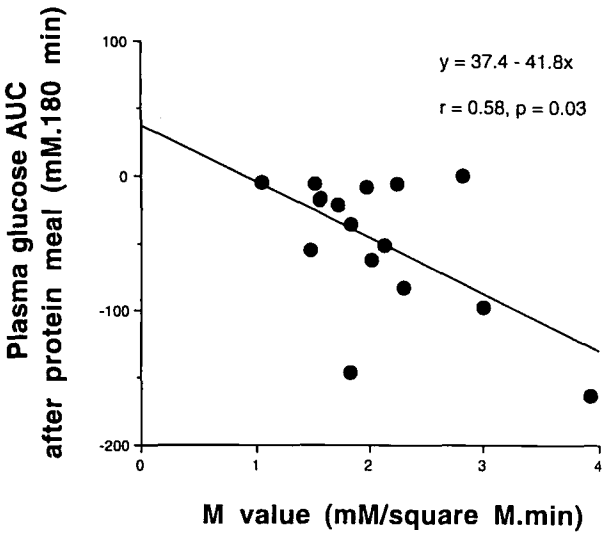

Fig 4. Relationship between insulin sensitivity (M value) and the decremental glucose AUC after 75 g protein (N = 16).

explained by reduced insulin secretion. To our knowledge, this result has not been reported elsewhere. However, the relatively small number of subjects ($N = 16$) and the wide scatter of data around the line of best fit ($r = .58$, $P = .03$) indicate that the results must be interpreted cautiously, requiring confirmation by further studies.

Our findings verify that ingestion of a protein meal results in significant insulinemia,[1-4] albeit less than that found after a glucose load. Furthermore, insulin remained above fasting levels even after 3 hours. Hence, 4- to 8-hour studies may be needed to quantify the full extent of insulinemia stimulated by protein. Nonetheless, the insulin AUC after the protein load was 1 third (34%) of the AUC after the same weight of glucose, a figure similar to that (28%) reported by Krezowski et al.[2]

One might expect that an increase in insulin after protein feeding would reduce plasma glucose levels at least to some degree. But protein also stimulates glucagon secretion,[9,10] which promotes hepatic glucose production and offsets any insulin-induced decline in glucose levels. However, in the present study, subjects who showed higher glucagon responses tended to be those with the greatest decline in plasma glucose ($r = -.6$, $P = .06$). This suggests that a higher glucagon response is actually a compensatory response to decreasing plasma glucose levels. Unfortunately, the intraassay and interassay variation was large in the case of both glucagon and insulin, making the interpretation more difficult.

Our study suggests that the net effect of insulin and glucagon secretion on plasma glucose levels after protein intake might depend on the degree of insulin resistance. Glycogenolysis or gluconeogenesis in the liver from either exogenous or endogenous amino acids may have been higher after the protein load in the more insulin-resistant subjects, thereby counteracting any insulin-induced decrease in plasma glucose. This implies that hepatic glucose production is more resistant to inhibition by insulin in these subjects, even under the physiological conditions of protein feeding. Although hepatic insulin resistance has been well documented in diabetics, it is not clear whether this is a primary or secondary feature of the disease.[9] The 4-fold variation in insulin sensitivity (M value) among our lean healthy subjects may be a function of environmental factors such as physical activity or of genetic inheritance of an insulin-resistant genotype. The varying degree of insulin sensitivity could not be explained by age, BMI, ethnic background, or family history of diabetes in this group of subjects. Any correlation between insulin sensitivity and metabolic responses to protein may be more evident in a population with a predisposition to type 2 diabetes.

The present study lends some support to our previously published hypothesis (the "carnivore connection") that hunter-gatherer diets based largely on animal (protein) foods selected for insulin-resistant individuals who could maintain glucose homeostasis more effectively than insulin-sensitive people.[15,16] Significant declines in plasma glucose after protein meals, as observed in the most insulin-sensitive individuals in the present study (eg, 1 subject had a decline to an absolute level of 2.6 mmol/L), may compromise survival and reproduction. Hypoglycemia may be chronic and accentuated if every meal is based mainly on protein, although further studies are needed to test this. Australian Aboriginals and Alaskan Eskimos (Inuit) provide examples of populations traditionally dependent on an animal protein–based diet and who also appear to be insulin-resistant even when young, lean, and healthy.[6,17] Changes in the environment and diet with westernization (including increases in carbohydrate intake) appear to worsen the insulin resistance to the point where β-cell function is compromised and impaired glucose tolerance and type 2 diabetes ensue.[9]

In summary, we measured insulin sensitivity by a euglycemic clamp in 16 healthy normal-weight Caucasians and compared glucose, insulin, C-peptide, and glucagon responses to 75-g protein and 75-g glucose loads given on separate occasions. Mean plasma glucose levels declined gradually after protein ingestion, reaching a nadir of −0.4 mmol/L between 60 and 120 minutes, but there was wide variation among the subjects. Those with the greatest insulin sensitivity (M value) experienced the greatest decrease in glycemia after protein ingestion. The more insulin-resistant individuals displayed more stable blood glucose levels after protein intake. We speculate that this metabolic characteristic may be an advantage if the diet is based largely on high-protein animal foods, as occurred for some population groups in our evolutionary past.[16] Our findings require confirmation in a larger number of individuals and within populations with greater insulin resistance.

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