Prediction of Glucose and Insulin Responses of Normal Subjects after Consuming Mixed Meals Varying in Energy, Protein, Fat, Carbohydrate and Glycemic Index

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ABSTRACT To see if both the amount and source of carbohydrate consumed determined postprandial glucose and insulin responses of mixed meals, eight nondiabetic subjects took five different mixed meals containing variable energy (1650–2550 kJ), fat (8–24 g), protein (12–25 g) carbohydrate (38–104 g) and glycemic index (43–99). Incremental glucose and insulin responses for the five meals varied over a 2.3-fold range. Amount of carbohydrate alone was not significantly related to the mean glucose and insulin responses. However, using previously derived equations, amount of carbohydrate and glycemic index explained ~90% of the variability of the observed mean glucose and insulin responses (P = 0.01). We conclude that both amount and source of carbohydrate determine the glucose and insulin responses of lean, young, nondiabetic subjects after different mixed meals with variable glycemic index. Variation in protein and fat intake, over the range tested here, appears to have a negligible effect on postprandial glucose and insulin. J. Nutr. 126: 2807–2812, 1996.

INDEXING KEY WORDS:
- humans • carbohydrate • glycemic index
- glucose • insulin

Postprandial blood glucose and insulin responses are influenced by the amount (Gannon et al. 1989) of carbohydrate consumed and its glycemic index (GI) (Jenkins et al. 1981, Wolever et al. 1994). Despite evidence to the contrary (Brand et al. 1991, Chew et al. 1988, Collier et al. 1986, Frost et al. 1994, Indar-Brown et al. 1992, Wolever and Jenkins 1986, Wolever et al. 1992), it is generally considered that the GI is of no practical utility (Hollenbeck et al. 1986, National Institutes of Health 1987) because differences among individual foods are obscured in normal mixed meals by the effects of protein (Estrich et al. 1967, Gannon et al. 1988, Nuttall and Gannon, 1990, Collier et al. 1984) and fat (Estrich et al. 1967, Welch et al. 1987) on insulin secretion and gastric emptying. This view is reflected in the current position of the American Diabetes Association (1994) on dietary carbohydrate which states: "... from a clinical perspective first priority should be given to the total amount of carbohydrate consumed rather than the source of the carbohydrate." By contrast, our hypothesis is that both the amount and source of carbohydrate consumed are important determinants of postprandial glucose and insulin responses. The primary purpose of this study was to test this hypothesis in nondiabetic subjects using normal mixed meals which varied in energy, protein, fat, carbohydrate and GI. We also compared the capillary blood glucose responses to the venous plasma glucose responses to the test meals.

SUBJECTS AND METHODS

Eight normal university students (4 male, 4 female, age 28.4 ± 1.3 y; body mass index 24.6 ± 1.1 kg/m²) were studied on five separate occasions in random order in the morning after an overnight fast using a protocol approved by the Human Subjects Review Commit-

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They mixed each Remained or oats cheese margarine cheese g heated blood energy sampling, finger-prick to mixed 15, 215, g increase and cheese, was 30, 120, 60, 90 and 120 min after subjects started to eat. Immediately after each venous blood sample, a finger-prick capillary blood sample was also obtained. To increase peripheral blood flow and facilitate blood sampling, subjects warmed their hands in electrically heated pads for ~5 min before each blood sample.

The test meals were intended to be representative of normal mixed meals and were designed to vary in energy content, macronutrient composition and GI. They consisted of a cheese omelette, bread and margarine [66 g egg, 40 g cheddar cheese, 100 g 2% milk, 45 g white bread, 10 g margarine]; spaghetti with tomato, cheese and lentils [60 g spaghetti, 50 g red lentils, 100 g tomato, 20 g cheddar cheese]; cornflakes, milk, bread and margarine [45 g cornflakes, 90 g white bread, 200 g 2% milk, 10 g margarine]; oatmeal, milk, bread and margarine [35 g rolled oats, 45 g white bread, 10 g margarine, 125 g whole milk]; and barley with tomato and cheese [barley meal; 60 g pearled barley, 50 g tomato, 20 g cheddar cheese, 50 g 2% milk]. Spaghetti, lentils, oats and barley were weighed dry and cooked by boiling in water according to package directions. Each meal also included 125 g orange juice. Subjects also chose to have coffee, tea or water with 30 mL 2% milk and/or aspartame sweetener if desired; the drink chosen remained constant for each test. The composition of the test meals is shown in Table 1. The GI of each test meal was calculated as previously described [Wolever and Jenkins 1986] as the weighted average of the GI of each food with the weighting based on the proportion of total meal carbohydrate contributed by the foods.

Venous blood samples were taken into 3-mL grey top vacutainers [Beckton-Dickenson, Rutherford, NJ], mixed and kept on ice before centrifuging at 600 × g for 10 min to separate the plasma. Capillary blood samples for glucose analysis were taken into tubes containing about 0.35 mg sodium fluoride and 0.28 mg potassium oxalate. Venous plasma and capillary blood glucose were measured using a YSI analyzer (model 2300, Yellow Springs, OH). Plasma insulin was measured by RIA using a commercially available kit (Pharmacia, Dorval, Quebec).

Results are expressed as means ± SEM. The incremental areas under the observed glucose and insulin response curves, ignoring area beneath the fasting level, were calculated as previously described [Wolever and Jenkins 1986]. Statistical analysis was by two-way ANOVA with subject and test meal as the variables, and the Neuman-Kuels method used to adjust for multiple comparisons [Snedecor and Cochran 1980]. The correlation coefficient, r, between amount of carbohydrate consumed and mean incremental glucose and insulin response areas was determined by linear regression analysis. The predicted effect of amount of carbohydrate and meal GI on glucose and insulin responses was calculated from equations previously derived in nondiabetic subjects who consumed various amounts of different foods [Wolever and Bolognesi 1996] as follows:

\[
GR = 1.5 \cdot GI \cdot (1 - e^{-0.018D}) + 13; \text{ and IR} = 2.9 \cdot (0.6 \cdot GI + 0.003 \cdot GI^2) \cdot (1 - e^{-0.0078D}) + 5,
\]

where GR and IR are the incremental areas under the response curves for glucose and insulin, respectively, expressed as a percentage of those after 50 g carbohydrate from white bread, GI is meal glycemic index and D is amount of carbohydrate in grams. The correlation coefficient, r, between the predicted relative glucose and insulin responses for the five test meals and the observed means was determined by linear regression analysis. The proportion of the variance of the dependent variable (observed glucose or insulin response) which was accounted for by variation of the independent variable (amount of carbohydrate or predicted response) was determined by \(r^2\).
PREDICTING GLUCOSE AND INSULIN RESPONSES

The glucose and insulin responses to the five test meals are shown in Figure 1. The overall mean fasting glucose concentration was greater in venous plasma than capillary blood [4.71 ± 0.05 vs. 3.92 ± 0.04 mmol/L; P < 0.001]. On the other hand, the mean incremental area under the glucose curves for all five meals was greater in capillary blood than in venous plasma [111 ± 13 vs. 91 ± 17 (mmol·min)/L; P < 0.05, Table 2]. The ranking of mean glucose responses for the five meals was similar for capillary or plasma glucose, but on two-way ANOVA, the proportion of total variance not accounted for by test meal and subject for capillary glucose response areas, 34%, was less than that for plasma glucose response areas, 47%. Thus, there was greater heterogeneity between the means for the capillary glucose responses [F(4,28) = 10.35, P < 0.001] than for the plasma glucose responses for the different meals [F(4,28) = 4.06, P < 0.02]. Capillary glucose responses after the omelette and barley meals were significantly less than those after the spaghetti and oatmeal meals, which in turn, were significantly less than that after cornflakes (Table 2). The only significant differences for plasma glucose were that the omelette and barley meal responses were less than that after cornflakes. The mean insulin responses ranked in the same order as the plasma glucose responses, with the responses after the omelette and barley meals being significantly less than that after cornflakes (Table 2).

Amount of carbohydrate consumed was not significantly related to the mean capillary glucose (P = 0.10), plasma glucose (P = 0.20) or plasma insulin (P = 0.11) responses (Fig. 2). By contrast, the predicted glucose responses, based on GI and amount of carbohydrate, were significantly related to the observed mean capillary (r = 0.930, P = 0.022) and plasma glucose responses (r = 0.950, P = 0.013; Fig. 2). Also, the predicted insulin responses were significantly related to the observed mean insulin responses (r = 0.957, P = 0.011, Fig. 2). The y-intercepts of the fitted regression equations of observed on predicted responses were not significantly different from 0 for either capillary glucose (−5 ± 25), plasma glucose (−2 ± 19) or plasma insulin (5.2 ± 2.4). Amount of carbohydrate explained only 65, 47 and 79%, respectively, of the variation of mean capillary glucose, plasma glucose and plasma insulin responses. However, the predictions based on amount and source of carbohydrate explained 86, 90 and 92% of the variance.

TABLE 2

<table>
<thead>
<tr>
<th>Meal</th>
<th>Capillary glucose</th>
<th>Plasma glucose</th>
<th>Plasma insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omelette</td>
<td>71 ± 8</td>
<td>62 ± 9</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Spaghetti</td>
<td>116 ± 12</td>
<td>89 ± 13</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Cornflakes</td>
<td>163 ± 16</td>
<td>135 ± 24</td>
<td>27 ± 7</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>128 ± 16</td>
<td>113 ± 27</td>
<td>20 ± 6</td>
</tr>
<tr>
<td>Barley</td>
<td>77 ± 11</td>
<td>59 ± 12</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. abc Means with different letter superscripts are significantly different (P < 0.05).
Thus, the relationship between the amount of carbohydrate consumed and responses for capillary glucose (left), plasma glucose (center) and plasma insulin (right) was determined. Predictions were based on amount of carbohydrate and meal GI (see Table 1). Points represent means ± SEM for observed incremental areas under the curve for eight normal subjects. Lines represent regression equations, extrapolated to show the y-intercept, but not forced through the origin. None of the y-intercepts is significantly different from 0.

**FIGURE 2** Determinants of postprandial glucose and insulin responses after five different mixed test meals taken by normal subjects. Top: relationship between amount of carbohydrate consumed and responses for capillary glucose (left), plasma glucose (center) and plasma insulin (right). Bottom: relationship between predicted and observed responses for capillary glucose (left), plasma glucose (center) and plasma insulin (right). Predictions were based on amount of carbohydrate and meal GI (see Table 1). Points represent means ± SEM for observed incremental areas under the curve for eight normal subjects. Lines represent regression equations, extrapolated to show the y-intercept, but not forced through the origin. None of the y-intercepts is significantly different from 0.

**DISCUSSION**

The results supported our hypothesis that both the amount and source of carbohydrate consumed are important determinants of postprandial glucose and insulin responses of mixed meals in nondiabetic subjects. Amount of carbohydrate alone was not significantly related to the glucose and insulin responses. Both the amount and source of carbohydrate consumed had to be taken into account to obtain significant correlations between the composition of the meal and the glucose and insulin responses. This is not consistent with the implication of the current position of the American Diabetes Association (1994) on carbohydrate that the amount of carbohydrate consumed is clinically more important than its source. One of the reasons for the discrepancy is that different commonly consumed foods may not have different GI. Bread, french fries, corn chips, canned pea soup, angel food cake and Cheerios™ have almost identical GI (Wolever et al. 1994). Thus, merely consuming a different source of carbohydrate does not necessarily result in a different glycemic response. For source to have an effect, the GI must be different. In practical terms, the hypothesis that both amount and source of carbohydrate are clinically important is supported by the results of a recent study showing that dietary advice based on both amount and source of carbohydrate resulted in better conformance to dietary guidelines and better clinical outcome in subjects with newly diagnosed diabetes, compared with standard advice based primarily on the amount of carbohydrate (Frost et al. 1994).

The omelette meal, high in protein and fat, and the spaghetti meal, high in protein, were expected to result in lower glucose and higher insulin responses than predicted because of the effect of protein in stimulating insulin secretion (Gannon et al. 1988). For the omelette meal, the mean glucose response was more than one SD below the regression line. However, this was not due to increased insulin, and the mean insulin response for this meal was also below the regression line. Similarly, the mean insulin and glucose responses for the spaghetti meal were both below the regression line. Thus, the variation in protein and fat among the different meals does not account for the differences between observed and predicted responses.

The predicted glucose and insulin responses of the meals were derived only from the amount of carbohydrate and meal GI, without taking into account differences in protein and fat. For single foods, the equations accounted for 92–94% of the variability of glucose responses and 85% of the variability of insulin responses (Wolever and Bolognesi 1996). The remainder of the variation, 6–15%, was due, at least in part, to experimental error, including within and between subject variation, order effects and analytic error. In the present study, in the setting of mixed meals, about 90% of the variability of the glucose responses and 85% of the variation of the insulin responses were explained by the prediction equations which do not account for the effects of protein and fat. Thus, if protein and fat influenced glucose and insulin responses, the predictions for mixed meals varying in protein and fat content would not be expected to be as accurate as for single foods. However, the proportion of the variance of observed responses explained by the predictions was virtually identical in the context of single foods and mixed meals. This suggests, at least in normal subjects over the range of intakes tested, that the carbohydrate component of meals is the primary determinant of postprandial glucose and insulin with variation in meal protein and fat appearing to have a negligible effect.

We do not intend the results of this study to be seen as denying the validity of the many excellent studies showing that protein and fat influence postprandial glucose and insulin responses (Collier et al. 1984, Estrich et al. 1967, Gannon et al. 1988, Gulliford et al. 1989, Nuttall and Gannon 1990, Nuttall et al. 1984, Spiller et al. 1987, Welch et al. 1987). These studies have been useful to understand the effects of protein...
and fat, and we agree that, under certain circumstances, the addition of protein to carbohydrate stimulates insulin secretion and reduces postprandial glucose responses, and the addition of fat delays gastric emptying and delays or reduces postprandial glucose responses. However, we question the extrapolation of these results to normal diets. Our major reservations about the widely held view that protein and fat influence postprandial glucose and insulin responses concern the nature of the test carbohydrate and the doses of protein and fat used. The effects of protein and fat have often been studied by adding 25–50 g protein and/or 40 g fat to 50 g glucose (Estrich et al. 1967, Gannon et al. 1988, Nuttall and Gannon 1990, Nuttall et al. 1984). In some studies 0.5–1 g fat and/or 1 g protein per gram of carbohydrate were added to lentils or potato (Collier et al. 1984, Gulliford et al. 1989, Welch et al. 1987), and in one study, 16–50 g protein was added to a liquid test meal containing 58 g sugars (Spiller et al. 1987).

The effects of varying fat and protein in normal mixed meals containing starchy foods may be different from those in which the test carbohydrate is glucose or sucrose because most normal meals to which protein or fat might be added are not protein or fat free. The effects of adding 25 g protein or fat to a meal already containing some protein and fat may be less than those observed if the protein or fat is added to a glucose or sucrose. Indeed, there is evidence that the dose-response effect of protein on insulin secretion is not linear. Adding 16 g protein to a liquid test meal containing no protein and 58 g carbohydrate from sugars reduced the glucose response by 40% and doubled the insulin response. When protein was increased to 50 g, the glucose response was reduced by a further 40%, but the insulin response was no greater than after the meal containing 16 g protein (Spiller et al. 1987).

When 25–50 g of fat is added to 50 g carbohydrate, 53–69% of the energy of the test meal is provided by fat. This is about twice the recommended intake of 30–35% fat, and nearly twice the amount of fat normally consumed in high fat North American diets, 35–40%. When 25–50 g protein is added to 50 g carbohydrate, 33–50% of energy is provided by protein, which again is about twice the 12–20% which is recommended and actually consumed. However, dietary recommendations apply to whole diets and not individual meals. Thus, individual meals may vary widely in protein and fat and still be compatible with dietary recommendations. It is easy to think of normal meals or snacks which are virtually protein and fat free (e.g., pop, juice or fruit) or virtually carbohydrate free (e.g., eggs, cheese or meat eaten alone). However, there are few data about what normal individuals eat in meals because most dietary surveys report data for the whole diet. Thus, while the meals tested here were meant to be “representative,” it is difficult to know exactly how applicable the results of the present study are to normal diets.

A number of studies determined the composition of breakfast in children and adolescents (Morgan et al. 1981 and 1986a), college students (Hammond and Chapman 1994), adults (Morgan et al. 1986b) and elderly persons (Morgan et al. 1986c). Breakfast cereal and milk with or without bread or juice, and eggs with or without bread, milk or juice are the most popular breakfast meals. Thus, our test meals, including a meal of eggs and bread and two meals of cereal and bread, were representative of normal breakfast meals. However, the composition of breakfast is extremely variable; for example, the approximate mean ± SD composition of breakfast of 1970 adults over the age of 62 who ate breakfast was as follows: energy 1795 ± 1255 kJ, protein 15 ± 12 g, fat 18 ± 18 g and carbohydrate 55 ± 35 g (Morgan et al. 1986c). The high SD suggest that the distribution of intakes is skewed, making it impossible from these data to determine what proportion of normal breakfast meals would be represented within the range of test meal composition fed here. The 1977–78 Nationwide Food Consumption Survey showed that ~20% of total daily energy and ~18% of total daily protein intake of the United States population is consumed at breakfast, with 24% of energy and 24% of protein at lunch and 44% of energy and 50% of protein at dinner (Kennedy et al. 1982). This suggests that the range of meals in this study may be similar to normal breakfast and lunch meals, but are probably not representative of normal dinner meals which on average, are twice the size of breakfast and lunch with a high protein content.

Previous studies have used different types of blood sampling to determine postprandial glucose responses. The present results confirm our previous study (Wolver and Bolognesi 1996) which showed that incremental glycemic responses have a greater magnitude and less variability when measured in capillary blood than in venous plasma. Thus, measuring glucose in capillary blood may be preferable to measuring it in venous plasma for detecting differences in glycemic response between different test meals.

We conclude that both the amount and source of carbohydrate consumed determine the glucose and insulin responses of lean, young, nondiabetic subjects after different mixed meals with variable GI. Variation in protein and fat intake, over the range tested here, appears to have a negligible effect on postprandial glucose and insulin responses.

LITERATURE CITED


