The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

In Vitro Starch Digestion Kinetics, Corrected for Estimated Gastric Emptying, Predict Portal Glucose Appearance in Pigs[1,2]

Theo A. T. G. van Kempen,[3,4] Prajwal R. Regmi,[5] J. Jacques Matte,[6] and Ruurd T. Zijlstra[5]*

[3]Provimi Research and Innovation Centre, B-1932 Brussels, Belgium; [4]North Carolina State University, Raleigh, NC 27695; [5]University of Alberta, Edmonton T6G 2P5, Alberta, Canada; and [6]Agriculture and Agri-Food Canada, Sherbrooke J1M 1Z3, Quebec, Canada

Abstract

In vitro starch digestion is used for predicting the in vivo glucose response, but their relationship has not been defined thoroughly. To clarify, in vitro starch digestion using a modified Englyst-assay was compared to portal glucose appearance in pigs. Four portal vein-catheterized pigs (43.2 ± 4.8 kg body weight) were fed 4 diets containing 70% purified starch ranging from slowly to rapidly digestible [maximal rate of in vitro digestion (%)/min: 0.22 (slowly), 0.38, 0.73, and 1.06 (rapidly)] for 7-d periods in a 4 × 4 Latin square. In vivo (R^2 = 0.964) and in vitro (R^2 = 0.998) data were modeled using a Chapman-Richards model that accurately described the sigmoidal glucose-release profiles. Across samples, the extent of glucose recovered was less in vivo than in vitro (69 vs. 42% of starch). The rate of glucose release adjusted for plateau effects was lower in vivo (0.35 vs. 0.89%/min), whereas the shape parameter adjusted for plateau effects (sigmoidal modifier) was higher in vivo (37.9 vs. 13.7). Consequently, peak glucose release in vivo occurred 69 min postprandial, whereas it occurred only 6 min into the second stage of digestion in vitro. Cumulative portal glucose appearance was strongly related (R^2 = 0.89; $P < 0.001$) to in vitro glucose release, although a nonlinear bias was observed. After correcting in vitro release with predicted gastric emptying, the relationship improved and became linear (R^2 = 0.95; $P < 0.001$). In conclusion, in vitro starch digestion kinetics predict portal glucose appearance up to 8 h postprandial accurately provided that in vitro data are corrected for gastric emptying. J. Nutr. 140: 1227–1233, 2010.

Introduction

For nutritional purpose, starch types are commonly described by 3 fractions based on the rate and extent of in vitro enzymatic digestion: *1*) rapidly digestible starch (RDS)[7] that acutely increases blood glucose; *2*) slowly digestible starch (SDS) that results in a drawn-out increase in blood glucose; and *3*) resistant starch (RS) that resists digestion by mammalian enzymes and thus does not yield glucose (1,2). The in vitro Englyst-assay (3) determines these fractions, RDS, SDS, and RS, as digested within 20 min, 20–120 min, and not digested within 120 min, respectively (1,2); has a good repeatability; and is thus widely used for predicting in vivo glycemic responses.

The in vitro-based fractions RDS and SDS are better related with the glycemic index (R^2 = 0.62), which ranks carbohydrate-containing foods according to their in vivo glucose response (4), than with actual carbohydrate components such as glucose, starch, sucrose, and fructose (R^2 = 0.17) (1,5). In addition, several studies (6–10), although not always consistent (11,12), have indicated that higher SDS and RS contents in diets will reduce the rate and extent of in vivo starch digestion and thus maintain sustained and lower postprandial glucose responses in peripheral circulation. Therefore, starches ranging in SDS and RS contents are used in many food preparations (13) for the management of diseases related to carbohydrate metabolism (13,14). Their actual contribution for maintaining the rate and extent of in vivo glucose absorption, however, is not clearly understood, because accurate extrapolation from in vitro to in vivo is not yet possible. Furthermore, whether in vitro RS is an accurate predictor of starch resistant to enzymatic digestion in the small intestine is controversial, because starch digestion may continue well beyond 120 min of in vitro incubation (7).

The nutrient absorption kinetics in healthy humans is difficult to measure for ethical and technical reasons. Hence, pigs, having similar digestive anatomy and physiology (15) and a similar profile of nutrients and hormones in blood circulation (16), can be a good model for understanding the kinetics of portal glucose appearance in humans. Therefore, a study was designed to determine the postprandial glucose response in portal vein-catheterized pigs fed diets containing purified

[1] Supported by Provimi Research and Innovation Centre (Brussels, Belgium), Alberta Pulse Growers, and Alberta Livestock Industry Development Fund. Penford Food Ingredients (Centennial, CO) provided purified corn starch.
[2] Author disclosures: T. A. T. G. van Kempen, P. R. Regmi, J. J. Matte, and R. T. Zijlstra, no conflicts of interest.
[7] Abbreviations used: $C_{plateau}$, shape parameter adjusted for plateau effects; K, rate of glucose release; $K_{plateau}$, K rate parameter adjusted for plateau effects; RDS, rapidly digestible starch; RS, resistant starch; S1 to S4, starch sources ranging from rapidly to slowly digestible; SDS, slowly digestible starch.
* To whom correspondence should be addressed. E-mail: ruurd.zijlstra@ualberta.ca.

Manuscript received January 7, 2010. Initial review completed January 28, 2010. Revision accepted April 5, 2010.
First published online May 5, 2010; doi:10.3945/jn.109.120584.

starches with a wide range in the in vitro digestion kinetics and to describe the relationship between these two. The hypothesis was that in vitro glucose release reflects the kinetics of in vivo glucose absorption.

Methods

In vitro digestion method validation and modification. A 2-h in vitro technique described by Englyst et al. (3) simulating gastric and small intestinal digestion is commonly used for determining in vitro digestibility of starch and for predicting the glycemic index of starchy foods (17). In the present study, the Englyst et al. (3) technique was used with 2 modifications. First, we observed that the difference between glucose content measured 1 d after compared with immediately after in vitro enzymatic digestion was greater ($P < 0.001$) when 66% compared with absolute ethanol was used to stop the incubation (0.7 ± 0.23 vs. 0.0 ± 0.01 mmol/L, respectively). This difference indicated that 66% ethanol as used by Englyst et al. (3) did not stop the enzymatic activity and thus the time of glucose measurement relative to when the assay was stopped affected the readings. Absolute ethanol, on the other hand, completely stopped the enzymatic activity after incubation; therefore, absolute ethanol was used in the present study. Second, the time points at which subsamples for glucose analysis were taken were changed and the assay was extended to 8 h. Specifically, the 20-min sampling time was moved forward to better characterize the sigmoidal digestion; 15 min was used as a compromise between what is practically feasible and what is desirable. The reason for extending the assay to 8 h was to not only match the in vivo time frame but also to ensure that all starch diets were digested to at least 95% of the plateau value. Going beyond 8 h, as done by, e.g., McCleary et al. (18), was deemed to not add value and may result in an increasingly unstable assay due to, e.g., microbial contamination. Thus, samples were taken at 0, 15, 30, 60, 120, 240, 360, and 480 min to properly characterize starch digestion.

In the modified technique, 1 g ground sample (1-mm screen, Retsch grinder, model ZM1, Brinkman Instruments) was added into a 50-mL tube. Samples were incubated in triplicate in 10 mL pepsin solution, containing 0.05 g pepsin (P-7000; Sigma-Aldrich) and 0.05 g guar gum in 0.05 mol/L HCl for 30 min to mimic gastric digestion. For mimicking small intestine digestion, 10 mL of 0.25 mol/L sodium acetate ($C_2H_3NaO_2$) solution and 5 mL of an enzyme mixture containing 0.7 g pancreatin before centrifugation (P-7545; Sigma-Aldrich), 0.05 mL amyloglucosidase (EC 3.2.1.3; 61–002; 200 kEU/L; Englyst Carbohydrate Service), and 3 mg invertase (P-57629; Sigma-Aldrich) in water were added to the digestion solution and further incubated for up to 480 min. Incubations were carried out at 39°C under horizontal agitation; glass beads were added to enhance the efficacy of agitation and to provide a grinding action. At each sampling, an aliquot of 0.5 mL was taken to which absolute ethanol was added for stopping the digestion of the starch. Glucose content was determined in this blend using a glucose oxidase kit (Megazyme).

Starch sources. Remyline AX-DR rice starch (Remy Industries), Remy B7 rice starch (Remy Industries), Nastar pea starch (Cosucra Group Warcoing), and Gelose 80 corn starch (Penford Food Ingredients) samples with an expected wide range in digestibility kinetics were used in the study. The starches were considered rapidly (S1), moderately rapid (S2), moderately slow (S3), and slowly digestible (S4) starches based on their in vitro kinetics (maximal in vitro rate of starch digestion, %/min; 1.06, 0.73, 0.38, and 0.22). The starch sources had a wide range in content of SDS (starch digested from 20 to 120 min in vitro; 11.4–68.1%) and RS (starch undigested after 120 min in vitro; 3.1–85.0%) (**Table 1**).

Animal experiment. The animal protocol was approved by the Animal Care and Use Committee for Livestock at the University of Alberta. Four female pigs (35.0 ± 0.2 kg initial body weight) were catheterized in the portal vein and carotid artery and a flow probe was installed around the portal vein (19). After recovery, pigs (43.2 ± 4.8 kg body weight) were fed 1 of the 4 diets containing purified starches (**Table 2**) in a 4 × 4 Latin square design. Pigs were fed 1200 g/d in 2 equal meals at 0800 and 2000.

TABLE 1 Kinetic parameters of in vitro starch digestion and portal glucose appearance in pigs of diets containing 4 starches[1]

	Starch diets				
Characteristic	S1	S2	S3	S4	Pooled error
In vitro kinetics parameter[2]					
Plateau,[3] *% of starch*	75.87	74.85	73.91	49.83	0.67
$K_{plateau}$,[4] *% of starch/min*	1.92	1.02	0.38	0.22	0.02
$C_{plateau}$[5]	38.90	15.67	0.00	0.00	1.12
Peak time, *min*	14.49	9.16	0.03	0.05	1.34
Maximum rate of glucose release, *%/min*	1.06	0.73	0.38	0.22	0.01
In vivo kinetics parameter[6]					
Plateau,[7] *% of starch*	48.1	51.6	46.9	31.4	5.42
$K_{plateau}$,[8] *% of starch/min*	0.63	0.25	0.34	0.16	0.12
$C_{plateau}$[9]	86.5	22.2	34.3	8.7	3.81
Peak time, *min*	78.2	73.7	76.1	48.8	9.89
Maximum rate of glucose release, *%/min*	0.29	0.15	0.18	0.10	0.05
Starch composition,[10] *% of starch*					
RDS	28.8	19.7	7.1	3.6	0.12
SDS	68.1	46.8	31.7	11.4	0.21
RS	3.1	33.5	61.2	85.0	0.22

[1] Values are mean and pooled SE in vitro analyses, $n = 3$, and mean and pooled SEM for in vivo analyses, $n = 4$.
[2] Determined by in vitro enzymatic digestion until 480 min (4).
[3] Peak in vitro starch digestion.
[4] Maximum rate of in vitro starch digestion.
[5] Shape parameter of in vitro starch digestion.
[6] Determined by glucose absorption until 480 min postprandial during present pig study.
[7] Peak net portal glucose appearance.
[8] Maximum rate of net portal glucose appearance.
[9] Shape parameter of net portal glucose appearance.
[10] Values are means, $n = 3$.

The nutrient composition of the diets met NRC requirements (20). On d 7 of each period, blood samples from the portal vein and carotid artery catheters were collected 15 min before morning feeding and 0, 15, 30, 60, 120, 240, 360, and 480 min after feeding. During each blood collection, portal blood flow rate was measured using a flow meter (Transonic Systems). Plasma flow rate was calculated from blood flow using the equation: plasma flow = blood flow × (1 − hematocrit). Plasma was analyzed for glucose (Glucose oxidase kit; Diagnostics Chemicals).

Glucose absorption was calculated using the formula q = (portal concentration – carotid artery concentration) × F (dt). Cumulative net glucose absorption was calculated subsequently using the formula $Q = \sum_{t0}^{t480} q$ (21). In these formulae, q is the amount of portal glucose appearance within time period dt, F is plasma flow in the portal vein, and Q is the amount of glucose absorbed from time t_0 to t_{480} (0 to 480 min postprandial). Percentage of starch that is digested and absorbed as glucose per minute is calculated and fitted into a model using Eq. 1 and 2 (provided below).

Model used. Using the data obtained from in vitro and in vivo studies, the digestion coefficient of starch was calculated using the following formula: digestion coefficient at time t = [glucose present at time t – 0 min glucose release) × 0.9]/total starch. A factor of 0.9 was used, because the molecular weight of glucose as incorporated into starch is 90% of that of free glucose. The Chapman-Richards model describes a complex nonlinear function where an independent variable can undergo different rates of change such as initial slow rate followed by rapid progression up to a peak or maximum rate (22). In vitro starch digestion and portal glucose appearance in the present study are examples of such variables and thus can be described by such a model. Hence, a modification of the Chapman-Richards model described by van Kempen et al. (23) (Eq. 1 and 2) was used to model in vitro starch digestion and glucose release and portal glucose appearance in the present study:

TABLE 2 Ingredient compositions of starch diets

Ingredient	g/kg of diet
Starch[1]	700
Casein[2]	140
Fish meal[3]	74
Cellulose[4]	40
Canola oil	10
Limestone	10
$CaHPO_4$	8
NaCl	3
Vitamin premix[5]	5
Mineral premix[6]	8
K_2CO_3	2

[1] Four sources: *1*) Remyline AX-DR rice (Remy Industries, Leuven-Wijgmaal, Belgium); *2*) Remy B7 rice (Remy Industries); *3*) Nastar pea (Cosucra Groupe Warcoing, Warcoing, Belgium); and *4*) Gelose 80 corn (Penford Food Ingredients, Centennial, CO) were used to prepare diets containing S1, S2, S3, and S4 starches, respectively;
[2] Calcium caseinate, American Casein Company, Burlington, NJ.
[3] Menhaden fish meal, Omega Protein, Hammond, LA.
[4] Solka-floc, International Fiber Corp., North Tonawanda, NY.
[5] Provided per kg diet: Zn, 100 mg as $ZnSO_4$; Fe, 80 mg as $FeSO_4$; Cu, 50 mg as $CuSO_4$; Mn, 25 mg as $MnSO_4$; I, 0.5 mg as $Ca(IO_3)_2$; Se, 0.1 mg as Na_2SeO_3.
[6] Provided per kg diet: retinol, 2.5 mg; cholecalciferol, 20.6 μg; α-tocopherol, 2.7 μg; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine,1 mg; D-biotin, 0.2 mg; vitamin B-12, 0.025 mg.

$$\text{Starch hydrolysis, \%} = A + B \times [1 - \exp(-K_{plateau}/\text{Plateau} \times \text{time})]^{(C\text{plateau}/\text{Plateau}+1)} \quad \text{(Eq.1)}$$

$$\text{Glucose release, \%/min} = (C_{plateau}/\text{plateau} + 1)((1 - \exp(-K_{plateau} \times \text{time}))\text{^}(C_{plateau} - 1) \times K_{plateau} \times \exp(-K_{plateau} \times \text{time})/0.9 \quad \text{(Eq.2)},$$

where A is the free glucose present in the sample before enzyme addition, B is the glucose released by exhaustive digestion, $K_{plateau}$ is the rate of glucose release corrected for plateau effects, plateau is the sum of A + B and thus the maximal glucose release as a percent of sample weight, and $C_{plateau}$ is the sigmoidal/shape modifier corrected for plateau effects. Compared to the standard Michaelis-Menten enzyme kinetics model, the modifications are that K is substituted by K corrected for plateau effects ($K_{plateau}$) and a shape parameter $C_{plateau}$ is added that is also corrected for plateau effects. These modifications were applied, because digestion was sigmoidal for some starches and because the rate and shape parameters were affected by the amount of starch incubated and the above correction eliminated this effect.

Correction of in vitro glucose release with gastric emptying. The link between the in vivo and in vitro data might improve with a correction of in vitro data for gastric emptying. Previously, gastric emptying has been explained by the formula: $y(t) = (1 - \exp^{-kt})^{\beta}$ (24,25). In this equation, k is the rate and β is the shape parameter. $\beta > 1$ indicates an initial delay in gastric emptying and $\beta < 1$ indicates initial rapid emptying. Hence, the following equation was tested in this study:

$$\text{In vitro starch hydrolysis corrected for gastric emptying} = D \times (1 - \exp^{-kt})^{\beta} \int (A + B \times [1 - \exp(-K_{plateau}/\text{plateau} \times \text{time})]^{(C\text{plateau}/\text{plateau}+1)}) \quad \text{(Eq.3)},$$

where D is a multiplier for converting absolute in vitro to in vivo data. D can thus account for glucose consumption by the gastrointestinal tract itself.

To test Eq. 3, 16 sequential time periods were created so that for each time period, an equal amount of starch left the stomach that each was assumed subsequently digested at the rates determined in vitro. By integrating these data across the 16 time periods, a glucose appearance curve in portal blood was created and compared with the actual in vivo data. Using an iterative process while attempting to minimize the root mean square error, the values for D, k, and β were obtained.

Statistical analysis. For in vivo data, pig was considered the experimental unit. The effect of starch diets on portal glucose appearance at different time points was analyzed using repeated measures in a mixed model of SAS (version 9.1; SAS Institute). The model included diet, time, and their interaction as fixed effects and pig and period as random effects. Means were separated using the probability of difference provided by the mixed model. For in vitro data, starch diet was considered the experimental unit. The SE was determined for triplicate analysis, but treatment effects were not analyzed statistically. The relationship of in vitro glucose release and portal glucose appearance was analyzed using the regression procedure of SAS. Least square means were used for all calculations and kinetics model development. Significance of difference was set at $P < 0.05$.

Results

In vitro starch digestion and glucose release. In vitro glucose release from starch diets could be modeled effectively with Eq. 1 (R^2 = 0.998) (**Fig. 1**). Digestion curves also clearly exhibited a lag phase that could be described with the sigmoidal shape parameter. Specifically, across starch diets, 0.33% (range: 0.25–0.46%) of total starch was present as free glucose at 0 min after the start of incubation. In vitro glucose release peaked at 15 min after the start of incubation and the peak glucose release was 1.35, 0.93, 0.52, and 0.35% of starch for S1, S2, S3, and S4 starch diets, respectively (Fig. 1*A*). By 60 min, glucose release dropped drastically for the S1 and S2 starch diets. The modeled

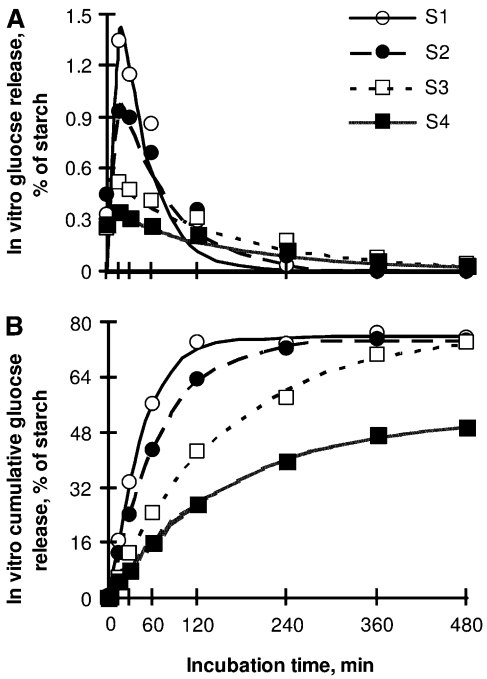

FIGURE 1 Absolute (*A*) and cumulative (*B*) release of glucose during in vitro digestion of 4 starch diets. A modified Chapman-Richards model was used to predict kinetics of glucose release from observed values (R^2 = 0.998). Values are means, n = 4. In A, the SE ranged from 0.00 to 0.02%; in B, the SE ranged from 0.02 to 2.03%.

in vitro glucose release had reached 95% of the plateau value after 95 min for the S1 starch diet, but the S4 starch diet required 400 min of digestion to reach 95% of plateau. Cumulative starch digestion at 120 and 480 min was 72.4 and 75.9% for S1, 63.0 and 74.9% for S2, 41.0 and 73.9% for S3, and 27.2 and 49.8% for S4 starch diets, respectively (Fig. 1*B*). The ranking of cumulative starch digestion was consistent for the entire incubation among starch diets. Out of total starch digested, 4.5 and 45.5% was digested from 120 to 480 min of the incubation, respectively, in the S1 and S4 starch diets (**Fig. 2**).

Portal glucose appearance. Net portal appearance of glucose peaked ~60 min after feeding the pigs and the peak was higher ($P < 0.05$) for the S1 starch diet (0.24%/min) than for the S2 (0.17), S3 (0.16), and S4 (0.10) starch diets (**Fig. 3***A*). Subsequently, glucose appearance dropped drastically for the S1 starch diet compared with other diets and from 270 min onward the rate of glucose release was similar ($P > 0.10$) among the diets. Cumulative portal appearance of glucose (percent of starch) at 120 min after feeding was lower ($P < 0.01$) for the S4 than the S1 starch diet and at 480 min after feeding was lower ($P < 0.001$) for the S4 compared with the S1, S2, and S3 starch diets (Fig. 3*B*). In vivo glucose release could be modeled effectively with Eq. 1 ($R^2 = 0.964$). Of the total glucose that appeared in portal circulation, 18.3 and 54.4% appeared from 120 to 480 min postprandial, respectively, in the S1 and S4 starch diets (Fig. 2).

Gastric emptying. The calculated time points at which equal amounts of starch diet left the stomach were 0, 6.7, 13.0, 19.6, 26.5, 33.8, 41.8, 50.0, 60.1, 70.9, 83.4, 98.0, 115.8, 138.7, 170.8, 225.5, and 480.0 (fixed to end of measurements) min. D converged to 0.85, k to 0.0075, and β to 0.76, suggesting a half-time for gastric emptying of 69 min and a lag phase of 4.6 min and rapid initial emptying.

Relation of in vitro starch digestion with portal glucose appearance. The in vitro starch digestion had a quadratic relationship with portal glucose appearance ($R^2 = 0.89$; $0.01x^2 + 0.07x + 0.53$; $P < 0.001$; **Fig. 4***A*). The in vitro starch digestion corrected for gastric emptying had a linear relation with portal glucose appearance ($R^2 = 0.95$; $y = 0.95x$; $P < 0.001$; Fig. 4*B*).

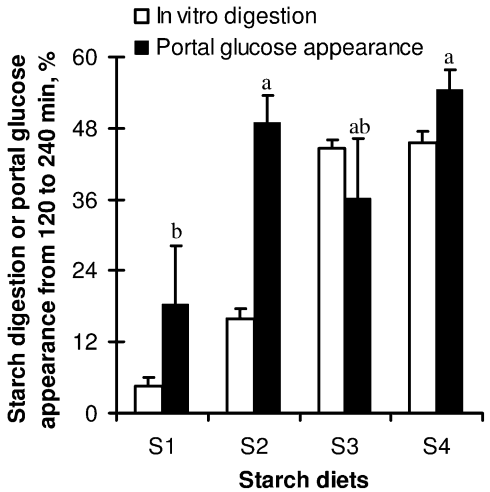

FIGURE 2 In vitro glucose release from 120 to 480 min of incubation and portal glucose appearance in pigs from 120 to 480 min after feeding from 4 starch diets. Values are means + SE, $n = 3$ (in vitro release) or 4 (portal appearance). For glucose appearance, means without a common letter differ, $P < 0.05$.

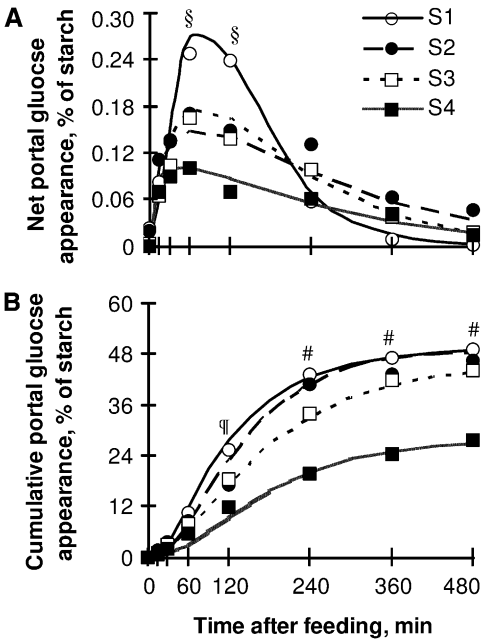

FIGURE 3 Absolute (*A*) and cumulative (*B*) portal appearance of glucose in pigs fed 4 starch diets. A modified Chapman-Richards model was used to predict kinetics of portal glucose appearance from observed values ($R^2 = 0.964$). Values are means, $n = 4$. In A, the SE ranged from 0.01 to 0.03; in B the SE ranged from 0.02 to 5.22%. Symbols indicate that means differ, $P < 0.05$: §, S1 > S2 = S3 > S4; ¶, S1 > S4; and #, S1 = S2 = S3 > S4.

Discussion

The current paradigm is that the in vitro-based starch fractions RDS, SDS, and RS reflect the in vivo glucose response (1,2). However, whether these fractions accurately translate to in vivo glucose absorption is controversial. For example, 30 min in vitro starch digestion of cooked rapidly and slowly digestible legume starch related weakly ($R^2 = 0.41$) with 0- to 60-min postprandial glucose responses (26). Glycemic index was moderately related with RDS ($R^2 = 0.54$) and SDS ($R^2 = 0.63$) from cereal products (5). Similarly, in vitro digestion was related ($R^2 = 0.76$) with rate of digestion in chickens fed cereal starches (9). Finally, the RS of rice, cornflakes, and baked beans measured after 15 h but not after 120 min in vitro digestion was related strongly ($R^2 = 0.94$) to in vivo RS in ileostomy patients (7). In vitro predictions of in vivo glucose release thus vary with method and starch source (2,7,27). Hence, an important proportion of the variation between in vitro starch digestion and in vivo glucose response is yet to be defined and should be considered to improve the accuracy of prediction equations.

Possible explanations for these discrepancies in previous studies between in vitro starch digestion and in vivo glucose response include: *1*) confounding effects of nutrients other than starch present in grains and diets; *2*) coverage of limited postprandial duration (typically 120 min); *3*) use of discrete glucose response observations as opposed to continuous glucose kinetics response curves; and *4*) observation of glucose response in peripheral circulation as opposed to portal circulation.

The present study was designed to avoid these shortcomings. First, possible confounding effects of starch-associated com-

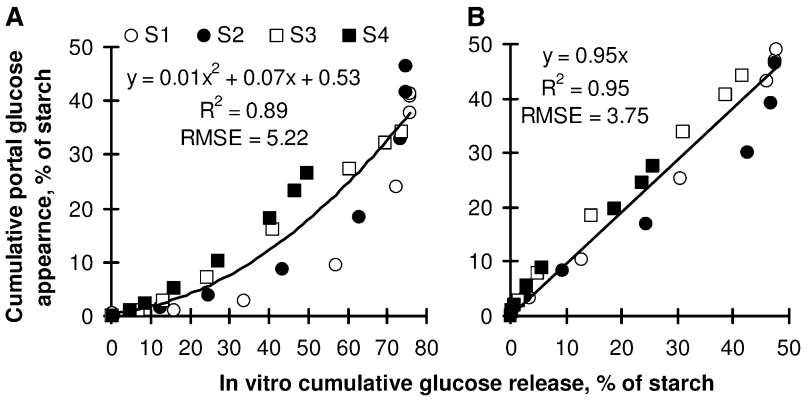

FIGURE 4 Prediction of modeled net portal glucose appearance in pigs fed 4 starch diets using in vitro cumulative glucose digestion (*A*) and in vitro cumulative glucose digestion corrected for predicted gastric emptying (*B*).

pounds such as protein, fat, and fiber were avoided by replacing unpurified starch in diets (28,29) with purified starch. Second, in vivo and in vitro responses were extended beyond 120 min, because a significant proportion of in vitro glucose release and portal glucose appearance takes place after 120 min in our and other studies (7,18). Third, to measure in vivo glucose response, postprandial net portal glucose appearance replaced peripheral glucose (11,26), because the latter does not account for hepatic glucose consumption (30) and, thus, under-represents true glucose uptake. Fourth, discrete glucose response observations were converted to continuous glucose kinetics response curves using a modified Chapman-Richards model (23).

Specifically for this last point, glucose data were modeled to facilitate the comparison between in vivo and in vitro. For this type of work, typically the first-order derivative of an association-type exponential model to determine the kinetics of starch digestion is used: starch hydrolysis = plateau × [1 − exp(–K × time)]. This model is based on the rate and maximum release of glucose. However, this model poorly fit the digestion curves in our study. First, this model does not represent the sigmoidal digestion pattern that was observed especially for rapidly digesting starch sources like wheat (23). A possible explanation for the sigmoidal digestion is that starch granules with type A crystalline patterns are digested inside-out (23,31). In vivo, the sigmoidal pattern is also reinforced by an initial delay in digesta transit (24,32). Second, the model does not consider the amount of free glucose initially present in the sample. The present study finding that samples contained up to 0.46% of glucose at the start of incubation with pancreatin justified the inclusion of initial glucose data in the model. The modified Chapman-Richards model uses the parameters of the first-order model and also describes the sigmoidal nature of starch digestion and initial glucose release. Thus, this model was used to determine the kinetics of in vitro starch digestion and net portal glucose appearance.

Portal glucose appearance was strongly related with in vitro starch digestion (R^2 = 0.89; Fig. 4A), a substantial improvement over most of the studies listed above. However, the relation was not linear, indicating that an important variable was missing and also had a high prediction error (root mean square error = 5.22). The resultant relationship was thus not satisfactory.

A major discrepancy between in vivo and in vitro digestion is that in vitro, the entire sample is incubated with an optimized enzyme cocktail at time 0. In contrast, in vivo, a meal is eaten over time (minutes) and subsequently the stomach acts as a buffer that releases the content slowly and nonlinearly (hours). Released digesta are mixed with pancreatic amylase, which is slowly and semicontinuously released under complex physiological control and brush-border disaccharidases (33). Finally, glucose present in the incubation solution determines in vitro glucose release, whereas glucose released in the intestinal lumen has to pass across the gut epithelium before reaching the portal vein (minutes). Glucose transport across the epithelium will delay appearance, whereas epithelial glucose use will reduce portal appearance of glucose (34). Indeed, the initial lag phase was longer in vivo and maximal glucose release rates occurred roughly 1 h later than in vitro. The subsequent decline was also notably slower in vivo, indicating a longer half-life. A logical extension for the above model is thus to take the digestive tract prior to the portal vein into consideration. Gastric emptying is likely the dominant factor, because it occurs over hours rather than minutes and hence was the focus for a correction factor.

Indeed, correction of in vitro data with gastric emptying yielded a much improved and linear fit between in vivo and in vitro data. The obtained gastric emptying parameters in the present study are in perfect agreement with values observed by Lefebvre et al. (35), who obtained a halftime of 74 min and a lag phase of 3 min in pigs of comparable size. We assumed that the 4 starch sources had an equal rate of gastric emptying but realize that variation may exist; determination of actual gastric emptying will likely further improve the prediction accuracy.

The D value of 0.85 indicates that 15% less glucose is recovered in vivo than in vitro, in line with differences between in vivo and in vitro plateau values. The likely explanation for this difference is glucose consumption by the intestinal microbiota and epithelium. Glucose is certainly not the preferred energy substrate of the epithelium but is nevertheless used as a fuel (36,37). The correction of in vitro starch digestion for gastric emptying and intestinal glucose utilization thus improved the relation between in vitro starch digestion and portal glucose appearance, decreased the prediction error, and changed the relationship from quadratic to linear. This provides strong mathematical evidence that gastric emptying and intestinal glucose utilization should be considered when extrapolating in vitro to in vivo data.

RS is defined as the sum of starch and starch degradation products that reach the large intestine of monogastric species and is predicted by Englyst et al. (2) as the amount of starch not digested after 120 min of incubation. A cutoff of 120 min is supported by data from Englyst et al. (38), who showed that 100% of in vitro-determined RS from retrograded cereals was recovered in ileal digesta in humans. Conflicting with this observation, in the present study, a significant proportion of starch digestion occurred from 120 to 480 min (in vitro, up to 45.5% of total glucose release; in vivo, up to 54.4% of total glucose appearance in portal circulation). Similar to the present study, in vitro RS from up to 360-min digestion was related

poorly with in vivo RS in humans eating diets containing 0.7–5.7% RS from ground rice, cornflakes, and baked beans (7). These findings indicate that the 120-min digestion values are inappropriate as a general recommendation for determining RS. Instead, by extrapolating in vitro digestibility data to infinity (plateau values), a good relation is obtained between in vivo and in vitro (R^2 = 0.95 in our study). Practically 95% of starch was digested in vitro by 400 min for even the S4 starch diet. Hence, in vitro incubations of 400 min or more, or, e.g., 16 h as described by McCleary et al. (18) are all adequate for determining RS if modeling of the data are not feasible. In vivo plateau values, though, were 13% lower across samples, indicative of intestinal consumption of glucose.

The RDS is commonly seen as the fraction with the strongest impact on blood glucose and insulin responses after eating a starch source and is linked with type-II diabetes. If starch digestion was nonsigmoidal, this interpretation would be warranted. However, findings from the present study and van Kempen et al. (23) indicate that starch digestion is sigmoidal in nature, especially sources high in RDS. Maximum rates of starch digestion were observed as late as 20 min into the in vitro assay (T.A.T.G. van Kempen, unpublished data); therefore, defining the first 20 min of in vitro digestion as RDS is inappropriate. The definition of SDS, digested between 20 and 120 min or between RDS and RS, also loses relevance if both RDS and RS are not defined properly. The substantial proportion of starch digestion and glucose absorption that occurs between 120 and 480 min postprandial indicates that the glycemic index, which is derived by measuring blood glucose from 0 to 120 min postprandial, has little biological relevance and cannot properly differentiate foods with different rates of starch digestion. Our proposal is to instead model digestibility curves using the modified Chapman-Richards model. This allows for the calculation of the maximal rate of starch digestion and thus glucose release as a predictor of insulin response, the lag time for this peak, the final extent of digestion, and the kinetics of starch digestion throughout.

In conclusion, starch digestion can be modeled accurately using the modified Chapman-Richards model that correctly describes the sigmoidal digestion curves observed in vivo and in vitro. In vivo portal glucose appearance data can be predicted effectively from in vitro digestibility data after correction for gastric emptying and correction for intestinal glucose utilization. The resultant model explained 95% of the in vivo variation when testing 4 heterogeneous starch sources. The fractions RDS, SDS, and RS appear to have less biological relevance and are thus of little value for predicting the in vivo responses to starch sources.

Acknowledgments

P.R.R., T.A.T.G.v.K., and R.T.Z. designed research; P.R.R. and J.J.M. conducted research; P.R.R. and T.A.T.G.v.K. analyzed data; P.R.R., T.A.T.G.v.K., and R.T.Z. wrote the paper; R.T.Z. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited

1. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH. Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. Am J Clin Nutr. 1999;69:448–54.
2. Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. Eur J Clin Nutr. 1992;46 Suppl 2:S33–50.
3. Englyst KN, Hudson GJ, Englyst HN. Starch analysis in food. In: Meyers RA, editor. Encyclopedia of analytical chemistry. Chichester (UK): John Wiley & Sons Ltd; 2000. p. 4246–62.
4. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. Am J Clin Nutr. 1991;54: 846–54.
5. Englyst KN, Vinoy S, Englyst HN, Lang V. Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. Br J Nutr. 2003;89:329–40.
6. Englyst HN, Veenstra J, Hudson GJ. Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. Br J Nutr. 1996;75:327–37.
7. Muir JG, O'Dea K. Validation of an in vitro assay for predicting the amount of starch that escapes digestion in the small intestine of humans. Am J Clin Nutr. 1993;57:540–6.
8. Garsetti M, Vinoy S, Lang V, Holt S, Loyer S, Brand-Miller JC. The glycemic and insulinemic index of plain sweet biscuits: relationships to in vitro starch digestibility. J Am Coll Nutr. 2005;24:441–7.
9. Weurding RE, Veldman A, Veen WA, van der Aar PJ, Verstegen MW. In vitro starch digestion correlates well with rate and extent of starch digestion in broiler chickens. J Nutr. 2001;131:2336–42.
10. O'Dea K, Snow P, Nestel P. Rate of starch hydrolysis in vitro as a predictor of metabolic responses to complex carbohydrate in vivo. Am J Clin Nutr. 1981;34:1991–3.
11. Priebe MG, Wachters-Hagedoorn RE, Heimweg JA, Small A, Preston T, Elzinga H, Stellaard F, Vonk RJ. An explorative study of in vivo digestive starch characteristics and postprandial glucose kinetics of wholemeal wheat bread. Eur J Nutr. 2008;47:417–23.
12. Kim WK, Chung MK, Kang NE, Kim MH, Park OJ. Effect of resistant starch from corn or rice on glucose control, colonic events, and blood lipid concentrations in streptozotocin-induced diabetic rats. J Nutr Biochem. 2003;14:166–72.
13. Grabitske HA, Slavin JL. Low-digestible carbohydrates in practice. J Am Diet Assoc. 2008;108:1677–81.
14. Behall KM, Scholfield DJ, Hallfrisch JG, Liljeberg-Elmstahl HG. Consumption of both resistant starch and beta-glucan improves postprandial plasma glucose and insulin in women. Diabetes Care. 2006;29:976–81.
15. Pond WG, Lei XG. Of pigs and people. In: Lewis AJ, Southern LL, editors. Swine nutrition. 2nd ed. New York: CRC Press; 2001. p. 3–18.
16. Spurlock ME, Gabler NK. The development of porcine models of obesity and the metabolic syndrome. J Nutr. 2008;138:397–402.
17. Germaine KA, Samman S, Fryirs CG, Griffiths PJ, Johnson SK, Quail KJ. Comparison of in vitro starch digestibility methods for predicting the glycaemic index of grain foods. J Sci Food Agric. 2008;88:652–8.
18. McCleary BV, McNally M, Rossiter P. Measurement of resistant starch by enzymatic digestion in starch and selected plant materials: collaborative study. J AOAC Int. 2002;85:1103–11.
19. Hooda S, Matte JJ, Wilkinson CW, Zijlstra RT. Technical note: an improved surgical model for the long-term studies of kinetics and quantification of nutrient absorption in swine. J Anim Sci. 2009;87: 2013–9.
20. NRC. Nutrient requirements of swine. 10th rev. ed. Washington, DC: National Academy Press; 1998.
21. Rérat AA, Vaissade P, Vaugelade P. Absorption kinetics of some carbohydrates in conscious pigs. 2. Quantitative aspects. Br J Nutr. 1984;51:517–29.
22. Fekedulegn D, Mac Siurtain MP, Colbert JJ. Parameter estimation of nonlinear growth models in forestry. Silva Fennica. 1999;33:327–36.
23. Van Kempen T, Pujol S, Tibble S, Balfagon A. In vitro characterization of starch digestion and its implications for pigs. In: Wiseman J, Varley MA, McOrist S, Kemp B, editors. Paradigms in pig science. Nottingham (UK): Nottingham University Press; 2007. p. 515–26.
24. Siegel JA, Urbain JL, Adler LP, Charkes ND, Maurer AH, Krevsky B, Knight LC, Fisher RS, Malmud LS. Biphasic nature of gastric emptying. Gut. 1988;29:85–9.
25. Camilleri M, Malagelada JR, Brown ML, Becker G, Zinsmeister AR. Relation between antral motility and gastric emptying of solids and liquids in humans. Am J Physiol. 1985;249:G580–5.
26. O'Dea K, Wong S. The rate of starch hydrolysis in vitro does not predict the metabolic responses to legumes in vivo. Am J Clin Nutr. 1983;38:382–7.
27. Champ M. Determination of resistant starch in foods and food products: interlaboratory study. Eur J Clin Nutr. 1992;46 Suppl 2: S51–62.

28. Annison G, Topping DL. Nutritional role of resistant starch: chemical structure vs physiological function. Annu Rev Nutr. 1994;14:297–320.
29. Thorne MJ, Thompson LU, Jenkins DJ. Factors affecting starch digestibility and the glycemic response with special reference to legumes. Am J Clin Nutr. 1983;38:481–8.
30. Woerle HJ, Meyer C, Dostou JM, Gosmanov NR, Islam N, Popa E, Wittlin SD, Welle SL, Gerich JE. Pathways for glucose disposal after meal ingestion in humans. Am J Physiol Endocrinol Metab. 2003;284: E716–25.
31. MacGregor AW, Ballance DL. Hydrolysis of large and small starch granules from normal and waxy barley cultivars by alpha-amylases from barley malt. Cereal Chem. 1980;57:397–402.
32. Ziessman HA, Fahey FH, Collen MJ. Biphasic solid and liquid gastric emptying in normal controls and diabetics using continuous acquisition in LAO view. Dig Dis Sci. 1992;37:744–50.
33. Liddle RA. Regulation of pancreatic secretion. In: Johnson LR, Barret KE, Gishan FK, Merchant JL, Said HM, Wood JD, editors. Physiology of the gastrointestinal tract. 4th ed. Maryland Heights (MO): Elsevier Inc.; 2006. p. 1397–435.
34. Huntington GB, Harmon DL, Richards CJ. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J Anim Sci. 2006;84 Suppl:E14–24.
35. Lefebvre RA, Dick JM, Guerin S, Malbert CH. Involvement of NO in gastric emptying of semi-solid meal in conscious pigs. Neurogastroenterol Motil. 2005;17:229–35.
36. Wu G, Knabe DA, Yan W, Flynn NE. Glutamine and glucose metabolism in enterocytes of the neonatal pig. Am J Physiol. 1995; 268:R334–42.
37. Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. quantitative importance of glutamine, glutamate, and aspartate. J Biol Chem. 1980;255: 107–12.
38. Englyst HN, Kingman SM, Hudson GJ, Cummings JH. Measurement of resistant starch in vitro and in vivo. Br J Nutr. 1996;75:749–55.