Starch with High Amylose and Low in Vitro Digestibility Increases Short-Chain Fatty Acid Absorption, Reduces Peak Insulin Secretion, and Modulates Incretin Secretion in Pigs1–3

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Abstract

Diets containing different starch types affect peripheral glucose and insulin responses. However, the role of starch chemistry in kinetics of nutrient absorption and insulin and incretin secretion is poorly understood. Four portal vein-catheterized pigs (35.0 ± 0.2 kg body weight) consumed 4 diets containing 70% purified starch [0–63.2% amylose content and 0.22 (slowly) to 1.06%/min (rapidly) maximum rate of in vitro digestion] for 7-d periods in a 4 × 4 Latin square. On d 7, blood was collected for 12 h postprandial with simultaneous blood flow measurement for determining the net portal appearance (NPA) of nutrients and hormones. The NPA of glucose, insulin, C-peptide, and glucose-dependent incretinotropic polypeptide (GIP) during 0–4 h postprandial were lower (P < 0.05) and those of butyrate and total SCFA were higher (P < 0.05) when pigs consumed the diet containing slowly digestible compared with rapidly digestible starch. The peak NPA of insulin occurred prior to that of glucose when pigs consumed diets containing rapidly digestible starch. The kinetics of insulin secretion had a linear positive relation with kinetics of NPA of glucose (R² = 0.50; P < 0.01). In conclusion, starch with high amylose and low in vitro digestibility decreases the kinetics of glucose absorption and insulin and GIP secretion and increases SCFA absorption and glucagon-like peptide-1 secretion. In conclusion, starch with high amylose content and a lower rate and extent of in vitro digestion decreased glucose absorption and insulin secretion and increased SCFA absorption. J. Nutr. 141: 398–405, 2011.

Introduction

Starch is the major dietary source of glucose for monogastric species. The kinetics (rate and extent) of starch digestion depends on starch chemistry, particle size, processing method, and association with other compounds such as lipids, protein, fiber, minerals, and antinutritional factors (1). Among starch chemistry characteristics, amylose content and crystallinity are important factors affecting starch digestibility and thus metabolic responses in vivo. Unlike amylpectin, which is highly branched, amylose polymers have less surface area and more intra-molecular hydrogen bonds (1) and can form complexes with surface compounds such as fatty acids (2). Thus, amylose polymers are digested at a lower rate and extent than amylpectin polymers due to decreased accessibility for α-amylase to the molecule. Conversely, the crystalline region of starch granules, which is formed by densely packed glucose molecules mainly from amylpectin, has lower accessibility for enzymes compared with the amorphous region and, thus, purportedly lower digestibility (1).

The kinetics of starch digestion influence postprandial glucose and insulin responses that are associated with the risk of metabolic diseases such as type II diabetes in humans (3). In addition, luminal glucose and SCFA, affected by starch digestibility, stimulate the secretion of incretins such as glucose-dependent insulinitropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (4). Increased blood glucose concentration...
stimulates insulin secretion and incretins potentiate glucose-stimulated insulin secretion (4). Although effects of amylase (5,6) and resistant starch (RS) (6–8) on peripheral glucose and insulin response have been reported, the association that allows starch chemistry to affect the absorption of starch-derived nutrients and secretion of insulin and incretins is not clear. In addition, the relationship between the kinetics of nutrient absorption and hormone secretion is not clear.

Therefore, diets containing 4 purified starches with a wide difference in starch chemistry were consumed by porto-arterial catheterized pigs to test the hypothesis that a higher content of amylase reduces glucose absorption, insulin, and incretin secretion and increases SCFA absorption via a lower starch digestibility. The objectives were to determine the role of starch chemistry in the kinetics of starch-derived nutrient absorption and insulin and incretins secretion and to understand the interrelationship of the kinetics of nutrient absorption and insulin and incretin secretion as affected by starch chemistry.

Materials and Methods

Starch characterization. Remyline AX-DR 0% amylase rice starch (Remy Industries), Remy B7 20% amylase rice starch (Remy Industries), Nestar 35.5% amylase pea starch (Cosucra Group Warcoing), and Gelose 80% amylase corn starch (Penford Food Ingredients) samples were used in the study (Table 1). Starch content was determined after enzymatic hydrolysis of samples using thermostable α-amylase and amyloglucosidase (Megazyme Int. Ireland). Amylose content was determined using a Megazyme amylose/amylopectin assay kit (Megazyme Int.). Starch granule size was determined using a scanning-electron microscopy (6301E; JEOL) (Fig. 1). Crystallinity was determined after X-ray diffraction of starches (Geigerflex 2173, Rigaku/MSC) using a cobalt X-ray source (Supplemental Fig. 1) (9). Crude protein content was determined using an N-analyzer (FP-2000 N Analyzer; Leco Instrument). Fat content was determined by extraction with ether (method 920.39; AOAC, 1990) using a Goldfisch Fat Extractor (Laconco). The starches were considered rapidly digestible (S1), moderately rapidly digestible (S2), moderately slowly digestible (S3), and slowly digestible (S4) based on the maximum rate of in vitro starch digestion based on a 2-stage in vitro digestion assay modified after Englyst [S1, 1.06; S2, 0.73; S3, 0.38; and S4, 0.22%/min (10)].

**TABLE 1** Characteristics of 4 starches used in the diets

<table>
<thead>
<tr>
<th>Characteristic (DM basis)</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch, g/kg</td>
<td>963</td>
<td>958</td>
<td>979</td>
<td>943</td>
</tr>
<tr>
<td>Maximum rate of in vitro digestion, g/kg-min</td>
<td>10.6</td>
<td>7.3</td>
<td>3.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Total in vitro starch digestion, g/kg</td>
<td>759</td>
<td>749</td>
<td>739</td>
<td>498</td>
</tr>
<tr>
<td>Free glucose, g/kg</td>
<td>2.3</td>
<td>2.5</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Amylose content, g/kg</td>
<td>&lt;5%</td>
<td>196</td>
<td>284</td>
<td>632</td>
</tr>
<tr>
<td>Crystallinity, %</td>
<td>40</td>
<td>36</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>Starch granule size</td>
<td>Width, μm</td>
<td>1.8–3.8</td>
<td>2.4–5.6</td>
<td>5.0–13.5</td>
</tr>
<tr>
<td></td>
<td>Length, μm</td>
<td>2.4–5.7</td>
<td>2.3–8.9</td>
<td>5.4–34.0</td>
</tr>
<tr>
<td></td>
<td>Crude protein, g/kg</td>
<td>17</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Crude fat, g/kg</td>
<td>5.1</td>
<td>5.6</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Ash, g/kg</td>
<td>1.8</td>
<td>2.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1 Determined by 8 h in vitro enzymatic digestion (10).
2 Determined by X-ray diffraction (Supplemental Fig. 1), and can be visualized as opaque area of starch particle observed under transmission electron microscopy (Supplemental Fig. 2).
3 Detection limit was 50 g/kg.

**Pig experiment.** The animal use protocol was approved by the Animal Care and Use Committee for Livestock at the University of Alberta. Four female pigs were surgically modified (11) and consumed 1 of the 4 diets containing 70% of the purified starches (Supplemental Table 1) in a 4 × 4 Latin square design. Blood sampling, portal blood-flow rate measurements, and plasma flow rate calculations were previously described and the same samples were used for the present study (10).

**Plasma analysis and calculations.** Plasma was analyzed for glucose ([intra- and inter-assay CV ≤ 2.0]; glucose oxidase kit, Diagnostics Chemicals), L-lactate ([intra- and inter-assay CV ≤ 2.0]; lactate dehydrogenase-based kit, State University of New York), and SCFA (acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate) using GC ([intra- and inter-assay CV ≤ 11.2] as described by Brighenti (12) with some modifications. Briefly, isocaproic acid (0.93 mg/L of water) was used as an internal standard (12.5 μL/100 μL of plasma) and plasma was deproteinized using 25% phosphoric acid solution (8 μL/100 μL of plasma) at 60°C for 30 min. The solution was centrifuged at 8000 × g for 30 min to remove the proteins. The supernatant was analyzed for SCFA by GC using He as a carrier gas, a 30-m × 0.53-mm × 1-μm size column, and an FID detector (Hewlett Packard). Insulin ([intra-assay CV = 7.3 and inter-assay CV = 8.8] and C-peptide ([intra-assay CV = 6.6 and inter-assay CV = 9.5]) were analyzed by using porcine RIA kits (Linco). For GIP analysis, 85 μg of aprotinin (Roche Diagnostics) was added to each milliliter of plasma before storage to prevent GIP degradation. GIP content in the plasma was determined (intra-assay CV = 10.0 and inter-assay CV = 13.5) by RIA (Peninsula Laboratories). For GLP-1 determination, the samples were extracted using alcohol and determined (intra-assay CV = 12.8 and inter-assay CV = 15.1) by RIA (Peninsula Laboratories).

Net portal appearance (NPA) of glucose, L-lactate, SCFA, insulin, C-peptide, GIP, and GLP-1 was calculated using the formula: NPA of nutrients or hormones = (portal concentration – arterial concentration) × plasma flow rate in the portal vein. Subsequently, cumulative 12-h NPA of nutrients was calculated. Peak NPA (Tmax) and time to reach peak NPA (Tmax) for glucose, insulin, and C-peptide and time required to halve the peak NPA (1/2Tmax) for insulin and C-peptide were calculated. Carotid plasma glucose half-life (time required to halve the peak concentration of glucose in carotid plasma), which reflects insulin effects on blood glucose concentration, was also calculated. The simultaneous glucose uptake was not separated from the arterial glucose pool. The area under the curve (AUC) was calculated based on the trapezium rule using the formula: AUC = 1/2 (t1 + t0) (yi+yi+1). In the formula, ti is a specific time point and y is the measurement (13).

The total NPA of starch-derived nutrients such as glucose, L-lactate, and total SCFA as a percentage of starch consumed was calculated. NPA of glucose was multiplied by a factor of 0.9, because the molecular weight of glucose as incorporated into starch is 90% of that of free glucose. Two moles each of L-lactate are considered to be produced from each mole of glucose (14). Because the NPA of SCFA is primarily from microbial fermentation of undigested starch and protein in the ileum and large intestine, the NPA of SCFA from protein was deducted from the total NPA of SCFA to calculate the NPA of SCFA from starch. The NPA of SCFA from protein was calculated using the NPA of branched-chain fatty acids (BCFA), isovalerate and isobutyrate, as a marker, because BCFA are solely produced from protein sources and 20% of the total SCFA production from protein was assumed to be BCFA (15). Each mole of glucose is considered to be converted to either 2 mol each of acetate, propionate, and isopropionate or either 1 mol of butyrate, valerate, and caproate (16).

**Modeling of kinetics of insulin secretion.** A Chapman-Richards model was used to describe the NPA of insulin based on measured insulin values. A modified model, corrected for plateau effect, was used to describe the NPA of glucose based on measured glucose values (10). Several models were evaluated for predicting the NPA of insulin based on the NPA appearance of glucose, but none yielded satisfactory results (data not shown). Instead, the NPA of insulin was modeled based on in vitro glucose release using a linear model: predicted NPA of insulin, I(t) = I(0) – I0 exp[-(t–ti)/τ]. In the formula, I(t) is the NPA of insulin, I(0) is the peak NPA of insulin, I0 is the area under the curve (AUC) of insulin, and τ is the time constant of insulin secretion.

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pmol/min = A × in vitro glucose release corrected for gastric emptying, % of starch, where A is a constant used to make the in vitro glucose release as the same scale as the NPA of glucose in mmol/min.

**Statistical analysis.** The NPA of nutrients, insulin, and incretins was analyzed using repeated measures in SAS (version 9.1; SAS Institute). The model included diet, time, and interaction of diet and time as fixed effects and pig and period as random effects. Preprandial observations were used as covariates to determine postprandial effects. Cmax, Tmax, and 1/2Tcmax for glucose, insulin, and C-peptide were analyzed using the mixed model in SAS with diet as a fixed effect and period as a random effect. Means were separated for diet using the PDIF statement in the Mixed model for individual time points after detecting a significant diet effect using SLICE/time. Pigs were considered the experimental units and significance of difference was set at \( P < 0.05 \). All variables were tested by the Kolmogorov-Smirnoff test for normal distribution. Values in the text are means ± SEM.

Principal component (PC) analysis was performed using JMP (version 8.0.2; SAS Institute). The loading plots of PC 1 and PC 2, the first 2 eigenvalues, were used to determine the correlation among the NPA of nutrients and hormones and starch characteristics. The angles between the lines were used to describe the interrelationship. Simple linear and multiple linear regression analyses were performed using JMP to determine relationships between and among variables. Log-transformed data were used for regression analyses to meet the assumption of linear relationships.

**Results.**

All pigs were healthy throughout the experiment as indicated by normal appetite and growth. Development of collateral circulation in the portal system was not observed during autopsy at the end of experiment. The blood flow rate was \( 1.2 \pm 0.09 \) L/min and did not differ \( (P > 0.05) \) when pigs consumed the different starch diets. The blood flow rate was higher \( (P < 0.05) \) from 0 to 4 h than from 4 to 10 h, followed by 10–12 h postprandial (data not shown).

**Glucose.** When pigs consumed the S4 diet, the peak (Table 2) and 12-h NPA (Table 3) of glucose were lower \( (P < 0.05) \) than when they consumed the other 3 diets. The time point for peak NPA of modeled glucose did not differ \( (P > 0.05) \) among pigs consuming the 4 starch diets and ranged from 63 to 92 min postprandial. The time required to halve the peak concentration of glucose in the carotid artery was shorter \( (P < 0.05) \); Table 2) when pigs consumed the S1 diet than when they consumed the S4 diet. When pigs consumed the S4 diet, the NPA of glucose was lower \( (P < 0.05) \) than when they consumed the S1 diet from 45 min to 3.5 h, than when they consumed the S2 diet at 30 and 45 min and at 1.5, 2, and 3.5 h, and than when they consumed the S3 diet at 2 and 3.5 h postprandial (Fig. 2A). Similarly, the NPA of glucose was lower \( (P < 0.05) \) when pigs consumed the S3 diet than when they consumed the S1 diet at 45 min and 1.5 and 2 h postprandial and was lower \( (P < 0.05) \) when pigs consumed the S2 diet than when they consumed the S1 diet at 1 and 2 h postprandial. The NPA of glucose was negative from 8 to 12 h postprandial when pigs consumed the S1 diet and at 12 h when they consumed the S2 and S3 diets and was positive for all time points when pigs consumed the S4 diet (Fig. 2A). The NPA of glucose differed among 4 postprandial periods: 0–2 h

![Figure 1](image1.jpg)

**FIGURE 1** Electron micrograph of the 4 starches (×350).

**TABLE 2** Kinetic parameters of NPA of glucose, insulin, and C-peptide in pigs consuming 4 starch diets differing in rate of digestion

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Starch diets</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>Glucose</td>
<td>Cmax, mmol</td>
<td>6.61a</td>
</tr>
<tr>
<td>T_{max}, min</td>
<td>62.6</td>
<td>92.3</td>
</tr>
<tr>
<td>Carotid plasma half-life, min</td>
<td>380a</td>
<td>375ab</td>
</tr>
<tr>
<td></td>
<td>1/2T_{cmax}, min</td>
<td>62.7</td>
</tr>
<tr>
<td>Insulin</td>
<td>Cmax, mmol</td>
<td>0.91a</td>
</tr>
<tr>
<td>T_{max}, min</td>
<td>47.4</td>
<td>75.5</td>
</tr>
<tr>
<td>1/2T_{cmax}, min</td>
<td>62.7</td>
<td>82.6</td>
</tr>
<tr>
<td>C-peptide</td>
<td>Cmax, mmol</td>
<td>1.68a</td>
</tr>
<tr>
<td>T_{max}, min</td>
<td>50.6</td>
<td>71.2</td>
</tr>
<tr>
<td>1/2T_{cmax}, min</td>
<td>83.8</td>
<td>95.4</td>
</tr>
</tbody>
</table>

1 Values are means and pooled SEM, \( n = 4 \). Labeled means in a row without a common superscript differ, \( P < 0.05 \).
TABLE 3  NPA of major starch-derived nutrients in 12 h in pigs consuming 4 starch diets differing in rate of digestion  

<table>
<thead>
<tr>
<th>Starch-derived nutrients</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>52.2 ± 3</td>
<td>49.6 ± 3</td>
<td>33.8 ± 3</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.186 ± 0.05</td>
<td>0.320 ± 0.05</td>
<td>0.264 ± 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Acetate</td>
<td>89.4 ± 3</td>
<td>84.4 ± 3</td>
<td>86.8 ± 3</td>
<td>57.9 ± 3</td>
<td>*</td>
</tr>
<tr>
<td>Propionate</td>
<td>2.26 ± 0.38</td>
<td>2.36 ± 0.38</td>
<td>2.50 ± 0.40</td>
<td>12.6 ± 4.79</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means and pooled SEM, n = 4. Means in a row without a common superscript differ, P < 0.05.
2 The NPA of starch-derived nutrients was calculated as a percentage of starch consumed using the following assumptions: glucose in starch = NPA of (free) glucose × 0.9; 1 mol of glucose = 2 mol of L-lactate; NPA of SCFA from starch = total NPA of SCFA – NPA of SCFA from protein; NPA of SCFA from protein = (isovalerate + isobutyrate) × 0.3; 1 mol each of butyrate, valerate, and caproate.

increased and peaked), 2–4 h (declined drastically), 4–10 h (declined further toward zero), and 10–12 h (negligible); hence, these time periods were used to calculate the AUC of NPA of nutrients and hormones in the present study. The AUC of NPA of glucose was lower (P < 0.05) when pigs consumed the S4 diet than when they consumed the S1 diet at 0–4 h and the S2 diet at 4–10 h postprandial (Supplemental Fig. 3A). Lactate and SCFA. When pigs consumed the S3 and S4 diets, the NPA of L-lactate was lower (P < 0.05) than when they consumed the S1 diet at 1 h postprandial and when they consumed the S2 diet at 45 min and 1 h postprandial (Fig. 2B). The NPA of L-lactate was higher (P < 0.05) when pigs consumed the S4 diet than when they consumed the S1 and S2 diets at 12 h. The AUC of NPA of L-lactate was lower (P < 0.05) than when they consumed the S1 diet than when they consumed the other 3 diets at 0–2 h postprandial (Supplemental Fig. 3C). At 4–10 h postprandial, the AUC of NPA of C-peptide was greater when pigs consumed the S2 diet than when they consumed the S4 diet. The AUC of insulin did not differ (P > 0.05) among pigs consuming the 4 diets.

Incretins. The NPA of GIP was higher (P < 0.05) when pigs consumed the S1 diet than when they consumed the S4 diet at 2.5 h postprandial (Fig. 3C). Although the NPA of GLP-1 did not differ (P > 0.05) among pigs consuming the 4 diets (Fig. 3D), the AUC of NPA of GLP-1 was higher (P < 0.05) when pigs consumed the S3 and S4 diets than when they consumed the S1 and S2 diets from 4 to 10 h postprandial (Supplemental Fig. 3F).

Relations of starch characteristics with NPA of nutrients, insulin, and incretins. PC analysis showed that PC 1 and PC 2 explained 79.2% of the total variability among starch characteristics and the AUC of NPA of nutrients, insulin, and incretins for different postprandial periods (Fig. 4). Total in vitro digestion corrected for gastric emptying (10) had a strong positive relation (small angle among variables) with NPA of glucose, insulin, C-peptide, and GIP and a strong negative relation (angle close to 180° among variables) with the NPA of total SCFA from postprandial (Fig. 3A). The NPA of C-peptide was greater (P < 0.05) when pigs consumed the S1 diet than when they consumed the S4 diet at 30 min and 1.5 h and than when they consumed the S2 and S3 diets at 1.5 h postprandial (Fig. 3B). The AUC of NPA of insulin and C-peptide was greater (P < 0.05) when pigs consumed the S1 diet than when they consumed the other 3 diets at 0–2 h postprandial (Supplemental Fig. 3C,D). At 4–10 h postprandial, the AUC of NPA of C-peptide was greater when pigs consumed the S2 diet than when they consumed the S4 diet. The AUC of insulin did not differ (P > 0.05) among pigs consuming the 4 diets.
The in vitro digestion rate and NPA of glucose were positively associated at 0–2 and 4–10 h postprandial. Amylose had a strong negative relation with insulin from 0 to 2 h postprandial and a weak relation from 2 to 4 h and 4 to 10 h postprandial. Crystallinity had a positive relation with the NPA of glucose and insulin from 0 to 10 h postprandial (Fig. 4).

**Relations among nutrients and hormones.** The kinetics of insulin secretion had a linear positive relation with kinetics of the NPA of glucose ($R^2 = 0.50; P < 0.01$), GIP ($R^2 = 0.42; P < 0.01$), and GLP-1 ($R^2 = 0.24; P < 0.01$) (Fig. 5). The $R^2$ value from multiple regression of kinetics of the NPA of glucose, L-lactate, total SCFA, GIP, and GLP-1 with kinetics of the NPA of insulin was not greater than that of kinetics of the NPA of insulin with kinetics of the NPA of glucose alone (data not shown). The $R^2$ of modeled NPA of insulin using a modified Chapman-Richards model and predicted NPA of insulin from modeled NPA of glucose was 0.55 ($P < 0.01$; Supplemental Fig. 4). When pigs consumed the S1 and S2 diets, the peak NPA of insulin was observed before the peak NPA of glucose, while the peak NPA of insulin was observed after the peak NPA of glucose when pigs consumed the S3 and S4 diets (Table 2; Fig. 6; A,B; Supplemental Fig. 4). In vitro starch digestion, therefore, predicted in vivo insulin release well. The kinetics of GIP secretion was related with the kinetics of NPA of glucose ($R^2 = 0.62; P < 0.01$) and the kinetics of GLP-1 secretion ($R^2 = 0.43; P < 0.01$). The kinetics of GLP-1 secretion was related with the kinetics of NPA of glucose ($R^2 = 0.47; P < 0.01$) (Fig. 5). The kinetics of insulin and C-peptide secretion were strongly related ($R^2 = 0.80; P < 0.01$). Similarly, the kinetics of NPA of glucose and L-lactate had a good relationship ($R^2 = 0.67; P < 0.01$) (Supplemental Fig. 5).

**Discussion**

The present study evaluated the effects of purified starches with a wide range in physicochemical properties using portal vein-catheterized pigs. Pigs, having a similar digestive physiology and anatomy (17) and profile of carbohydrate-derived nutrients and insulin from 0 to 2 h postprandial and a weak relation from 2 to 4 h and 4 to 10 h postprandial. Crystallinity had a positive relation with the NPA of glucose and insulin from 0 to 10 h postprandial (Fig. 4).

![FIGURE 3](https://example.com/fig3.png) NPA of insulin (A), C-peptide (B), GIP (C), and GLP-1 (D) in pigs consuming starch diets differing in rate of digestion. Values are means, $n = 4$. Symbols indicate that means differ, $P < 0.05$: *, S1 > S4; ¥, S1 = S2 > S4.

![FIGURE 4](https://example.com/fig4.png) Loading plots of the first 2 eigenvalues (PC 1 and PC 2) showing the correlations among starch characteristics (dashed arrows) and AUC of the NPA of starch-derived nutrients and insulin and incretin secretion (solid arrows) at 0–2 h (A), 2–4 h (B), 4–10 h (C), and 10–12 h (D) postprandial in pigs consuming starch diets differing in rate of digestion: 1, glucose; 2, lactate; 3, total SCFA; 4, insulin; 5, C-peptide; 6, GIP; 7, GLP-1; 8, rate of in vitro digestion; 9, total in vitro digestion; 10, amylose; and 11, crystallinity. From 0 to 2 h; 2 to 4 h; 4 to 10 h; and 10 to 12 h postprandial, the NPA of glucose, respectively, increased and peaked, declined drastically, declined further toward zero, and was negligible; hence, the postprandial period may represent 4 distinct physiological phases.
pancreatic and gut hormones as humans (18), can be used to understand the kinetics of nutrient absorption and pancreatic and gut hormone secretion. This model worked well, as indicated by sustained catheters patency throughout the study, blood flow rate (11,19), and total portal glucose recovery (20), similar to previous studies.

Generally, starches with high amylose and RS content are considered to reduce in vivo glucose and insulin responses; however, the in vivo response was not always consistent with pigs and humans (21,22). Reasons for the inconsistent response include: 1) inconsistent and inadequate starch characterization; 2) confounding effects of starch-associated compounds such as protein, fat, and fiber present in grains or diets; 3) observation of glucose response in peripheral circulation as opposed to portal circulation; and 4) coverage of limited postprandial duration (typically 120 min) to study in vivo responses.

We addressed these issues using 4 approaches. First, starch sources were characterized in depth and in vitro starch digestion was used as a link between physicochemical properties of starch and in vivo responses (10). Second, purified starch instead of intrinsic starch was used in diets to avoid confounding effects of starch-associated compounds. Third, the NPA of starch-derived nutrients, insulin, and incretins was used instead of their peripheral concentrations as a measure of in vivo starch effects, so that net absorption of nutrients and secretion of insulin and incretins was properly measured and their hepatic metabolism was avoided. Fourth, in vivo responses were observed up to 12 h postprandial replacing the typical 120 min in vivo response, because a major proportion of starch digestion can take place after 120 min postprandial (10,23).

**Starch effect on glucose, insulin, and C-peptide.** Differences in postprandial glucose and insulin responses were detected when pigs (6) and humans (5) consumed starches with a wide range in amylose and RS content. In the present study, greater peak glucose absorption was associated with greater secretion of insulin and C-peptide that resulted in a lower arterial plasma half-life of glucose. Although peak glucose absorption was greater when pigs consumed S2 and S3 diets than when they consumed the S4 diet, peak insulin secretion did not differ when pigs consumed these 3 diets. This indicated that insulin responses do not exactly parallel glucose absorption or, more likely, the portal vein-catheterized pig model with 4 observations/treatment is not sensitive enough for detecting minor differences in hormone responses (24).

Modeling indicated that secretion of insulin was delayed 5–15 min after glucose stimulation (25). In contrast, modeled insulin secretion peaked prior to peak glucose absorption when pigs consumed the S1 and S2 diets and peaked after peak glucose absorption when pigs consumed the S3 and S4 diets in the

**FIGURE 5** Relationships between NPA kinetics of insulin and glucose ($R^2 = 0.50$; root mean square error (RMSE) = 0.30; $P < 0.001$) (A), insulin and GIP ($R^2 = 0.42$; RMSE = 0.32; $P < 0.001$) (B), insulin and GLP-1 ($R^2 = 0.24$; RMSE = 0.37; $P < 0.001$) (C), GIP and glucose ($R^2 = 0.62$; RMSE = 0.19; $P < 0.001$) (D), GIP and GLP-1 total ($r = 0.43$; RMSE = 0.23; $P < 0.001$) (E), and GLP-1 and glucose ($R^2 = 0.49$; RMSE = 0.08; $P < 0.001$) (F) in pigs consuming starch diets differing in rate of digestion. Values are log-transformed means: insulin, GIP, and GLP-1 as log(pmol+2); glucose as log (mmol+2), $n = 4$.

**FIGURE 6** $T_{max}$ for NPA of glucose and insulin in pigs consuming 4 starch diets differing in rate of digestion and peak in vitro glucose release. (A) The NPA of glucose and insulin in pigs consuming the S1 diet and in vitro glucose release corrected for gastric emptying. A modified Chapman-Richards model (10) was used to model the variables from measured values (NPA of glucose, $R^2 = 0.97$; NPA of insulin, $R^2 = 0.87$). A constant was used to make the in vitro glucose release data (% of starch) on the same scale as NPA of glucose data (mmol/min). Values are predicted means, $n = 4$. (B) Difference in $T_{max}$ of glucose and that of insulin was based on observed values; insulin peaked prior to glucose in pigs consuming S1 and S2 starch diets, whereas glucose peaked prior to insulin in pigs consuming S3 and S4 starch diets. Values are means and SEM, $n = 4$. 

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present study. Earlier peak insulin secretion when pigs consumed rapidly digestible starch might be due to conditioned insulin secretion or stimulation of chemo-sensory proteins such as T1R sweet taste and G protein gustducin receptors in the tongue, stomach, and proximal small intestine (26–28) in the presence of free glucose (29). In fact, stimulation of these receptors triggers entero-pancreatic neurons that cause preabsorptive insulin secretion (30). The neural stimulation from cephalic and postprandial phases of food ingestion contributed up to 48% of total peripheral appearance of insulin (30).

**Starch effects on lactate and SCFA.** In the present study, total absorption of L-lactate was 9–12% of ingested starch and slightly higher than the 7% of ingested starch measured in pigs consuming diets containing 37% starch (21). Glucose absorption and the NPA of L-lactate were closely related. The conversion of glucose into L-lactate in the portal-drained viscera is thus proportional to glucose absorption during periods of high glucose availability in the gut. Previously, the starch effect on peripheral plasma lactate was inconsistent (6).

Total SCFA production in the gut, plasma concentration (31), total absorption (7,32), and fecal butyrate concentration (33) were higher after feeding diets containing more RS or amylose in previous studies. In the present study, total SCFA and butyrate absorption was much greater when pigs consumed the S4 diet than when they consumed the other 3 diets and did not differ when pigs consumed the S1, S2, and S3 diets. A major amount of RS is thus required to increase SCFA production and low amounts of amylose can still be converted to glucose. Therefore, only the starch with very high amylose content and lower in vitro digestibility is available in major quantities for microbial fermentation.

When pigs consumed the S1 diet, 12-h glucose was higher and 12-h total SCFA absorption was lower than when they consumed the S4 diet, but 12-h NPA of total starch-derived nutrients did not differ when pigs consumed the 4 starch diets. Thus, although much starch was not converted to glucose for the S4 diet, considerable undigested starch was fermented in the gut and absorbed as SCFA. Because energy production from SCFA is at least 14% lower than from glucose (34,35), high amylose starch is energetically less efficient than low amylose starch. Moreover, 30–35% of starch, which was not recovered in portal vein as glucose, lactate, and SCFA might have been partly used by PDV as energy, partly absorbed as alanine or intermediate metabolites of glycolysis and the Kreb’s cycle, and partly fermented in the large intestine beyond 12 h postprandial.

**Starch effects on incretins.** The cross-talk between intestine and pancreatic β-cells occurs partly via incretins, which play an important role in the regulation of blood glucose via mechanisms such as amplification of glucose-induced insulin secretion (36). Incretin-stimulated insulin secretion accounts for at least 50% of insulin secreted after oral glucose consumption (37). Duodenal and proximal jejunal K cells release GIP, whereas distal jejunal, ileal, and colonic L cells release GLP-1 (38). Glucose in the lumen stimulates signal transduction via the apical membrane of K and L cells that facilitates incretin secretion (39). Similar to the present study, peripheral GIP during the absorptive phase was positively correlated with plasma glucose in humans (40). GLP-1 was, however, not affected by starches during 0–4 h postprandial; rather, it was greater when pigs consumed S3 and S4 diets than when pigs consumed S1 and S2 diets during 4–10 h postprandial. Previously, peripheral GLP-1 was either positively correlated (8,41) or unaffected (40) during 0–2 h postprandial or negatively correlated (40) from 2 to 4 h postprandial in humans with a high compared with low plasma glucose profile. Glucose and SCFA act as potent stimulators of GLP-1 production (42,43). Therefore, SCFA-induced GLP-1 production might have balanced the glucose-induced GLP-1 production from 0 to 4 h postprandial and when pigs consumed S3 and S4 diets from 4 to 10 h postprandial in the present study.

**Starch characteristics and metabolic responses.** PC analysis revealed that the relationship of starch characteristics with the NPA of carbohydrate-derived nutrients, insulin, C-peptide, and incretins changed at different postprandial periods. Total in vitro digestion (corrected for gastric emptying) was strongly related with glucose, insulin, C-peptide, GIP, and total SCFA from 0 to 10 h postprandial and is thus a better indicator of total amount of starch available for enzymatic digestion and gut microbial fermentation than other starch characteristics. The current paradigm is that crystallinity reduces the rate of starch digestion (1); however, starch crystallinity was positively related with glucose absorption and insulin secretion from 0 to 4 h postprandial in the present study. Crystallinity effects of starch might have been confounded by effects of amylose content. Overall, our data indicate that in vitro starch characteristics can better predict nutrient absorption and insulin and incretin secretion than starch physicochemical characteristics.

**Relationships among nutrients and hormones.** Digesta passage, glucose absorption, and SCFA production affect incretin secretion (38), and blood glucose and incretin secretion affects insulin secretion (44). Peripheral insulin kinetics has been described using peripheral glucose kinetics using several models based on differential equations following oral and parenteral challenges with glucose and insulin (45). However, such models were not useful for the present study, because, in contrast to glucose infusion or intake at specific time points, glucose was absorbed continuously following starch digestion.

In the present study, kinetics of insulin secretion was better related with kinetics of glucose absorption than other starch-derived nutrients. The kinetics of GIP and GLP-1 secretion and L-lactate absorption had a good relation with kinetics of insulin secretion. However, the addition of these 3 variables in the regression model did not improve the prediction of kinetics of insulin secretion beyond a prediction based solely on glucose absorption, indicating that the relation of glucose with L-lactate and incretins is not independent of the glucose and insulin relation. Glucose alone described one-half of the variation in the NPA of insulin (\( R^2 = 0.55;\) \( P < 0.01 \)). Thus, kinetics of glucose absorption and other factors such as chemo-sensing in mouth or gut are major factors responsible for insulin secretion following intake of high dietary starch.

In conclusion, starch with high amylose content and lower rate and extent of in vitro digestion decreased glucose absorption and insulin secretion and increased SCFA absorption. Combining in vitro digestion and starch physicochemical characteristics describes in vivo starch effects well. Glucose absorption is an important modulator of insulin secretion; however, other physiological mechanisms such as chemo-sensing in the digestive tract must exist to explain the insulin response peaking prior to peak glucose uptake into the portal vein. Overall, the present study updated our current understanding of starch effects on the kinetics of nutrient absorption and insulin and incretin absorption and the information can be useful for the management of metabolic diseases and gut health in humans using functional foods.
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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. analyzed data; P.R.R., T.A.T. G.V.K., J.J.M., and R.T.Z. wrote the paper; and R.T.Z. had primary responsibility for final content. All authors read and approved the final manuscript.

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