ABSTRACT

Background: Previously we observed that the consumption of pasta and bread resulted in a similar glycemic response, despite a slower intestinal influx rate of glucose from the pasta. Underlying mechanisms of this effect were not clear.

Objective: The objective was to investigate the differences in glucose kinetics and hormonal response after consumption of products with slow and rapid in vivo starch digestibility but with a similar glycemic response.

Design: Ten healthy male volunteers participated in a crossover study and consumed 13C-enriched wheat bread or pasta while receiving a primed-continuous D-[6,6-2H2]glucose infusion. The dual-isotope technique enabled calculation of the following glucose kinetics: rate of appearance of exogenous glucose (RaE), endogenous glucose production, and glucose clearance rate (GCR). In addition, postprandial plasma concentrations of glucose, insulin, glucagon, and glucose-dependent insulinotropic polypeptide (GIP) were analyzed.

Results: GIP concentrations after pasta consumption were lower than after bread consumption and strongly correlated with the RaE (r = 0.82, P < 0.01). The insulin response was also lower after pasta consumption (P < 0.01). In accordance with the low insulin response, the GCR was lower after pasta consumption, which explained the high glycemic response despite a low RaE.

Conclusions: Slower intestinal uptake of glucose from a starchy food product can result in lower postprandial insulin and GIP concentrations, but not necessarily in a lower glycemic response, because of a slower GCR. Even without being able to reduce postprandial glycemia, products with slowly digestible starch can have beneficial long-term effects. These types of starchy products cannot be identified by using the glycemic index and therefore another classification system may be necessary. This trial was registered at controlled-trials.com as ISRCTN42106325. Am J Clin Nutr 2012;96:1017–24.

INTRODUCTION

The consumption of foods with slowly digestible starch compared with those with rapidly digestible starch may benefit the management of type 2 diabetes and may decrease the risk of the development of obesity, insulin resistance, and type 2 diabetes (1–6). The glycemic index (GI)4, which is based on the postprandial glycemic response, is often used to derive information about the rate of starch digestion of carbohydrate-rich foods (7) by considering a high-GI product as containing rapidly digestible starch and a low-GI product as containing slowly digestible starch. However, the glycemic response is not only determined by the rate of glucose absorption from the ingested food but also by endogenous glucose production (EGP) and glucose uptake into tissues.

The use of stable isotopes enables the distinction between these processes, and studies show that the rate of appearance of glucose into the systemic circulation and the glycemic response of a starchy food product do not always correspond well. Previously, bread and pasta consumption were compared by using the dual-isotope technique to obtain an insight into the in vivo digestive behavior of these starchy foods and how this relates to the glycemic response (8). Despite an apparent difference in the rate of appearance of exogenous glucose (RaE), which is much slower for pasta, consumption of bread and pasta resulted in the same glycemic response. On the other hand, in a single-label study that investigated the underlying glucose kinetics of breakfast cereals with a low and a high GI, the difference in glycemic response was explained not by a difference in glucose appearance but by a difference in glucose uptake into tissues, which is reflected by the glucose clearance rate (GCR) (9). These studies showed the importance of determining glucose kinetics, because it is possible that a low-GI product contains rapidly digestible starch and that a product that contains slowly digestible starch results in a relatively high glycemic response. Both may be beneficial but via a different mechanism.

Several hormones play a role in the regulation of postprandial glucose kinetics and metabolism. The EGP is mainly regulated by the opposing actions of the hormones glucagon and insulin.

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4 Abbreviations used: EGP, endogenous glucose production; GCI/IRMS, gas chromatography combustion isotope ratio mass spectrometry; GCR, glucose clearance rate; GI, glycemic index; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; iAUC, incremental AUC; RaE, rate of appearance of exogenous glucose; RaT, rate of appearance of total glucose.

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Glucose uptake into tissue is greatly influenced by insulin, because it regulates the presence of the glucose transporter GLUT4 on the membrane of muscle cells and adipocytes as well as the subsequent glucose transport into the cells. In addition to responding to elevated glucose concentrations in the circulation, the insulin response is greatly potentiated (≈70%) by the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), which are released from the small intestine after an oral glycemic load (10–12). The incretins are thereby also important regulators of glucose kinetics. In a previous study that used corn products, we showed plasma GIP concentrations to correlate strongly with the RaE (13) and related GIP release to the rate of intestinal glucose absorption.

To explain why the glycemic response is not different after the consumption of bread and pasta, despite a large difference in RaE, as previously reported (8), our study also examined the EGP and GCR and analyzed postprandial insulin, glucagon, and GIP concentrations in plasma samples.

SUBJECTS AND METHODS

Subjects

Ten healthy men [age: 21 ± 0.5 y; BMI (in kg/m²): 23 ± 0.6 (mean ± SEM)] were recruited. The criteria for exclusion were as follows: use of medication, blood donation or use of antibiotics in the past 3 mo, gastrointestinal surgery or dysfunction, inflammatory diseases, and diabetes mellitus. Approval was obtained from the Medical Ethics Committee of the “Beoordeling Ethiek Biomedisch Onderzoek” Foundation, Assen, Netherlands. Each subject provided written informed consent for the study. This trial was registered at trials.com as ISRCTN42106325. A part of the data obtained in this trial was previously published (8).

Experimental design

The study was performed in a crossover manner, with ≥1 wk between each study day. The subjects were asked to refrain from consuming 13C-enriched foods, such as cane sugar, corn products, and pineapple, for 3 d preceding the experiments and from alcohol consumption and strenuous exercise for 24 h before each study day. Food intake on the day before each experiment was individually standardized by using a diary. A standard evening meal was provided at the commercial research facility (QPS Netherlands BV), where the subjects stayed overnight. In the evening a venous catheter was inserted in each subject’s forearm for blood collection and for infusion of D-[6,6-2H₂]glucose (98% 2H atom percent excess) (Isotec). Subjects fasted overnight but were allowed to drink water. In the morning (t = −120 min), 26.7 mL D-[6,6-2H₂]glucose solution (80 × 0.07 mg/kg body weight) was infused, and a continuous infusion of 0.07 mg/kg body weight D-[6,6-2H₂]glucose per minute was started and maintained for 8 h. Two hours after the start of the infusion (t = 0) the test meal was ingested. During the study period, physical activity was limited.

Sample collection

Blood was collected into 2-mL BD Vacutainer fluoride tubes and 3-mL BD P700 Blood Collection Systems containing 13C-enriched by the addition of 12% 13C-labeled wheat [Triticum aestivum var Paragon (1.359 atom% 13C)] cultured in a 13CO₂-enriched atmosphere, as described previously (14). Both test meals consisted of 50 g available carbohydrates and were consumed together with 10 g light margarine (4 g fat), 2 slices lean ham (5 g fat, 6 g protein), and 250 mL tap water within 20 min.

The bread was prepared with 1110 g unlabeled white wheat flour [T. aestivum Kolibri/Ibis (ratio: 70:30) (1.085 atom% 13C)], 180 g 13C-labeled wheat flour, 210 g wheat bran, 975 g water, 25 g yeast, and 30 g salt. After kneading, the dough was allowed to rise for 30 min and was baked for 30 min at 240°C. Portions of 132 g bread were stored at −20°C until use. Pasta was freshly prepared with 739 g wheat flour (T. durum de Cecco), 120 g 13C-labeled wheat flour, 141 g wheat bran, 400 g water, and 20 g salt. Portions of 119 g were stored at 5°C until use and cooked for 6.5 min in 2 L water before consumption.

In vitro analysis of starch fractions and determination of 13C abundance of the test meals were previously described (8).

Measurement of plasma glucose, insulin, glucagon, and GIP concentrations

Plasma glucose concentrations were measured on a Roche/Hitachi Modular automatic analyser (Roche Diagnostics, Hitachi) by using a glucose hexokinase method. The within- and between-batches CVs were ≤2%. The ARCHITECT insulin assay (Abbott Laboratories) was used to determine insulin concentrations in plasma. The total CV of this chemiluminescent microparticle immunoassay was ≤7%. Glucagon was determined by using a radioimmunoassay (KGN1; Siemens). The plasma concentrations of total GIP were measured on the Luminex 100 Total System (Luminex Corporation) by using the Bio-Plex Pro Human Diabetes Panel (BioRad Laboratories).

13CO₂ analysis in breath samples

Analysis of 13C abundance in breath carbon dioxide was performed by using gas chromatography isotope ratio mass spectrometry (Delta Plus XL; Thermo Fisher Scientific) measuring the 13C/12C ratio compared with the international standard Pee Dee Belemnite (δ13C_PDB, in ‰).
Analysis of isotopic enrichment by gas chromatography–mass spectrometry and gas chromatography combustion isotope ratio mass spectrometry

Analysis of isotopic enrichment by gas chromatography requires the derivatization of plasma glucose to glucose penta acetate or an alternative suitable derivative. The sample preparation is described in detail elsewhere (13, 15). The $^2\text{H}$ enrichment of plasma samples was measured by gas chromatography–mass spectrometry as previously described (15), and the $^{13}\text{C}:{^{12}\text{C}}$ isotope ratio was measured in plasma by using gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) as previously described (16, 17), both with some modifications (8).

Calculation of glucose kinetics

The molar percentage enrichment of [6,6-$^2\text{H}_2$]glucose and the $^{13}\text{C}$ atom% were calculated as previously described (15), except that the data were not smoothed. The rate of appearance of total glucose [RaT; glucose from exogenous (meal) and endogenous (hepatic) sources] was calculated from total plasma glucose concentrations and $^3\text{H}$-enrichment data by using the non–steady state equation of Steele et al (18) as modified by De Bodo et al (19). It was assumed that labeled and unlabeled glucose molecules showed identical behavior. The effective volume of distribution was considered to be 200 mL/kg and the pool fraction to be 0.75 (20). The systemic RaE was calculated from the RaT and $^{13}\text{C}$-enrichment data, as described by Tissot et al (20). The EGP was calculated by subtracting the RaE from the RaT (20). The GCR, which reflects the tissue glucose uptake, was calculated as described by Schenk et al (9).

Incremental AUC

To determine differences in glucose kinetics and in plasma glucose, insulin, glucagon, and GIP concentrations, the 0–2-h incremental AUC (iAUC) was calculated by using the trapezoidal rule (21). The averages of fasting measurements were used as baseline values, and areas below baseline were not included. For the iAUC calculations of RaT, RaE, and GCR, the values were multiplied by body weight. The iAUC of RaE was expressed as a percentage of the administered dose of glucose equivalents (cumulative dose %). Because EGP and glucagon were suppressed after the test meals, the area beneath baseline (decremental AUC) was calculated by using the mirrored graphs of these variables.

Statistical analysis

Data are presented as means ± SEM ($n = 9; n = 8$ for glucagon). A mixed model was used to test for time × treatment effects. If a significant interaction was found ($P < 0.05$), partial tests between treatments at time point were performed. A Bonferroni correction was used to correct for multiple comparisons. If necessary, data were log-transformed before analysis. The analyses were performed with the software package SAS (release 9.1; SAS Institute). For additional indexes (fasting and peak values, time to peak, and iAUC), differences between meals were assessed by using a 2-tailed paired Student’s $t$ test. The within-subject relation (correlation) between variables was tested by regression analysis according to the method of Bland and Altman (22). These analyses were performed by using SPSS 16.0 for Windows (SPSS Inc). A $P$ value $<0.05$ was considered significant.

RESULTS

Characterization of test meals

Results from the in vitro analysis of starch fractions and determination of $^{13}\text{C}$ abundance of the test meals were previously reported (8).

Postprandial glucose response

Data were available for 9 subjects, because one person did not consume the entire pasta meal. No differences were found in fasting plasma glucose concentrations ($P = 0.42$), peak values ($P = 0.55$), time to peak ($P = 0.35$), or iAUC ($P = 0.22$) after the consumption of bread and pasta (Table 1; Figure 1).

Glucose kinetics

The results are summarized in Table 1. The RaE was slower after pasta consumption compared with after bread consumption (Figure 2), resulting in a lower 0–2-h iAUC ($P < 0.0001$). The EGP was more suppressed after consumption of pasta in comparison with bread (decremental AUC, $P = 0.03$). Comparison of the GCR after both test meals showed that glucose after pasta consumption was cleared from the circulation at a lower rate (Figure 3). The 0–2-h iAUC was >50% lower after pasta ($P = 0.0002$).

Postprandial insulin, glucagon, and GIP response

Insulin concentrations were lower after pasta consumption (Figure 4), which was indicated by a 48% smaller iAUC (0–2 h) compared with after bread consumption ($P < 0.01$) (Table 1). Glucagon concentrations after pasta were not significantly different compared with after bread (data not shown). Postprandial GIP concentrations were lower after pasta consumption than after bread consumption (Figure 5), which was also apparent from the lower iAUC ($P = 0.002$) (Table 1).

$^{13}\text{CO}_2$ excretion in breath

For $^{13}\text{CO}_2$ excretion in breath, reflecting the rate of oxidation of the $^{13}\text{C}$-labeled substrate, a significant time × treatment interaction was found ($P < 0.0001$). $^{13}\text{CO}_2$ excretion was higher after bread consumption from $t = 90$ until $t = 180$ min ($P < 0.001$) and was higher after pasta consumption at $t = 330$ min ($P = 0.003$) (data not shown). Peak values were the same after both meals ($P = 0.68$), but the time to peak was significantly later after pasta consumption ($P = 0.007$) (Table 1).

Correlations

Correlations between glucose, insulin, GIP, and RaE (all time points, 0–2 h; $P < 0.01$) are presented in Table 2. RaE and GIP showed the strongest correlation.
DISCUSSION

Previously, we reported that bread and pasta consumption resulted in a similar glycemic response in healthy men, despite the slower glucose influx rate from the intestine (RaE) after pasta consumption (8). These findings emphasize that the glucose response does not reflect the influx of starch-derived glucose from the food product and is the result of several metabolic processes occurring simultaneously. Because the lower RaE did not lead to a lower glycemic response, it was expected that the EGP was less suppressed after pasta compared with after bread, thus “counter-balancing” the slow RaE. Therefore, the EGP and GCR were studied in detail together with the hormones influencing these processes.

The similar glycemic response after bread and pasta consumption could not be explained by the difference in EGP, because EGP was somewhat more suppressed after pasta consumption. However, the GCR was much slower after the consumption of pasta, enabling the glucose to remain in the circulation for longer. Thus, the slower GCR after pasta consumption “counterbalances” the slow RaE, explaining the similar glycemic response after the slowly and rapidly digestible test meals. In accordance with the lower GCR, insulin concentrations were lower after pasta than after bread consumption. However, the insulin response to pasta was much lower, as would be expected from the glycemic response, because both responses often resemble each other after the consumption of starchy food products (23–25). Nevertheless, this

![FIGURE 1. Mean (±SEM) changes from baseline in plasma glucose concentrations after ingestion of 132 g 13C-enriched control bread (●) and 119 g 13C-enriched pasta (△) in healthy men (n = 9). There was a significant time × treatment interaction (P = 0.0190), but no differences between treatments per time point were found after Bonferroni correction.](https://academic.oup.com/ajcn/article/96/5/1017/4576956 by guest on 18 March 2022)
discrepancy was also observed after intake of certain types of breads, as discussed below.

The insulin response is potentiated by the incretin hormones, and therefore the low GIP concentrations after the consumption of pasta were as expected. The low GIP response is likely explained by slower digestion of starch in pasta, which is reflected by the slow RaE. In the present study the individual patterns of RaE and GIP closely resembled each other, which is in accordance with one of our previous studies that used corn products, in which GIP and RaE also showed a strong correlation (13). Duodenal perfusion studies showed that GIP release dose-dependently responds to the rate of glucose delivery in the duodenum as well (26–28). Together, this means that the slow digestion of starch in pasta results in a low GIP and a subsequent low insulin response. This in turn leads to slower GCR and by that to a relatively high glycemic response.

The question then remains why some products with slowly digestible starch result in a low glycemic response (13, 23, 29), despite this apparent counterbalancing effect. The answer could possibly be derived from 2 intraduodenal perfusion studies that compared glucose, insulin, and incretin responses during glucose infusion ranging from 1 to 4 kcal/min (26, 28). An increase in glucose load from 1 to 2 kcal/min considerably increased the glycemic response, but there was hardly any further increase in glycemic response when rates of 3 kcal/min (28) or 4 kcal/min (26) were administered. The absence of a further increase in glucose concentrations was explained by the substantially greater incretin and insulin responses to the higher glucose infusion rates (26, 28). GIP was suggested to account for the effect on insulin in the lower infusion range, whereas GLP-1 mainly contributed in the highest infusion range (26). The responses to the 3–4-kcal/min and 2-kcal/min infusion rates are in line with our observations after bread and pasta consumption. In accordance with the infusion rate of 3–4 kcal/min, our bread (high RaE) resulted in a high glucose, GIP, and insulin response, whereas, consistent with the infusion rate of 2 kcal/min, our pasta (low RaE) resulted also in a high glucose response but a lower GIP and insulin response. Thus, it can be expected that a product with an even slower RaE than our pasta would elicit a response comparable to that of the 1-kcal/min infusion rate, namely a very low GIP and insulin response and a low glycemic response. We hypothesize that in this condition the reduction of GCR is not sufficient to counterbalance the very low RaE, resulting in a lower glycemic response (Figure 6). Further investigation is needed to determine the role of GLP-1 and the validity of this hypothesis when there are other factors present in food that could influence GIP, insulin, or GCR independently from the rate of starch digestion. For example, protein is capable of enhancing postprandial GIP and insulin secretion (30, 31) and...
when added to a starchy food product is likely to affect the glycemic response. It must also be noted that this study was conducted in healthy subjects, and results might be different in subjects with, for example, decreased glucose tolerance.

Our findings could shed light on the yet unexplained discrepancy between glucose and insulin response after the consumption of certain types of bread. For instance, several rye breads elicit a glycemic response comparable to that of white wheat bread, whereas the insulin and incretin responses were significantly lower (32–34). Glucose kinetics after consumption of these rye breads were not studied; however, on the basis of the observed correlations between glucose influx and GIP concentrations (13, 26, 28), it is very likely that the lower GIP, GLP-1, and insulin concentrations after consumption of these rye products can be explained by a lower RaE. Indications for a slower digestibility of starch in these products were the lower hydrolysis indexes in comparison with white wheat bread (32, 33). This was explained by a decreased accessibility of starch by degrading enzymes due to a different (microscopic) structure of rye breads (33). In addition, it can be expected that the lower insulin concentrations after rye bread result in a reduced GCR, thus showing the same counterbalancing effect as in the present study and which explains the similar glycemic response after the white wheat and rye breads. Similarly, the consumption of several differently processed wheat breads also resulted in a similar glucose response but a lower GIP (35) or insulin response (36) in comparison with conventional breads. Here, the differences in bread preparation were suggested to have decreased the availability of starch, as indicated by the lower content of rapidly available glucose (35) or a more rigid bread structure (36).

The consumption of slowly digestible products, although it does not always result in a low glycemic response, might have several beneficial effects. Because glucose enters the circulation at a slower rate, less insulin is secreted to keep glucose...
concentrations beneath an acceptable limit, which would be less demanding for the pancreatic β cells. The modest insulin response also prevents a decrease in glucose concentrations beneath baseline, which is associated with increased food intake (1). The effect of a low insulin response on inflammatory status, which is associated with an increased risk of the development of type 2 diabetes, was studied in a 12-wk intervention study in individuals with the metabolic syndrome. A slightly positive modulation of inflammation markers after the low-insulin diet was found (37), whereas the high-insulin diet upregulated genes related to inflammation and oxidative stress (38). With the knowledge of the present study in mind, these findings might also be explained by a slower RaE, GCR, and/or oxidation rate after consumption of the low-insulin diet, which likely results in less oxidative stress and inflammation. Low postprandial GIP concentrations might also be favorable, because several studies have indicated that GIP might be involved in the development of obesity due to its anabolic effects in adipose tissue, such as stimulation of fatty acid synthesis and enhancement of insulin-stimulated incorporation of fatty acids into triglycerides (39–42).

In conclusion, a high glycemic response after pasta consumption, despite slow starch digestion, can be explained by slower uptake of glucose into tissues. This is likely the result of a low insulin response caused by the low GIP response attributed to slow influx of glucose from the pasta meal. Foods with slowly digestible starch, even without being able to reduce postprandial glycemia, are preferable to those with rapidly digestible starch because of their expected beneficial long-term effects. These products cannot be identified with the GI because they would fall into the high-GI category. Therefore, another classification system may be necessary that would be able to reflect these more subtle differences. On the basis of our study results, insulin or GIP seem to be promising candidates.

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