Food Amylose Content Affects Postprandial Glucose and Insulin Responses

Kay M. Behall1,2 and Daniel J. Scholfield1

ABSTRACT

Beneficial reduction in glycemic response has been observed after consumption of some high-amylose foods. This study examined the effect of varying the moisture content and particle size of the starch in a test food. Twelve men and 12 women consumed corn chips or corn muffins made with starch or starch plus cornmeal from standard corn (30% amylose, 70% amylopectin) or high amylose (70%) corn. Half of the subjects were hyperinsulinemic based on a prestudy glucose challenge. No gender differences were observed. The hyperinsulinemic subjects had significantly higher insulin and glucose responses and area under the curve as compared with the normal responders. Average glucose, insulin and glucagon were usually lower after muffins compared with chips or foods containing cornmeal. Insulin and glucose responses, but not glucagon, were significantly lower after the consumption of foods made with high-amylose compared with standard corn starch and in control versus hyperinsulinemic subjects. Average plasma glucose and insulin area under the curves after high-amylose foods were approximately half those after standard corn starch. The presence of cornmeal (increasing the particle size of the starch) had less effect on the response of glucose, insulin or glucagon than the type of starch or food consumed.

METHODS AND MATERIALS

Twenty-four volunteers (12 men and 12 women), age 25–57, who were within 20% of their desirable weight for height completed this study (Table I). The study design was approved by the Human Studies Committees of the U.S. Department of Agriculture and Georgetown University. Written informed consent of the volunteers was obtained before the study. Subjects were screened before the study with a glucose tolerance test consisting of 1 g of glucose/kg of body weight and physician evaluation. Based on the simplified rating system utilized by Reiser et al (1981), half of the subjects selected had higher than normal insulin responses (hyperinsulinemic) to the glucose and half were normal responders (controls). Physicians from Georgetown University, who provided medical supervision throughout the study, evaluated the health history and clinical screening values to eliminate those with overt disease before subjects were accepted for study participation. None of the subjects were taking drugs known to affect glucose or insulin metabolism. Subjects maintained their typical diets throughout the study. The four test foods consisted of muffins or cheese-flavored corn chips made with food-grade corn starch alone or combined with cornmeal that was either high-amylose (70% amylose, 30% amylopectin) or standard corn (30% amylose, 70% amylopectin) and potato flour (low amylose) (Table II). The foods were consumed in a Latin square design. Starches and high-amylose corn were obtained from American Maize Products Co. (Hammond, IN). High-amylose corn was ground to meal by the USDA Northern Regional Research Center in Peoria, IL.

Test foods were weighed to give a load dose of 1 g of available carbohydrate/kg of body weight. The muffins and chips made with the corn starchy with and without cornmeal were analyzed for total dietary fiber (TDF) and RS content (Table II) which were used to calculate available carbohydrate. TDF in the chips and muffins was determined by using AOAC TDF method 985.29 (Lee et al 1992). The amount of RS in the chips and muffins was determined using AOAC method 991.43 (Lee et al 1992) with and without pretreatment with dimethyl sulfoxide. Starch was calculated from the glucose content in enzyme hydrolyzate as determined by high-performance anion-exchange chromatography (Li 1999). All fiber and starch analysis (with and without dimethyl sulfoxide) were performed in duplicate on duplicate samples. Calories, protein, and fat were calculated from the food ingredients (U.S. Department of Agriculture 1976–1988).

Both the muffins and chips were made the day before the foods were consumed as a meal tolerance test. Ingredients for each food were weighed and mixed, then weighed again in the cooking utensil.

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Muffin dough was placed in the tins to a consistent depth for even baking. Chip dough was rolled on the baking sheet at a measured thickness prior to baking. Muffins were baked at 350°F for 20 min; chips were baked at 300°F for 20 min. After foods were baked and cooled, they were weighed again to determine moisture loss. Moisture content averaged 13.8% for the cooked chips and 34.1% for the muffins. The cooked weight of the food and the available carbohydrate in the recipe were used to determine the amount of food to be fed as a tolerance meal. Foods were stored at room temperature overnight and, in the case of the muffins, briefly warmed in a microwave before consumption. A standardized amount of water (3 mL/kg of body weight) was given to each subject to consume with starch food. No further water was allowed until 1 hr after the blood drawing.

Blood samples were collected before the test foods were consumed and at 0.5, 1, 2, and 3 hr after. A protease inhibitor was added to the EDTA containing blood collection tubes. Plasma glucose was determined by an automated enzymatic method (Baker Instruments Corp., Allentown, PA). Plasma insulin and glucagon were measured by radioimmunoassay using commercially available kits (ICN Biomedicals, Costa Mesa, CA, and Diagnostics Products Corporation, Los Angeles, CA, respectively).

Data were statistically analyzed by using a mixed models procedure for repeated-measures analysis of variance (PCSAS, v. 8.0, SAS Institute, Cary, NC). Data were evaluated for the main effects of group (hyperinsulinemic vs. control), starch type (high-amylose vs. low-amylose), food (chips vs. muffins), sex, time, and interactions between the main effects. Data are reported as least squares means ± SEM. The critical level of significance used was P < 0.05.

### RESULTS

No significant differences were observed between the mean and female subjects. Also, no significant differences were observed in fasting values. Average concentrations for glucose, insulin, and glucagon were significantly lower after test foods containing high-amylose than after test foods containing low-amylose starch. Average glucose concentration was significantly lower after muffins compared with the chips made with cornmeal and both foods with cornmeal were significantly lower than the muffins and chips made with only corn starch (test food, P < 0.0001). Average glucose concentration (Table III) after the muffins and chips made with high-amylose starch with cornmeal were significantly lower than the test foods made with low-amylose starch and chips made with low-amylose starch alone, and had significantly higher average glucose than all other test foods. When all muffins were compared with all chips, average glucose was significantly lower after the muffins (P < 0.014), especially those made with high-amylose (P < 0.0001). When all test foods made with cornmeal were compared with those without, average glucose was significantly lower with than without the presence of cornmeal.

Overall hyperinsulinemic subjects had higher mean glucose levels (P < 0.0001) (Fig. 1). Hyperinsulinemic subjects had significantly higher average glucose response after the chips than after the muffins and higher responses after the foods made with only starch. The average glucose response of the control subjects after the four test foods were not significantly different. Glucose responses to the test foods varied with the starch and group (Fig. 1). Significant differences between the two starches and between control and hyperinsulinemic subjects occurred primarily 30 min
after the test foods, regardless of the food consumed. Plasma glucose levels of the hyperinsulinemic subjects 1 hr after consumption of the high-amylose test foods were lower than those after low-amylose foods.

Plasma glucose area under the curve (AUC) (Table IV) showed significant differences between the groups, starch types, and the group-by-starch interaction. Average plasma glucose AUC was higher after the low-amylose than after the high-amylose starch for the same test food. Muffins tended to have lower AUC than chips, but the difference was not significant. Hyperinsulinemic subjects had the highest glucose response area; at least twice that of the controls after either starch.

Mean insulin concentrations (Table III) were significantly lower after the muffins made with or without cornmeal compared with the chips made with cornmeal and chips made with only corn starch (food, \( P < 0.002 \)). Average insulin concentration (Table III) after the muffins and chips made with high-amylose starch were significantly lower than other test foods while chips made with low-amylose starch with or without cornmeal had significantly higher average insulin than all other test foods.

Because subjects were grouped based on their insulin responses to a glucose tolerance test it was expected that the hyperinsulinemic subjects would have significantly higher (\( P < 0.0001 \)) insulin response than the controls. Mean insulin levels were lower after the high-amylose test foods than those observed after low-amylose, regardless of group. The highest response was after the low-amylose cornmeal chips and starch muffins in the hyperinsulinemic subjects, while the controls had their highest response to the low-amylose starch.

Significant time, time-by-group, time-by-starch, and time-by-group-by-starch interactions were observed in insulin response (Fig. 2). Difference between the two starchy and subject type occurred at 2 and 1 hr after the consumption of the test foods with significant differences between the two starchy and between control and hyperinsulinemic subjects at each time point.

Plasma insulin concentrations of the hyperinsulinemic subjects 2 hr after the low-amylose test foods were still higher than the subjects after high-amylose test foods or the control subjects. Control subjects had relatively flat insulin response after both high-amylose test foods.

![Figure 1. Glucose responses of 12 hyperinsulinemic (HI) and 12 control subjects to amylose (high-AM) and standard (low-AM) corn starch foods. Least-square means ± SEM. Glucose was significantly affected by group (HI > C, \( P < 0.0001 \)), starch (low-AM > high-AM, \( P < 0.0001 \)), time (\( P < 0.0001 \)), and starch x time interaction (\( P < 0.003 \)) and group x time interaction (\( P < 0.05 \)).](image)

![Figure 2. Insulin responses of 12 hyperinsulinemic (HI) and 12 control subjects to amylose (high-AM) and standard (low-AM) corn starch foods. Least-square means ± SEM. Insulin was significantly affected by group (HI > C, \( P < 0.0001 \)), starch (low-AM > high-AM, \( P < 0.0001 \)), time (\( P < 0.0001 \)), and group x time interaction (\( P < 0.001 \)), and group x time x time interaction (\( P < 0.024 \)).](image)

**TABLE III**

<table>
<thead>
<tr>
<th>Test Food</th>
<th>Mean Glucose (mmol/L)</th>
<th>Mean Insulin (mmol/L)</th>
<th>Mean Glucagon (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High-Amylose</td>
<td>Low-Amylose</td>
<td>High-Amylose</td>
</tr>
<tr>
<td>Chips</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>4.68 ± 0.12bc</td>
<td>5.19 ± 0.12a</td>
<td>370.3 ± 62.7bc</td>
</tr>
<tr>
<td>Cornmeal + starch</td>
<td>4.47 ± 0.08d</td>
<td>4.87 ± 0.08b</td>
<td>254.5 ± 48.6d</td>
</tr>
<tr>
<td>Muffins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>4.73 ± 0.12bc</td>
<td>4.80 ± 0.12bc</td>
<td>343.7 ± 62.2cd</td>
</tr>
<tr>
<td>Cornmeal + starch</td>
<td>4.48 ± 0.08d</td>
<td>4.58 ± 0.08cd</td>
<td>281.9 ± 48.3d</td>
</tr>
</tbody>
</table>

\( ^{a} \) Means ± standard error of the means. Values within a parameter (glucose, insulin, or glucagon) followed by different letters are significantly different at \( P < 0.05 \).

\( ^{b} \) High-amylose (70% amylose, 30% amylopectin); standard corn (30% amylose, 70% amylopectin, low-amylose).

\( ^{c} \) ANOVA for glucose: test food, \( P < 0.0001 \); starch, \( P < 0.0001 \); test food x starch, \( P < 0.016 \).

\( ^{d} \) ANOVA for insulin: test food, \( P < 0.023 \); starch, \( P < 0.0001 \); test food x starch, \( P < 0.002 \).

\( ^{e} \) ANOVA for glucagon: test food, \( P < 0.001 \); starch, \( P < 0.09 \); test food x starch, \( P < 0.005 \).
Plasma insulin AUC (Table IV) showed significant differences between the groups, starch types, test foods, fiber-by-starch, and group-by-starch interactions. Average plasma insulin AUC after low-amylose muffins was higher than after the high-amylose muffins but areas after the low-amylose chips were more than double those after high-amylose chips. Insulin AUC after the chips made with low-amylose were significantly higher than all other test foods. Hyperinsulinemic subjects had the highest insulin response area. Response AUC of the hyperinsulinemic subjects after test foods made with high-amylose were reduced to levels similar those of the controls consuming low amylose.

Glucagon varied with time (P < 0.001) but no interactions with group, fiber, or starch was observed (Fig. 3). Average glucagon concentration (Table III) was significantly lower after the muffins than after the chips. Muffins made with the low-amylose starch had the highest average glucagon concentration (food, P < 0.001). When all muffins were compared with all chips, no significant differences were observed in average glucagon. Hyperinsulinemic subjects averaged higher levels than the controls and had the highest average concentrations after the low-amylose starch chips and muffins. Highest levels of control foods were after the low-amylose starch chips and starch muffins, and lowest levels after the low-amylose cornmeal chips (food-by-group-by-starch, P < 0.05).

### DISCUSSION

Research has shown that the source and the form of the carbohydrate consumed make a difference in the glucose response to the food in control or diabetic subjects (Behall et al 1989, 2002; Liljeberg et al 1992; Björck et al 1994; Behall and Howe 1995; Foster-Powell and Miller 1995; Granfeldt et al 1995). The physical form of the food rather than the fiber (white flour vs. wholemeal) content and particle size was considered to be the major factor reducing the glycemic and insulin responses after spaghetti compared with bread (D’Emden et al 1987). Similar to our results, consumption of high-amylose muffins by NIDDM subjects resulted in lower glucose levels and insulin responses than did the corn flakes low-amylose muffin (Krezowski et al 1987). Standard corn flakes (low-amylose) resulted in lower insulin response but glucose response similar to that after the low-amylose muffin. Liljeberg et al (1992) reported lower responses in glucose and insulin when kernels were present in the bread compared with bread made with flour alone.

Several previous studies have shown that size of food particles has a significant effect on insulin and glucose responses. Foods consumed in large pieces or without chewing resulted in lower postprandial glucose levels than observed after the same food had been thoroughly chewed (Collier and O’Dea 1982; Read et al 1986). Consumption of the same food differing in fiber content or in coarseness of the flour or grain particles more commonly occurs. A few studies have reported increased corn fiber in the tolerance meal had little or no significant effect on glucose or insulin (Walker and Walker 1984; Feldman et al 1995). No significant increase in either peak response nor AUC of glucose occurred after an increase in the particle size of whole wheat flour (Heaton et al 1988; Leclere et al 1993; Behall and Halffrisch 1999). However, lower peak glucose response and AUC after coarse flour, cracked grains, or whole grains has also been reported compared with fine ground flour (O’Donnell et al 1989; Holt and Miller 1994). Lower peak insulin response and AUC after coarse flour, cracked grains, or whole grains was reported compared with fine ground flour (Heaton et al 1988; Holt and Miller 1988). Particle size appears to exert the greatest effect on glucose and insulin response when large food or grain particles are present although we did observe some reduction when cornmeal was present in our test foods.

**Fig. 3. Glucagon responses of 12 hyperinsulinemic (HI) and 12 control subjects to amylose (high-AM) and standard (low-AM) corn starch foods. Least-square means ± SEM. Glucagon was significantly affected only by time (P < 0.0001).**

### TABLE IV

<table>
<thead>
<tr>
<th>Food</th>
<th>Glucose (mmol x min/L)</th>
<th>Insulin (mmol x min/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High-Amylose</td>
<td>Low-Amylose</td>
</tr>
<tr>
<td>Chips</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>51.2 ± 17.0bc</td>
<td>96.3 ± 17.0a</td>
</tr>
<tr>
<td>Cornmeal + starch</td>
<td>25.1 ± 10.4c</td>
<td>85.6 ± 10.4a</td>
</tr>
<tr>
<td>Muffins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornstarch</td>
<td>34.1 ± 14.9bc</td>
<td>63.1 ± 27.4ab</td>
</tr>
<tr>
<td>Cornmeal + starch</td>
<td>26.4 ± 10.2c</td>
<td>61.9 ± 10.2ab</td>
</tr>
<tr>
<td>Subject Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.3 ± 11.0c</td>
<td>53.4 ± 11.9b</td>
</tr>
<tr>
<td>Hyperinsulinemic</td>
<td>48.0 ± 11.0b</td>
<td>100.7 ± 11.9a</td>
</tr>
</tbody>
</table>

| a | Means ± standard error of the means. Mean values of chips and muffins within a parameter (glucose or insulin) followed by different letters are significantly different at P < 0.05. Means of subject groups within a parameter (glucose or insulin) followed by different letters are significantly different at the P < 0.05. |
| b | ANOVA for glucose area: test food, P < 0.01; group, P < 0.014; starch, P < 0.0001; group x starch, P < 0.003; test food x starch, P < 0.01. |
| c | ANOVA for insulin area: test food, P < 0.05; group, P < 0.0003; starch, P < 0.0001; group x starch, P < 0.0016; test food x starch, P < 0.006. |

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In the present study, both the high-amylose chips and the muffins made with or without fiber showed significantly lower glucose and insulin response compared with low-amylose test foods, while glucagon response to the starch type was not significantly different. Hyperinsulinemic and control subjects showed similar patterns of response. Previous research in this laboratory (Behall et al. 1988, 1989, 1995, 2002) showed significant decreases in the insulin and glucose responses with nonsignificant decreased in glucagon after crackers or bread made with high-amylose compared with low-amylose corn starch. These foods were made with corn starch without the addition of commel. Others have reported lower glucose or insulin after foods made with high-amylose starch compared with comparable food with standard starch. (Krezowski et al. 1987; Weststrate and van Amelsvoort 1993).

Behall and Howe (1995) reported that normal and hyperinsulinemic subjects fed high-amylose bread had an average of 13 g of RS/tolerance meal, (Behall and Howe 1996) had significantly reduced insulin concentration and insulin AUC compared with values after low-amylose bread (<1.0 g of RS/tolerance meal). The greatest reduction in insulin occurred in the hyperinsulinemic subjects consuming the high-amylose bread. Abnormal insulin and glucose levels have been used as the primary indicators for carbohydrate sensitivity and NIDDM (Beebe and Rubenstein 1987; Kuczmaszki et al. 1994). Overweight hyperterglyceridemic men and women fed muffins containing 60 g of total carbohydrate and 5.8 g (high-amylose) or 1.3 g (low-amylose) of RS had plasma glucose 7% lower and insulin 25% lower after the high-amylose muffin compared with the low-amylose muffin (Noaks et al. 1996). Only the insulin change was statistically significant. Hoebler et al. (1999) fed white bread (93 g of TDF and 1.3 g of RS) and high-amylose corn starch bread (89 g of TDF and 16.5 g of RS) as part of a meal. Plasma glucose and insulin were significantly lower at 90–105 min and 90–200 min, respectively, after the high-amylose than after the white bread meal. Similar to the results in this study, significant decreases in glucose or insulin have been reported when the test meal contained over 12 g of RS (Behall and Howe 1995; Hoebler et al. 1999; Behall et al. 2002).

Granfeldt et al. (1995) fed normal subjects three tolerance meals made with either standard corn (providing 46.8 g of available carbohydrate and 1.8 g of RS) or high-amylose corn starch (providing either 29 or 45 g of carbohydrate and 15.9 or 24.7 g of RS). Both tolerance meals containing the high-amylose corn resulted in lower area under the curves for glucose and insulin compared with the standard corn even when the available carbohydrate consumed was the same. The glucose and insulin responses after the two high-amylose tolerance meals were similar even though the carbohydrate, TDF, and RS content was higher in one of the tolerance meals. Similar to the results of Granfeldt et al. (1995), the type of starch had a greater effect than the fiber or RS content.

Behall et al. (2002) fed subjects breads varying in amylose content. Subjects consumed an average of 8 g of RS in the tolerance meal with 50% of the starch as amylose, the level at which glucose was reduced, and 11.5 g in the test with 60% amylose, the level at which both glucose and insulin were reduced. Our results indicate that the amylose content of the starch used in tolerance meals needs to be >50% or the RS content in the tolerance meal needs to be >8 g (AOAC) to significantly reduce of plasma glucose and insulin in men and women.

Of the four other studies that reported RS content of the tolerance meal, all were fed 0.6–1.8 g of RS in their low-amylose food, which was similar to our 30% amylose bread. Noaks et al. (1996) fed 5.8 g of RS (similar to our 40% amylose bread tolerance meal) as their high level with the same results we observed (no significant difference). Other researchers (Behall and Howe 1995; Granfeldt et al. 1995; Hoebler et al. 1999) fed 13, 15.9, and 16.5 g of RS, respectively, as a higher level (similar to our 60% amylose bread tolerance meal) and reported significant differences between the low and higher intake amounts. Granfeldt et al. (1995) also had a higher RS level (15.9 vs. 24.7 g of RS), similar to our 60% and more than our 70% amylose bread tolerance meal analyzed by their method. This was not significantly different from our results.

CONCLUSIONS

Overall, particle size had less effect on glucose, insulin, or glucagon response than did the type of food or starch included in foods tested. Average glucose concentration was lowest after the high-amylose muffins and chips with commel and the highest was after the low-amylose starch muffins. Mean insulin concentrations were lower after the muffins than after the chips, regardless of the commel content. All high-amylose foods were lower than that observed for low-amylose. It was concluded that RS development in the moister food had a greater effect on glycemific response than the change of particle size of the starch by the addition of commel to the food.

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LITERATURE CITED


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