ABSTRACT

Background: Growth in normal and malignant tissues has been linked to hyperinsulinemia and insulin-like growth factors (IGFs). We hypothesized that IGF and IGF-binding protein (IGFBP) responses may be acutely affected by differences in the glycemic index (GI) of foods.

Objective: We compared the postprandial responses of IGFs and IGFBP to 2 foods of similar macronutrient composition but with greatly different GIs—pearled barley (GI: 25) and instant mashed potato (GI: 85).

Design: Ten young lean subjects consumed 50-g carbohydrate portions of the 2 foods or water (extended fast) in random order after an overnight fast. Capillary blood was collected at regular intervals over 4 h for measurement of blood glucose, insulin, and components of the IGF system.

Results: Serum IGFBP-1 declined markedly after both meals, but the mean (±SEM) change at 4 h was significantly (P < 0.01) more prolonged after the low-GI meal (−55 ± 20 ng/mL) than after the high-GI meal (−13 ± 15 ng/mL). Conversely, the change in serum IGFBP-3 concentration at 4 h was significantly (P < 0.05) higher after the low-GI meal (251 ± 102 ng/mL) than after the high-GI meal (−110 ± 96 ng/mL); the same pattern was observed at 2 h. Changes in IGFBP-2, free IGF-1, and total IGF-1 responses were minimal and did not differ significantly from those during the 4-h fast.

Conclusion: Acute changes in IGFBP-3 after low-GI and high-GI foods may provide a biologic mechanism linking cell multiplication with greater consumption of high-GI carbohydrates. Am J Clin Nutr 2005;82:350–4.

KEY WORDS Glycemic index, insulin, insulin-like growth factors, cancer

INTRODUCTION

There is increasing recognition that cancer growth may be promoted by hyperinsulinemia (1). Known risk factors for colorectal cancer, eg, physical inactivity, obesity, diabetes, and low-fiber diets, can be linked through a common association with high blood concentrations of insulin. Hyperinsulinemia could promote tumor development by increasing the activity of insulin-like growth factors (IGFs), which in turn stimulate cell proliferation and inhibit apoptosis (programmed cell death) (2). Postprandial hyperglycemia per se, as assessed by high 2-h postchallenge blood glucose concentration, has also been linked to a greater risk of colon cancer (3). Refined sugar and starchy foods that increase postprandial glycemia have been positively associated with colorectal and breast cancer (4, 5). Conversely, fiber, which has variable effects on the rate of carbohydrate absorption, has been associated with a lower cancer risk in some studies (6) but not in others (7). Because total carbohydrate intake per se shows no relation to cancer risk, the glycemic nature of the carbohydrate may be more important than its absolute amount.

The degree of postprandial glycemia is influenced by both the quality and quantity of carbohydrate in the meal (8). The glycemic index (GI) of a food is a summative measure of the carbohydrate quality or “glycemic potential” on a scale on which the reference food (glucose or white bread) by definition has a GI of 100. The GI permits comparisons of carbohydrates in different foods on a gram-for-gram (or weight-for-weight) basis. The average GI of whole diets has been linked to increased cancer risk in several case-control and observational studies (9). The concept of glycemic load (GL), defined as the product of the GI and the carbohydrate content, was introduced to derive a global estimate of postprandial glycemia and insulin demand. In the Women’s Health Study, GL was independently related to colorectal cancer with a relative risk of 2.85 between the highest and lowest quintiles after adjustment for known confounders (10). Similarly, in an Italian case-control study, persons in the highest quintile of GL had a relative risk of 1.8 after adjustment for age, body mass index, physical activity, fiber, and other risk factors (11). Breast cancer development may also be sensitive to hyperglycemia and insulinemia. In the same Italian study (12), the highest quintile of GI and GL had an adjusted relative risk of 1.4 and 1.3, respectively.

The association between dietary GI or GL and cancer risk may be mediated by the IGF system (9). IGF-I circulates while bound to 1 of 6 IGF-binding proteins (IGFBP-1 to -6) that together coordinate cell growth (13). Food intake has direct effects on all aspects of the IGF system. Prolonged fasting and protein-restricted diets are associated with reductions in plasma IGF-I, whereas meal feeding is associated with an acute decline in circulating IGFBP-1 (14). We hypothesized that the IGF axis may be responsive to differences in the GI of foods and thus provides a mechanism by which the growth of both normal and...
aberrant cells may be promoted. The aim here was to measure changes in blood glucose, insulin, IGF-I, and IGFBP-1, -2, and -3 after consumption of 2 foods with different GIs.

### SUBJECTS AND METHODS

#### Study population

Ten young lean subjects were recruited from the University of Sydney student population (Table 1). Inclusion criteria were that subjects have a BMI (in kg/m²) of 19–25 and that they be 18–30 y old, of European white origin, nonsmoking, engaged in moderate physical activity, and not taking medication known to alter glucose tolerance.

All subjects gave written informed consent. The protocol was approved by the University of Sydney human ethics committee.

#### Research design

Volunteers attended the Human Nutrition Unit on 6 separate occasions after a 10-h overnight fast. In randomized order, 2 visits involved the administration of a 50-g carbohydrate portion of the high-GI food, 2 visits involved the administration of a 50-g carbohydrate portion of the low-GI food, and 2 visits involved an extended period of fasting (water only). The high-GI challenge occurred after a 10-h overnight fast. In randomized order, 2 occasions after a 10-h overnight fast. In randomized order, 2 visits involved the administration of a 50-g carbohydrate portion of the high-GI food, 2 visits involved the administration of a 50-g carbohydrate portion of the low-GI food, and 2 visits involved an extended period of fasting (water only). The high-GI challenge was an 82-g serving of instant mashed potato reconstituted with 285 mL boiling water (published GI: 83 ± 1; 15). The low-GI challenge was a 160-g serving of cooked barley (published GI: 25 ± 2; 15). Whereas the GI of instant mashed potato was more than 3 times that of pearled barley, the meals were comparable in energy and macronutrients (Table 2) and were consumed within 13 min along with 250 mL water.

During each visit, a total of 11 fingerprick capillary blood samples (≈1 mL each) were collected at 0 time (just before the start of eating) and at 15–30-min intervals over 4 h. On 3 visits (one for each treatment), the samples were collected into Eppendorf tubes containing heparin for analysis of glucose and insulin. In the other 3 sessions, blood samples were collected into tubes not containing heparin for the assay of IGFBP-1 and IGFBP-2 at all 11 time points and the assay of IGFBP-3 and free and total IGF-1 at 120 and 240 min. Plasma and serum were immediately separated by centrifugation, and the samples were stored at −20 °C until analysis.

#### Plasma and serum metabolite and hormone measurements

Plasma glucose concentrations were assayed by using the hexokinase glucose-6-phosphate dehydrogenase enzymatic ultraviolet method (Roche Diagnostics, Basel, Switzerland). Precimat glucose standards (Boehringer Mannheim, Mannheim, Germany) were used to construct the standard curve. The intraassay and interassay CV was 1.0% and 0.5%, respectively. The Coat-A-Count Insulin kit (Diagnostic Products Corporation, Los Angeles, CA) was used to measure plasma insulin concentrations. Total IGF-I was measured by using a radioimmunoassay (RIA) with antiserum Tr10 as previously described (16), except that [125I]des(1–3)IGF-I was used as the radioligand. Free IGF-I was measured by using the immunoradiometric assay kit from Diagnostic Systems Laboratories (Webster, TX). IGFBP-1 and -2 were measured by using specific RIAs as described previously (17–19), although in this instance IGFBP-1 was assayed by using 50 μL of sample or standard. Serum IGFBP-3 was measured by using an enzyme-linked immunosassay and reagents supplied by the Kolling Institute of Medical Research (St Leonards, Australia). Briefly, goat anti-rabbit polyclonal antibody (at a concentration of 1:50) was used to capture IGFBP-3 antiserum R-100 at a final concentration of 1:20 000, which in turn captured biotinylated IGFBP-3. Antibody-bound biotinylated IGFBP-3 was detected by a streptavidin-horseradish peroxidase conjugate that underwent a color change when tetramethyl benzidin was added. The intraassay and interassay CV was 3.6% and 1.0%, respectively.

#### Statistical analysis

Changes from baseline in plasma glucose and insulin concentrations and in serum IGF-I and IGFBP concentrations were calculated. Cumulative changes in plasma glucose and insulin responses were quantified as the incremental area under the curve (AUC) that was truncated at the fasting value. Significant differences between the 2 meals were determined by using 2-factor analysis of variance for peak changes and AUC values, with foods as a fixed factor and subjects as a random factor. Significance was attained when P was < 0.05. Data were analyzed by using MINITAB statistical software (version 13; Minitab Inc, State College, PA). All data are presented as means ± SEs unless otherwise indicated.

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.8 ± 10.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5 ± 1.8</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.73 ± 0.01</td>
</tr>
</tbody>
</table>

| All values are x ± SD. HOMA-IR, homeostasis modeling assessment of insulin resistance: (fasting glucose x fasting insulin)/22.5.

### TABLE 2

<table>
<thead>
<tr>
<th>Food</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Fat</th>
<th>Dietary fiber</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearled barley, boiled</td>
<td>50 (79)</td>
<td>9.2 (15)</td>
<td>1.3 (5)</td>
<td>5.1 (4)</td>
<td>1018</td>
</tr>
<tr>
<td>Instant mashed potato</td>
<td>50 (78)</td>
<td>5.5 (9)</td>
<td>4.5 (16)</td>
<td>6.6 (5)</td>
<td>1023</td>
</tr>
</tbody>
</table>

Foods were prepared with the addition of water as directed by the manufacturer. Percentage of total energy in parentheses.
TABLE 3
Concentrations of glucose, insulin, and components of the insulin-like growth factor (IGF) system during the extended 4-h fast

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.2</td>
<td>5.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>7.5</td>
<td>6.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Free IGF-1 (ng/mL)</td>
<td>0.72</td>
<td>0.62</td>
<td>0.68</td>
</tr>
<tr>
<td>Total IGF-1 (mmol/L)</td>
<td>65.2</td>
<td>53.9</td>
<td>51.9</td>
</tr>
<tr>
<td>IGFBP-1 (ng/mL)</td>
<td>80</td>
<td>103</td>
<td>108</td>
</tr>
<tr>
<td>IGFBP-2 (ng/mL)</td>
<td>137</td>
<td>124</td>
<td>135</td>
</tr>
<tr>
<td>IGFBP-3 (ng/mL)</td>
<td>4360</td>
<td>4320</td>
<td>4190</td>
</tr>
</tbody>
</table>

All values are x ± SEM; n = 10. IGFBP, IGF-binding protein. None of the changes over time were significant (2-factor ANOVA with food as a fixed factor and subject as a random factor).

DISCUSSION

To our knowledge, this is the first study to investigate the acute effect of various carbohydrate sources on the IGF axis. As postulated, the greater glycemic and insulin responses to the high-GI meal elicited changes in the IGF system that may be clinically
important. Whereas both meals acutely reduced IGFBP-1 concentrations, the reduction was sustained for significantly longer after the low-GI meal (ie, barley). In contrast, IGFBP-3 concentrations rose after the barley meal, fell after the high-GI potato meal, and remained different at 4 h. Because both meals were closely matched for total energy, carbohydrate, fat, protein, and fiber, differences in macronutrient composition are unlikely to confound the interpretation of the results.

The changes in IGFBP-3, although quantitatively small (rising or falling by ≈6%), were 5-fold those in IGFBP-1, which suggested that high-GI carbohydrates may contribute to a metabolic environment that is conducive to tumor growth. IGFBP-3 is the major IGF-binding protein in serum and is expressed in many tissues, including normal and malignant breast epithelium. There is increasing evidence that IGFBP-3 has intrinsic antiproliferative and proapoptotic effects on human cancer cells. In case-control studies, lower serum IGFBP-3 concentrations are associated with greater risks of lung, bladder, and pancreatic cancer (20–22). However, the subject is controversial, because some studies show that IGFBP-3 can both augment and inhibit the mitogenic actions of IGF-1 and IGF-2 in breast cancer cells (23).

Acute changes in serum IGFBP-3 probably reflect stability or clearance rather than production (24). Most circulating IGF-I forms a complex comprising IGF-1, IGFBP-3, and an acid-labile subunit. In this form, the half-life of circulating IGF-1 is increased from a few minutes up to several hours because it traverses the capillary endothelium poorly (25). For this reason, a decrease in IGFBP-3, as occurred after the consumption of the high-GI food, suggests that a greater amount of free, biologically active IGF-1 may be available to the tissues (26). Conversely, the rise in IGFBP-3 concentrations after the low-GI meal implies less availability of free IGF-1. Because IGF-1 is a potent glucose-lowering peptide, circulating IGFBP-3 concentrations may fall in order to restore glucose homeostasis. Indeed, hypoglycemia induced by IGF-1 administration can be blocked by the coadministration of IGFBP-3 (19). Whereas we did not detect changes in free IGF-1 per se in the current study, its measurement, based on changes in total IGF and IGFBP-3, is at best an approximation (24). Free IGF-1 concentrations will depend on changes in all 6 IGFBPs.

In previous studies, carbohydrate consumption was shown to induce an acute decrease in serum concentrations of IGFBP-1 (27). This was also the case in the current study: by 2 h, both meals produced a decrease of ≈50 ng/mL in IGFBP-1. However, the effect was short-lived after the high-GI meal: by 4 h, the IGFBP-1 concentration had returned to the fasting concentration. In contrast, after the low-GI meal, the decrease was sustained for the remainder of the 4-h period of study. This result was somewhat surprising, given the smaller insulin response to the barley (28). However, our findings might be explained by the dynamics of the rise and fall in insulin. Because the high-GI meal elicits both a rapid rise and a rapid fall in insulin concentrations, the IGFBP-1 concentration may mirror this activity, falling and rising in quick succession. In contrast, the small but sustained rise in glycemia and insulinemia after consumption of the slowly digested food may depress IGFBP-1 concentration longer. Whether the magnitude of these changes is important in a clinical sense remains to be seen. IGFBP-1 has been reported to both inhibit and potentiate IGF-1 action, depending on cellular target and posttranslational modifications (26).

The strengths and weaknesses of the study should be considered. Only 2 foods were compared as single meals rather than as part of mixed meals on a chronic basis. Although the meals were matched in nutrient composition as closely as possible, they were not identical: the low-GI meal contained ≈4 g more protein and 3 g less fat than did the high-GI meal. Such small differences, however, are unlikely to elicit the differences in hormonal responses that we observed. In practice, most meals contain much greater amounts of fat and protein than were fed here. Nonetheless, differences in GI have been found to predict the glycemic response to realistic mixed meals and daylong glycemia (29, 30). In persons with diabetes, low-GI diets have been found to lower glycated hemoglobin, a measure of the average blood glucose concentration over the previous 2–3 mo (31). Our subjects were young and lean and mainly female, and the findings may not apply to persons who are overweight or more insulin resistant. However, despite the small number of subjects, the study had sufficient power to show statistically and perhaps biologically important differences in glycemia, insulinemia, and IGF system responses.

Increased consumption of high-GI carbohydrates could be considered one of the more subtle changes in the food supply over the past 50 y. As nations westernize and industrialize, traditional foods such as minimally processed whole grains and legumes are replaced by more highly processed and digestible foods. In general, traditional foods contain carbohydrates that are slowly digested because the starches and sugars remain closely embedded in the plant’s original botanical structure, where they are surrounded by bran and other barriers that inhibit starch gelatinization (32, 33). In contrast, modern methods of processing that involve the production of fine flours and the use of high temperatures and pressures increase starch gelatinization and hence the rate of digestion in vivo. Together with refined sugars, high-GI starches will increase the glycemic and insulinotropic potency of the diet.

We speculate that the findings of the current study may therefore be relevant to normal growth (34). Small increases in height, weight, and rate of maturation continue to occur over time, even in highly developed nations where nutrition standards have been high for decades (35). The differential effects of carbohydrate foods on the IGF axis might also influence the number of adipocytes and thus overweight and obesity. Pawlak et al (36) showed that rats fed a high-GI diet over an 18-week period gained 70% more body fat than did rats of equal body weight fed a low-GI diet. Increased consumption of milk (a low-GI food) has been associated with lower risk of weight gain over time (37) and, in boys, with higher IGFBP-3 concentrations (38). Milk was also the most consistent dietary correlate with IGFBP-3 in a study of >1000 healthy women (39).

The novel findings of the current study require confirmation in daylong and long-term studies using mixed diets with high- or low-GI carbohydrate sources. Further studies will also be necessary to ascertain whether changes in the IGF system are sensitive to postprandial glycemia and insulinemia in other population groups, particularly those with greater insulin resistance.
University of Sydney (www.glycemicindex.com), and is a coauthor of a series of books under the rubric The New Glucose Revolution (New York: Marlowe and Co).

REFERENCES