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# Digestion rate of dietary starch affects the systemic circulation of lipid profiles and lipid metabolism-related gene expression in weaned pigs

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## Abstract

The present study was conducted to investigate the effect of digestion rate of dietary starch on postprandial systemic circulating glucose, insulin and lipid profiles, and the activity and gene expression of lipid metabolism-related enzymes in weaned pigs. A total of twenty-four weaned pigs, surgically fitted with a catheter in the jugular vein, were randomly assigned to three dietary treatment groups, representing the high digestion rate starch (HDRS) group, the moderate-digestion rate starch (MDRS) group and the low-digestion rate starch (LDRS) group. The amylopectin:amylose ratios in the diets of each group were 27·6:1, 27·6:8·5 and 1:27·6, respectively. The serum concentrations of glucose, TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol in the HDRS group were increased to the peak point at postprandial 1·5, 2·5, 2·5, 1·5 and 1·5 h, those in the MDRS group were at postprandial 2·5, 3·5, 3·5, 3·5 and 3·5 h and those in the LDRS group were at postprandial 2·5, 3·5, 3·5, 1·5 and 3·5 h, respectively. The serum concentration of insulin in the HDRS group was higher ($P<0·05$) than those in the MDRS group, and those in the MDRS group was also higher ($P<0·05$) than those in the LDRS group at postprandial 0·5, 1·5 and 2·5 h, respectively. The serum concentrations of acetate, propionate and butyrate in the HDRS group were higher ($P<0·05$) than those in the MDRS group, and those in the MDRS group were higher ($P<0·05$) than in the LDRS group in each feeding cycle, in turn, respectively. The activity of fatty acid synthase (FAS) in the liver and abdominal adipose tissues, that of acetyl CoA carboxylase (ACC) in the myocardium and interscapular brown adipose tissues and that of the ATP-citrate lyase (ATP-CL) in the liver and interscapular brown adipose tissues in pigs of the HDRS group were higher ($P<0·05$) than that of the MDRS group. The mRNA levels of FAS in the myocardium, liver and interscapular brown adipose tissues of pigs in the HDRS group were higher ($P<0·05$) than those of the MDRS group. The activities and mRNA levels of FAS, ACC and ATP-CL in the myocardium, liver, abdominal and interscapular brown adipose tissues of the HDRS group were higher than those of the LDRS group. We conclude that the digestion rate of dietary starch affected not only the postprandial systemic circulating levels of glucose and insulin but also the lipid metabolism in weaned pigs. Dietary starch with higher digestion rate produces higher blood glucose and insulin response, ameliorates the blood lipid profiles and up-regulates the activity and gene expression profile of lipid metabolism-related genes in weaned pigs.

**Key words: Digestion rate: Starch: Lipogenic enzymes: Gene expression profile: Weaned pigs**

Starch is the main energy-yielding component of the daily diet for most mammals[1]. The major physiological properties of starch are characterised in the release of glucose as a source of energy for the body and the timeline of digestion in the small intestine[2]. Generally, starch is thought to be a mixture of amylose and amylopectin, or resistant and digestible starch[3,4]. High resistant starch level is associated with a high level of amylose[5]. Our previous *in vitro* digestibility trial indicated that starch with an amylopectin:amylose ratio of 1:27·6 was digested only 45·83 % within 4 h, whereas those with ratios of 27·6:8·5 and 27·6:1 were digested up to 90·95 or 98·77 % in the same time period[6]. *In vivo,* the digestion rate of dietary starch will affect the circulating level of glucose, as well as other absorbed nutrients such as small

**Abbreviations:** ACC, acetyl CoA carboxylase; ATP-CL, ATP-citrate lyase; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDRS, high digestion rate starch; LDRS, low digestion rate starch; MDRS, moderate digestion rate starch.

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peptides and free amino acids postprandially[7–9], and a periodical variation in the circulating levels of glucose, insulin and other nutrients has previously been observed in response to the 'two-time intake/d'[9] or 'three-time intake/d' feeding procedures[10,11]. Furthermore, the variation in the circulating levels of these metabolites was in accordance with the digestion rate of dietary starch well within postprandial 4 h, and the faster the starch digested, the higher the serum concentrations of these metabolites increased. Because the retention time of the digesta in the small intestine is limited (about 4 h from the proximal duodenum to the distal ileum)[12], the availability of dietary starch is also consequently affected by the retention time of the digesta in the small intestine. The unabsorbed glucose and undigested starch will flow into the large intestine, where they are fermented by microbes and absorbed into the portal blood stream in the form of SCFA[13,14], thus affecting lipid metabolism in the whole body[15]. Previous *in vivo* and *in vitro* experiments strongly support the view that glucose and insulin are the potent signal molecules that up-regulate the levels of mRNA and the activities of lipogenic enzymes in rats[16,17], as well as in pigs[18,19]. In addition, an increased glucose metabolism is necessary for the expression of insulin effects on fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) mRNA accumulation in white adipose tissues[20,21]. The objectives of the present study were to investigate how and to what extent the digestion rate of dietary starch affects the systemic circulating levels of glucose, insulin and lipid profiles and lipid metabolism-related gene expression in different tissues when pigs consumed their meals under a 'six-time intake/d' feeding procedure.

## Materials and methods

### Animals, experimental design and diets

The protocol for the animal experiment was approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences.

A total of twenty-four barrows, weaned at age 21 d with an average initial body weight of 6·25 (SEM 0·82) kg, were allocated on the basis of weight and litter of origin to three dietary treatments in a randomised complete block design. The dietary treatment groups were as follows: the high-digestion rate starch (HDRS) group, the moderate-digestion rate starch (MDRS) group and the low-digestion rate starch (LDRS) group. The amylopectin:amylose ratios in the diets of each treatment were 27·6:1, 27·6:8·5 and 1:27·6, respectively. The dietary starches were commercially available from the Changsha food market (Changsha, Hunan, China). There were eight pigs in each group, with one pig per metabolism crate. Each pig was surgically fitted with a catheter in the jugular vein, according to previous protocols after a 3 d adaptation period to the new environment[22,23]. The preparation of catheters and the pre- and post-operative care were as described previously [23]. The pigs were returned to the metabolism crates immediately after surgery. Each crate was equipped with a suspended water line fitted with a low-pressure nipple and wire flooring. All pigs were trained to adapt to a new feeding procedure as described previously[6]. Briefly, all pigs were fed six times daily (04.00, 08.00, 12.00, 16.00, 20.00 and 24.00 hours) and trained to consume their meals within 10 min. The daily feed allowance to each pig was strictly limited and equal to 5 % of their body weight. Water was freely available. The temperature was kept at 26 ± 2 °C and relative humidity was maintained between 60 and 75 %. Following a 7 d recovery, all pigs were fed the experimental diets.

Dietary crude protein, nutritional indispensable amino acids, vitamins and minerals were supplemented to meet or exceed the National Research Council nutritional requirements for swine[24], with a body weight of 5–10 kg. The ingredients and nutrient levels of the diets are summarised in Table 1.

### Sample preparation

Venous blood samples were taken from each pig via the catheter into 5 ml heparin-free vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) once per hour, from 08.30 to 15·30 hours on day 7 after the experiment started. All samples were centrifuged at 750 ***g*** (Biofuge 22R centrifuge; Heraeus Instruments, Hanau, Germany) for 10 min at 4 °C, the supernatant (serum) was immediately collected and placed into test-tubes and stored at −20 °C for later analysis. The pigs still consumed their meals six times daily, according to the feeding procedure during the sample collection period. On day 8, after the experiment started, all pigs consumed their meals at 08.00 hours and were

**Table 1.** Ingredient and chemical composition of the experimental diets

| | Treatment | | |
|---|---|---|---|
| Items | HDRS | MDRS | LDRS |
| Ingredient (%) | | | |
| Amylopectin | 68·44 | 54·18 | 2·48 |
| Amylose | 2·48 | 16·74 | 68·44 |
| Zein (crude protein 90 %) | 18·00 | 18·00 | 18·00 |
| Soyabean oil | 3·00 | 3·00 | 3·00 |
| L-Lys–HCl | 1·20 | 1·20 | 1·20 |
| DL-Met | 0·06 | 0·06 | 0·06 |
| L-Trp | 0·20 | 0·20 | 0·20 |
| Calcium phosphate | 0·60 | 0·60 | 0·60 |
| Calcium carbonate | 0·74 | 0·74 | 0·74 |
| Acidifier | 1·00 | 1·00 | 1·00 |
| Flavour | 0·10 | 0·10 | 0·10 |
| Choline chloride (50 %) | 0·08 | 0·08 | 0·08 |
| Premix* | 4·00 | 4·00 | 4·00 |
| $TiO_2$ | 0·10 | 0·10 | 0·10 |
| Analysed composition (%) | | | |
| DM | 94·79 | 95·53 | 94·60 |
| Total starch | 68·92 | 69·31 | 68·34 |
| Crude protein | 17·48 | 17·62 | 17·34 |
| Total Ca | 0·92 | 0·90 | 0·93 |
| Total P | 0·54 | 0·52 | 0·53 |

HDRS, high digestion rate starch; LDRS, low digestion rate starch; MDRS, moderate digestion rate starch.

*Supplied/kg of diet: vitamin A, 6 mg; vitamin $D_3$, 8 mg; vitamin E, 30 mg; vitamin K, 3 mg; vitamin $B_2$, 27 mg; vitamin $B_6$, 2 mg; vitamin $B_{12}$, 30 μg; biotin, 80 μg; folic acid, 8 mg; nicotinic acid, 24 mg; Na (NaCl), 3 g; Zn ($ZnSO_4$), 165 mg; Fe ($FeSO_4$), 165 mg; Mn ($MnSO_4$), 33 mg; Cu ($CuSO_4$), 165 mg; I ($CaI_2$), 297 μg; Se ($Na_2SeO_3$), 297 μg.

euthanised at 10.00 hours. About 5 g of the myocardium, liver, abdominal adipose and interscapular brown adipose tissues were collected and immediately frozen in liquid $N_2$, and stored at −80 °C for the determination of the activities of lipogenic enzymes, respectively. The tissue samples (2 g) were cut into approximately 5 × 5 × 1–2 mm pieces and placed immediately in RNAlater (Applied Biosystems, Austin, TX, USA), and stored at room temperature for a few hours, respectively, then frozen at −20 °C until further processing for RNA extraction and complementary DNA synthesis.

### Serum analyses

The serum concentrations of glucose, TAG, total cholesterol, LDL-cholesterol, HDL-cholesterol and SCFA (including acetate, propionate and butyrate) were determined using the Automatic Biochemical Analyser (Beckman, Miami, FL, USA) with corresponding kits (commercially available from Leadman Biochemistry Technology Company, Beijing, China). The serum insulin concentration was analysed using a commercially available $^{125}$I Radio Immunoassay Analyser kit (Beijing North Institute of Biological Technology, Beijing, China) with the γ-calculating instrument GC-300 (Zhongjia Company, Beijing, China), according to the manufacturers' instructions.

### Lipogenic enzyme activity analyses

The protocol for lipid metabolism-related enzyme activity analyses in the myocardium, liver, abdominal adipose and interscapular brown adipose tissues was as described previously[25]. Briefly, approximately 1 g of the tissue sample was rinsed, blotted dry, weighed and placed in 8 ml ice-cold 20 mM-riffs HCl buffer (pH 7·8) containing 0·2 % Triton X-100. The tissue was minced finely with scissors, homogenised manually with a Teflon-pestle homogeniser and centrifuged at 30 000 ***g*** for 20 min. After removal of the fat cake, the resulting supernatant was decanted, and the pellet was re-suspended in 5 ml isolation medium, re-homogenised and centrifuged as before. The supernatant was combined with that obtained after the first centrifugation step and used for enzyme assay. The activities of FAS (*EC* 2.3.1.85) and ATP-citrate lyase (*EC* 4.1.3.8) were measured as described previously[26]. All assays were performed in duplicate at 37°C using a Biochrom ultrosprc 3100 spectrophotometer (Biochrom, Science Park, Cambridge, UK). The absorbance change both against time and enzyme concentration was linear. ACC (*EC* 6.4.1.2) activity was measured by the $H^{14}CO_3$-fixation assay according to the method of Salati & Clarke[27]. All assays were conducted in the range of linearity with respect to the amount of enzyme and time. Soluble protein in the tissue supernatants was measured according to the method as described previously[28], using bovine serum albumin as the standard. All reagents were obtained from Sigma (St Louis, MO, USA).

### Gene expression profiles of lipogenic enzymes

The gene expression profile of lipid metabolism-related enzymes was determined according to the following protocol. Briefly, approximately 100 mg of the tissue sample was homogenised using a JT-B homogeniser (Luohe Jintian Institute of Test Equipment, Luohe, Henan, China). Total RNA was isolated from each homogenised tissue by using TRIZOL reagent (Invitrogen, Inc., Carlsbad, CA, USA) and then treated with DNase I (Invitrogen) as described by the manufacturer. The RNA quality was checked by 1 % agarose gel electrophoresis, stained with ethidium bromide (10 μg/ml). The RNA with an optical density 260/280 ratio between 1·8 and 2·0 was performed with Oligo (dT) 20 and Superscript II reverse transcriptase (Invitrogen)[29]. The quantitative PCR assays were performed using the Brilliant SYBR Green quantitative PCR Master Mix (Stratagene, La Jolla, CA, USA) and a Stratagene MX4000 Thermal Cycler (Stratagene). Briefly, a 10 μl volume reaction system contained 0·4 μl of the complementary DNA template, 5 μl of the SYBR Green Mix and 0·3 μl of each of the forward and reverse primers and 4 μl of double distilled water. The amplification programme started at 95°C for 30 s followed by forty cycles of 95°C for 5 s, the gene-specific annealing temperature for 30 s and extension at 72°C for 30 s. Fluorescence measurements were collected after each annealing during the cycles. PCR primers targeting FAS and ACC were as described previously[30], while those targeting ATP-CL and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed with the Primer Premier (Premier Biosoft International, Palo Alto, CA, USA) based on the available porcine GenBank sequences (Table 2). GAPDH was used as an internal reference gene to normalise target gene transcript levels. The identity of each product was confirmed by the dideoxy-mediated chain termination sequencing at Sangon Biotechnology, Inc. (Shanghai, China). The relative expression ratio (*R*) of mRNA was calculated by

**Table 2.** Quantitative PCR primers

| Gene | GenBank accession no. | Amplicon size (bp) | Annealing temperature (°C) | Sequence (5′ → 3′) | References |
|---|---|---|---|---|---|
| FAS | AY183428 | 196 | 58 | F: AGC CTA ACT CCT CGC TGC AAT<br>R: TCC TTG GAA CCG TCT GTG TTC | Zhao *et al.*[30] |
| ACC | EF618729 | 133 | 60 | F: ATG TTT CGG CAG TCC CTG AT<br>R: TGT GGA CCA GCT GAC CTT GA | Zhao *et al.*[30] |
| ATP-CL | NM001105302 | 94 | 61 | F: ATC TGG GAG GTG TCA ACG AGC<br>R: GAG AGG ATG GTC TTG GCA TAG TC | Present study |
| GADPH | AF017079 | 100 | 60 | F: ACTCACTCTTCTACCTTTGATGCT<br>R: TGTTGCTGTAGCCAAATTCA | Present study |

FAS, fatty acid synthase; F, forward; R, reverse; ACC, acetyl CoA carboxylase; ATP-CL, ATP-citrate lyase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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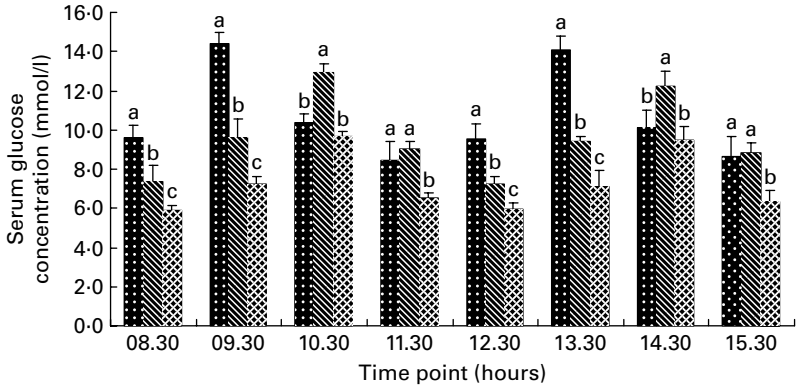


**Fig. 1.** Variation in postprandial serum systemic circulating glucose in two feeding cycles. ▩, High-digestion rate starch group; ▨, moderate-digestion rate starch group; ⊠, low-digestion rate starch group. [a,b,c] Mean values within the same sampling time with unlike letters were significantly different ($n$ 8, $P<0·05$).

$R = 2^{(CT(GAPDH)-CT(test))}$ (31). The amplification efficiency ($E$) of the quantitative PCR was acquired by the amplification of a dilution series of complementary DNA according to the equation $E = 10^{(-1/slope)}$ and was consistent between the target mRNA and GAPDH rRNA. Negative controls were performed in which complementary DNA was replaced with water[32].

### *Statistical analyses*

All physico-chemical analyses were performed at least in duplicate. The data on the serum biochemical parameters were analysed as a split-plot design for repeated measures using the generalised linear model procedure of SAS 9.13 (SAS Institute, Inc., Cary, NC, USA). The statistical model included the effect of treatment as the main plot (tested by the animal within treatment variance) and the effects of sampling time and the treatment × sampling time interaction as the subplot. The comparisons among treatments within sampling time were made when a significant $F$ test ($P<0·05$) for the treatment × sampling time interaction was observed. The data on the variation in the activities and mRNA expression of FAS, ACC and ATP-CL were also analysed as a split-plot design for repeated measures. The statistical model included the effect of treatment as the main plot and the effects of tissues and the treatment × tissue interaction as the subplot. The comparisons among treatments within tissues were made when a significant $F$ test ($P<0·05$) for the treatment × tissue interaction was observed. The Duncan's multiple comparison test was used to determine the differences among the means of treatment groups. A value of $P<0·05$ was considered to be statistically significant.

## Results

### *Serum circulating glucose and insulin*

The variation in postprandial systemic circulating glucose and insulin is summarised in Figs. 1 and 2, respectively. Both glucose and insulin were affected ($P<0·05$) by the sampling time as well as by the treatment × sampling time interaction, and changed periodically in response to the present feeding procedure. Furthermore, the serum level of glucose in pigs of the HDRS group was increased sharply to the peak point (at postprandial 1·5 h), while that in the MDRS and LDRS groups were increased slowly to their peak points at postprandial 2·5 h, respectively. The peak level of glucose in the HDRS group was higher ($P<0·05$) than that in the MDRS group, and that in the MDRS group was also higher ($P<0·05$) than in the LDRS group. The serum insulin levels in the three dietary treatment groups were increased quickly to the peak point, and

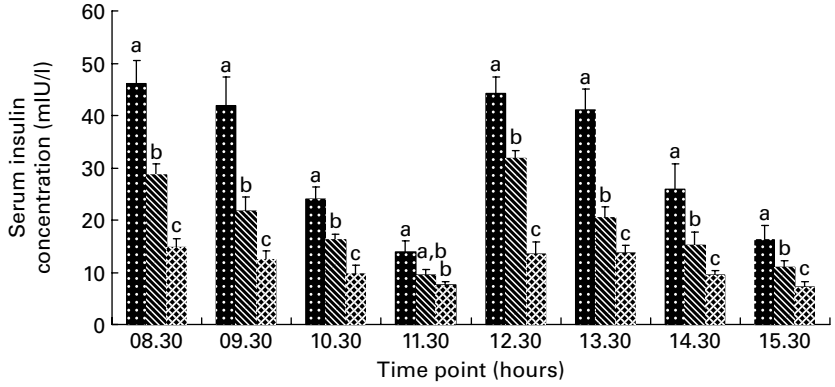


**Fig. 2.** Variation in postprandial serum systemic circulating insulin concentration in two feeding cycles. ▩, High-digestion rate starch group; ▨, moderate-digestion rate starch group; ⊠, low-digestion rate starch group. [a,b,c] Mean values within the same sampling time with unlike letters were significantly different ($n$ 8, $P < 0·05$). 1 mIU = 6·945 μmol.

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then decreased gradually within each feeding cycle; however, the variation in insulin level did not respond simultaneously to the postprandial blood glucose levels. The serum insulin concentration in the HDRS group was higher ($P<0·05$) than that in the MDRS group, and that in the MDRS group was also higher ($P<0·05$) than in the LDRS group at postprandial 0·5, 1·5 and 2·5 h, respectively. During the second feeding cycle, from 12.30 to 15.30 hours, the variation in postprandial systemic circulating glucose and insulin was similar to that observed during the first feeding cycle, from 08.30 to 11.30 hours.

### *Serum circulating lipid profiles and SCFA*

The postprandial serum concentrations of lipid and SCFA were also affected ($P<0·05$) by both the sampling time and the treatment × sampling time interaction (Tables 3 and 4), and changed periodically in response to the present feeding procedure. The serum levels of TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol in the HDRS group were increased to the peak point at postprandial 2·5, 2·5, 1·5 and 1·5 h, those in the MDRS group were at postprandial 3·5, 3·5, 3·5 and 3·5 h and those in the LDRS group were at postprandial 3·5, 3·5, 1·5 and 3·5 h, respectively. Furthermore, the serum TAG level in the HDRS group was higher ($P<0·05$) than that in the MDRS group at postprandial 1·5 and 2·5 h, and was even higher ($P<0·05$) than that in the LDRS group at different time points postprandially, and that in the MDRS group was higher ($P<0·05$) than that in the LDRS group at postprandial 3·5 h in the first feeding cycle. The serum TAG level in the MDRS group was lower ($P<0·05$) than that in the MDRS group at postprandial 3·5 h, and was higher ($P<0·05$) than that in the LDRS group at postprandial 1·5, 2·5 and 3·5 h; the serum TAG level in the MDRS group was higher ($P<0·05$) than that in the LDRS group at different time points postprandially in the second feeding cycle, respectively. The serum levels of total cholesterol and HDL-cholesterol in the HDRS group were higher ($P<0·05$) than those in the MDRS group at postprandial 0·5, 1·5 and 2·5 h, and those in the MDRS group were higher ($P<0·05$) than those in the LDRS group at different time points postprandially. The serum LDL-cholesterol level in the HDRS group was lower ($P<0·05$) than that in the MDRS group at postprandial 0·5, 2·5 and 3·5 h, and was higher ($P<0·05$) than that in the LDRS group at postprandial 1·5 h, and that in the MDRS group was higher ($P<0·05$) than that in the LDRS group at different time points postprandially in the first feeding cycle; the serum LDL-cholesterol level in the MDRS group was lower ($P<0·05$) than that in the MDRS group at postprandial 2·5 and 3·5 h, and was higher ($P<0·05$) than that in the LDRS group at postprandial 1·5 h, and that in the MDRS group was higher ($P<0·05$) than that in the LDRS group at postprandial 0·5, 2·5 and 3·5 h, respectively. The serum acetate, propionate and butyrate levels in pigs of the HDRS and MDRS groups increased to the peak point sharply, at postprandial 1·5 h; those in the LDRS group increased to the peak points at postprandial 2·5 h, respectively. Similar results were observed during the second feeding cycle, from 12.30 to 15.30 hours.

**Table 3.** Serum lipid profiles and SCFA concentration after the first feeding
(Mean values with their pooled standard errors, *n* 8)

| | Time (hours) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 08.30 | | | 09.30 | | | 10.30 | | | 11.30 | | | | |
| Items | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | Pooled SEM | Time effect (*P*) |
| Lipid profiles (mmol/l) | | | | | | | | | | | | | | |
| TAG | 0·37$^{a}$ | 0·36$^{a,b}$ | 0·34$^{b}$ | 0·45$^{a}$ | 0·37$^{b}$ | 0·35$^{b}$ | 0·50$^{a}$ | 0·38$^{b}$ | 0·39$^{b}$ | 0·44$^{a}$ | 0·46$^{a}$ | 0·40$^{b}$ | 0·004 | < 0·001 |
| TC | 2·44$^{a}$ | 2·39$^{a}$ | 1·82$^{b}$ | 2·53$^{a}$ | 2·51$^{a}$ | 1·93$^{b}$ | 2·78$^{a}$ | 2·67$^{a}$ | 2·22$^{b}$ | 2·36$^{a,b}$ | 2·73$^{a}$ | 2·19$^{b}$ | 0·070 | < 0·001 |
| LDL-C | 0·92$^{b}$ | 1·02$^{a}$ | 0·91$^{b}$ | 1·08$^{a}$ | 1·06$^{a}$ | 0·97$^{b}$ | 0·88$^{b}$ | 1·11$^{a}$ | 0·93$^{b}$ | 0·86$^{b}$ | 1·15$^{a}$ | 0·88$^{b}$ | 0·020 | 0·007 |
| HDL-C | 1·07$^{a}$ | 0·85$^{b}$ | 0·80$^{b}$ | 1·14$^{a}$ | 0·87$^{b}$ | 0·83$^{b}$ | 1·10$^{a}$ | 0·88$^{b}$ | 0·87$^{b}$ | 1·08$^{a}$ | 0·98$^{a,b}$ | 0·91$^{b}$ | 0·031 | 0·004 |
| SCFA (μmol/l) | | | | | | | | | | | | | | |
| Acetate | 84·44$^{c}$ | 127·68$^{b}$ | 170·65$^{a}$ | 92·08$^{c}$ | 140·04$^{b}$ | 183·15$^{a}$ | 87·85$^{c}$ | 125·76$^{b}$ | 200·75$^{a}$ | 83·81$^{c}$ | 118·32$^{b}$ | 188·00$^{a}$ | 3·476 | 0·001 |
| Propionate | 24·72$^{c}$ | 34·12$^{b}$ | 47·43$^{a}$ | 39·03$^{b}$ | 44·82$^{a,b}$ | 53·26$^{a}$ | 22·72$^{c}$ | 38·10$^{b}$ | 54·14$^{a}$ | 7·57$^{c}$ | 24·84$^{b}$ | 50·52$^{a}$ | 1·442 | 0·001 |
| Butyrate | 1·50$^{c}$ | 7·46$^{b}$ | 27·24$^{a}$ | 2·37$^{c}$ | 9·87$^{b}$ | 30·92$^{a}$ | 1·38$^{c}$ | 8·27$^{b}$ | 31·40$^{a}$ | 0·46$^{c}$ | 5·35$^{b}$ | 29·26$^{a}$ | 0·044 | < 0·001 |

HDRS, high digestion rate starch; MDRS, moderate digestion rate starch; LDRS, low digestion rate starch; TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol.
$^{a,b,c}$Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

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**Table 4.** Serum lipid profiles and SCFA concentration after the second feeding (continued from Table 3)
(Mean values with their pooled standard errors, *n* 8)

| | Time (hours) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12.30 | | | 13.30 | | | 14.30 | | | 15.30 | | | |
| Items | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | Pooled SEM | Time effect (*P*) |
| Lipid profiles (mmol/l) | | | | | | | | | | | | | | |
| TAG | 0·41$^{a}$ | 0·38$^{a,b}$ | 0·30$^{b}$ | 0·42$^{a}$ | 0·39$^{a}$ | 0·34$^{b}$ | 0·48$^{a}$ | 0·45$^{a}$ | 0·35$^{b}$ | 0·41$^{b}$ | 0·48$^{a}$ | 0·37$^{c}$ | 0·004 | < 0·001 |
| TC | 2·42$^{a}$ | 2·30$^{a}$ | 1·79$^{b}$ | 2·60$^{a}$ | 2·34$^{b}$ | 1·85$^{c}$ | 2·74$^{a}$ | 2·50$^{a}$ | 1·92$^{b}$ | 2·42$^{b}$ | 2·72$^{a}$ | 2·11$^{c}$ | 0·070 | < 0·001 |
| LDL-C | 0·93$^{b}$ | 0·97$^{a}$ | 0·92$^{b}$ | 1·06$^{a}$ | 1·01$^{a,b}$ | 0·96$^{b}$ | 0·87$^{c}$ | 1·08$^{a}$ | 0·95$^{b}$ | 0·83$^{b}$ | 1·15$^{a}$ | 0·89$^{b}$ | 0·020 | 0·007 |
| HDL-C | 1·02$^{a}$ | 0·82$^{b}$ | 0·80$^{b}$ | 1·17$^{a}$ | 0·91$^{b}$ | 0·89$^{b}$ | 1·09$^{a}$ | 0·94$^{b}$ | 0·90$^{b}$ | 1·06$^{a}$ | 0·97$^{a,b}$ | 0·86$^{b}$ | 0·031 | 0·004 |
| SCFA (μmol/l) | | | | | | | | | | | | | | |
| Acetate | 82·54$^{c}$ | 122·92$^{b}$ | 175·75$^{a}$ | 94·08$^{c}$ | 143·34$^{b}$ | 186·75$^{a}$ | 85·28$^{c}$ | 128·94$^{b}$ | 202·68$^{a}$ | 80·81$^{c}$ | 120·76$^{b}$ | 191·25$^{a}$ | 3·476 | 0·001 |
| Propionate | 23·22$^{c}$ | 33·68$^{b}$ | 49·34$^{a}$ | 39·82$^{b}$ | 43·76$^{b}$ | 54·13$^{a}$ | 27·65$^{c}$ | 39·28$^{b}$ | 56·02$^{a}$ | 6·75$^{c}$ | 25·83$^{b}$ | 52·92$^{a}$ | 1·442 | 0·001 |
| Butyrate | 1·41$^{c}$ | 7·26$^{b}$ | 28·60$^{a}$ | 2·42$^{c}$ | 9·65$^{b}$ | 31·40$^{a}$ | 1·68$^{c}$ | 8·62$^{b}$ | 32·50$^{a}$ | 0·41$^{c}$ | 5·54$^{b}$ | 30·72$^{a}$ | 0·044 | < 0·001 |

HDRS, high digestion rate starch; MDRS, moderate digestion rate starch; LDRS, low digestion rate starch; TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol.
$^{a,b,c}$Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

### *Activities of lipogenic enzymes*

The activities of the lipogenic enzymes were affected ($P<0·05$) by the tissue and the treatment × tissue interaction (Table 5). The activity of FAS in the myocardium, liver, abdominal adipose and interscapular brown adipose tissues of pigs of the HDRS and MDRS groups was higher ($P<0·05$) than that of the LDRS group; that in the liver and abdominal adipose tissues of the HDRS group was also higher ($P<0·05$) than that of the MDRS group. The activity of ACC in the myocardium, liver, abdominal adipose and interscapular brown adipose tissues of pigs of the HDRS group and that in the myocardium, liver and abdominal adipose tissues of the MDRS group were higher ($P<0·05$) than that of the LDRS group; the activity of ACC in the myocardial adipose and interscapular brown adipose tissues of the HDRS group was higher ($P<0·05$) than that of the MDRS group. The activity of ATP-CL in the myocardium, liver, abdominal adipose and interscapular brown adipose tissues of pigs of the HDRS group and that in the myocardium and liver of the MDRS group were higher ($P<0·05$) than that of the LDRS group; the activity of ATP-CL in the liver and interscapular brown adipose of the HDRS group was higher ($P<0·05$) than that of the MDRS group.

### *mRNA levels of lipid metabolism-related genes*

The mRNA levels of the lipid metabolism-related genes were affected ($P<0·05$) by the tissue and the treatment × tissue interaction (Table 6). The mRNA level of FAS in the myocardium, liver, abdominal adipose and interscapular brown adipose tissues of pigs of the HDRS group and that in the myocardium, abdominal adipose and interscapular brown adipose tissues of the MDRS group were higher ($P<0·05$) than that of the LDRS group; the mRNA level in the myocardium, liver and interscapular brown adipose tissues of the HDRS group was also higher ($P<0·05$) than that of the MDRS group. The mRNA level of ACC in the myocardium, liver, abdominal adipose and interscapular brown adipose tissues of pigs of the HDRS and MDRS group was higher ($P<0·05$) than that of the LDRS group; that in the myocardium and abdominal adipose tissue of the HDRS group was higher ($P<0·05$) than that of the MDRS group. The mRNA level of ATP-CL in the myocardium, liver, abdominal adipose and interscapular brown adipose tissues of the HDRS and MDRS group was higher ($P<0·05$) than that of the LDRS group; that in the liver, abdominal adipose and interscapular brown adipose tissues was higher ($P<0·05$) than that of the MDRS group.

## Discussion

Animal nutrition researchers have been trying to develop novel strategies to get maximal deposition of carbon, nitrogen as well as other nutrients in food animals, of which elevating the postprandial circulating levels of insulin, glucose and amino acids has been proved to be practical in mammals[33,34]. An important observation from the present study was that the postprandial circulating levels of glucose and insulin were changed periodically in response to the 'six-time intake/d'

**Table 5.** Activity of the lipogenic enzymes after pigs consumed the experimental diets (nmol/min per mg protein)
(Mean values with their pooled standard errors, *n* 8)

| | Tissues | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Myocardium | | | Liver | | | Abdominal adipose | | | Interscapular brown adipose | | | | |
| Lipogenic enzymes | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | Pooled SEM | Tissue effect (*P*) |
| FAS | 4·04$^{a}$ | 3·41$^{a}$ | 1·39$^{b}$ | 6·87$^{a}$ | 4·78$^{b}$ | 2·36$^{c}$ | 34·34$^{a}$ | 23·88$^{b}$ | 11·81$^{c}$ | 24·10$^{a}$ | 21·94$^{a}$ | 12·47$^{b}$ | 0·773 | 0·006 |
| ACC | 3·35$^{a}$ | 2·61$^{b}$ | 0·61$^{c}$ | 4·36$^{a}$ | 4·19$^{a}$ | 1·04$^{b}$ | 21·79$^{a}$ | 20·95$^{a}$ | 5·20$^{b}$ | 25·68$^{a}$ | 11·20$^{b}$ | 7·79$^{b}$ | 1·020 | 0·045 |
| ATP-CL | 3·33$^{a}$ | 2·71$^{a}$ | 1·34$^{b}$ | 6·67$^{a}$ | 4·61$^{b}$ | 2·55$^{c}$ | 33·33$^{a}$ | 23·05$^{a,b}$ | 12·77$^{b}$ | 17·48$^{a}$ | 10·18$^{b}$ | 7·88$^{b}$ | 1·231 | 0·004 |

HDRS, high digestion rate starch; MDRS, moderate digestion rate starch; LDRS, low digestion rate starch; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; ATP-CL, ATP-citrate lyase.
$^{a,b,c}$ Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

feeding procedure. Besides, about 81·90, 47·17 and 30·14 % of dietary starch were digested in the anterior jejunum of pigs in the HDRS, MDRS and LDRS groups, respectively[6]. The higher starch digestibility in the anterior small intestine results in higher and rapid postprandial circulating glucose and insulin responses[35]. Since insulin is secreted primarily in response to the elevated blood glucose concentration, stimulated by the feeding frequency and activity[36,37], and even controlled by the central nervous system through some signal pathways[38], the difference in the postprandial serum insulin levels in the present study should be a combined result of both the feeding activity and the efficiency of glucose absorption. In other words, there is a clear correlation between the starch digestibility and the insulin response for the three diets.

Lipogenesis is a process by which simple sugars such as glucose are converted to fatty acids[39], which is regulated by a wide array of interdependent factors, including nutrients, hormones, nuclear transcription factors and lipogenic enzymes, of which glucose and insulin are two important regulating molecules[40,41]. In this regard, the postprandial circulating lipid profile may reflect how blood glucose and insulin affected the lipid metabolism. An important finding from the present study is that the postprandial circulating levels of lipids and SCFA were also changed periodically. Interestingly, the higher the starch digestion rate was, the faster the postprandial circulating levels of TAG, total cholesterol and HDL-cholesterol responded, and the lower the circulating levels of SCFA responded. Insulin promotes the synthesis of fatty acids in the liver and inhibits the breakdown of fat in the adipose tissue by inhibiting the intracellular lipase that hydrolyses TAG to release fatty acids[42], and high glucose induces adipogenic differentiation in porcine[40], as well as in human tissues[43]. Therefore, the higher level of postprandial glucose and insulin stimulated the process of adipogenesis and elevated the circulating lipid profiles. SCFA are the major end products of bacterial metabolism in the large intestine of animals[44,45], which stimulate adipogenesis via the G protein-coupled receptor 43 pathway[46]. In the present study, lower levels of postprandial circulating lipid profiles were observed in pigs of the LDRS group with higher levels of fatty acids at the same time. The lower availability of glucose would result in higher oxidation of other nutrients, such as protein and lipids, for energy requirement[47,48], which may explain the lower concentration of serum lipids in pigs of the LDRS group, and a similar phenomenon was observed previously in rats[49], as well as in mice[50].

Lipids are considered to be crucial in the metabolic process of mammals[51,52]. There are two sources of lipids for metabolism, exogenously derived (dietary) lipids and endogenously synthesised lipids[53]. The biosynthesis of the latter (lipogenesis) depends on well-known enzyme-regulated processes, of which FAS, ACC and ATP-CL are the primary lipogenic enzymes, and were considered as rate-limiting enzymes of lipogenesis in pigs[54,55]. A novel finding of the present study was that the activities of FAS, ACC and ATP-CL in the myocardium, liver, abdominal adipose and interscapular brown adipose in pigs of the HDRS group were higher than those in the MDRS and LDRS groups, and these results are, at least partially, in accordance with the gene expression profiles of these enzymes. Metabolic regulation in mammals relies

**Table 6.** Relative key lipogenic enzyme gene expression profiles after pigs consumed the experimental diets (arbitrary units)
(Mean values with their pooled standard errors, *n* 8)

| | Tissues | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Myocardium | | | Liver | | | Abdominal adipose | | | Interscapular brown adipose | | | | |
| Lipogenic enzymes | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | HDRS | MDRS | LDRS | Pooled SEM | Tissue effect (*P*) |
| FAS | 1·17$^{a}$ | 0·84$^{b}$ | 0·49$^{c}$ | 1·23$^{a}$ | 0·93$^{b}$ | 0·85$^{b}$ | 1·45$^{a}$ | 1·32$^{a}$ | 0·25$^{b}$ | 1·28$^{a}$ | 0·89$^{b}$ | 0·44$^{c}$ | 0·026 | <0·001 |
| ACC | 1·14$^{a}$ | 0·97$^{b}$ | 0·80$^{c}$ | 1·38$^{a}$ | 1·25$^{a}$ | 0·38$^{b}$ | 1·88$^{a}$ | 0·82$^{b}$ | 0·57$^{c}$ | 1·55$^{a}$ | 1·49$^{a}$ | 0·37$^{b}$ | 0·015 | 0·005 |
| ATP-CL | 0·91$^{a}$ | 0·78$^{a}$ | 0·32$^{b}$ | 1·31$^{a}$ | 1·13$^{b}$ | 0·92$^{c}$ | 1·82$^{a}$ | 1·06$^{b}$ | 0·82$^{c}$ | 1·49$^{a}$ | 0·37$^{b}$ | 0·94$^{a}$ | 0·022 | <0·001 |

HDRS, high digestion rate starch; MDRS, moderate digestion rate starch; LDRS, low digestion rate starch; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; ATP-CL, ATP-citrate lyase.
$^{a,b,c}$ Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

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partly on transcriptional control of the expression level of key enzymes[56], and gene expression analysis is useful for inferring transcriptional activity. Glucose is known to directly affect lipogenic gene expression by altering the transcriptional repressor level via the lipid synthesis pathway in a range of tissues[57], while insulin regulates the lipogenic gene expression indirectly, either by increasing glucose transportation and metabolism through the translocation of the insulin-responsive GLUT-4 to the plasma membrane in adipocytes or by speeding up glucose metabolism in the liver through the activated glucokinase pathway[58]. In the present study, the mRNA levels of FAS, ACC and ATP-CL in the myocardium, liver, abdominal adipose and interscapular brown adipose in pigs of the HDRS group were higher than those of the LDRS group, corresponding with the postprandial circulating glucose and insulin responses in these groups. However, an interesting observation was that not only the mRNA levels but also the activities of these rate-limiting lipogenic enzymes in the same tissue were significantly different. Furthermore, the mRNA level and the activity of the same lipogenic enzyme gene in different tissues were also different. These significant differences may be due to the different functions of these lipogenic enzymes, different responses of these enzymes to blood glucose and insulin and different metabolic patterns of lipids in these tissues[59–62].

In summary, the present study shows that dietary starch with a higher digestion rate significantly elevated the postprandial blood glucose and insulin response, and also the profiles of circulating lipids but not of SCFA. Glucose and insulin have a priority in regulating lipogenesis in different tissues over that of SCFA. The activity of lipogenic enzymes and their gene expression were affected by the postprandial circulating glucose and insulin in weaned pigs.

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