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In vitro starch digestion kinetics of diets varying in resistant starch and arabinoxylan compared with *in vivo* portal appearance of glucose in pigs

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ABSTRACT

The current study was undertaken to investigate the relationship between the *in vitro* starch digestion kinetics and the *in vivo* portal glucose appearance in pigs used as models for humans. *In vivo* data were obtained from a previous study where the portal glucose appearance was obtained from six catheterised pigs equipped with permanent catheters in an artery and the portal vein and with a flow probe attached to the portal vein for monitoring the blood flow rate. Three experimental diets were studied — a low dietary fibre (DF), western-style diet (WSD) and two high-DF diets containing resistant starch (RSD) or arabinoxylan (AXD). A modified Englyst-assay involving gradual glucose hydrolysis over a time frame of 6 h was used *in vitro*. The *in vitro* starch digestion kinetics was modelled using a mechanistic growth model ($R^2 > 0.995$), whereas the *in vivo* data were better described by a sigmoid Gompertz model ($R^2 > 0.997$). The estimated plateau values were higher *in vitro* than *in vivo* but the diets were similarly ranked; ~95% for AXD and WSD and 81.8% for RSD *in vitro* and ~86% and 76.6% for the same diets *in vivo*. The rate of glucose release *in vitro* was much faster than the portal glucose appearance *in vivo* (0.0347–0.0705 *versus* 0.0136–0.0197% starch/min) with the starch in RSD being the most slowly degradable. This difference was most likely an effect of gastric retention. In conclusion, the *in vitro* method ranked the three diets in a similar relative manner as *in vivo* but the rate of glucose release was much faster *in vitro* than *in vivo*. It was only when the starch structure set the limit for the starch hydrolysis, however, that similar relative results were obtained.

1. Introduction

The high consumption of processed cereals in affluent societies has been considered as one of the reasons for the development of overweight and obesity (Brouns, van Buul, & Shewry, 2013; Hu et al., 2001; Tuomilehto et al., 2001). Obese subjects have an augmented risk of developing several lifestyle diseases, which has led to increased attention to prevention and treatment through diet (Fardet, 2010; Lindstrom et al., 2006; Ludwig, 2002; Ludwig et al., 1999).

A common feature of refined cereals is that glucose is made rapidly available through the digestion processes and continuous consumption of food rich in rapidly digestible carbohydrates may lead to insulin resistance due to constant fluctuations in blood sugar levels and the resulting high insulin responses (Hodge, English, O'Dea, & Giles, 2004). Thus a slower release of carbohydrates is associated with health benefits, whereas an increased glycaemic response is considered unfortunate under most circumstances (Jenkins et al., 2002; Ludwig, 2002; Montonen, Knekt, Jarvinen, Aromaa, & Reunanen, 2003).

Carbohydrate availability and rate of digestion *in vivo* are dependent on the food structure, physico-chemical characteristics of the food, associated meal components, and individual genetics (Englyst & Englyst, 2005; Englyst, Vinoy, Englyst, & Lang, 2003; Fardet, Leenhardt, Lioger, Scalbert, & Remesy, 2006). The main source of digestible carbohydrate in cereals is starch present in granules. These are composed of two distinct polysaccharide fractions, amylose and amylopectin, forming the semicrystalline granule structure. Amylose is a long, primarily linear, polymer consisting of 1,4-linked D-glucose units, whereas amylopectin is a highly branched polymer with 5% 1,6-linkages creating the numerous branch points of the molecule. The granule is composed of amorphous and crystalline lamella based on amylopectin branch points and parallel linear amylopectin chains respectively. The crystallite material yields X-ray diffraction patterns classifying the starch as either an A or B type polymorph or an intermediary C type polymorph containing a mix of the former two (Oates, 1997). A type polymorphic starches are typically associated with cereals, whereas B types are often are found in tubers such as potatoes but also in high amylose maize (Cheetham & Tao, 1998).

The rate of digestion is dependent on granule composition, the ratio of amylose and amylopectin as well as size and surface properties (Chiotelli & Le Meste, 2002; Singh, Dartois, & Kaur, 2010). Associated

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matrix components may interfere with the enzymatic access to the granule surface (Svihus, Uhlen, & Harstad, 2005; Vasanthan & Bhatty, 1996). This is the case in wheat granules, which are surrounded by a matrix of glutenin and gliadin (Hamaker & Bugusu, 2003; Singh et al., 2010). The food structure, *i.e.* the degree of grinding and associated matrix components, may be contributing factors that provide a physical barrier to the digestive enzymes (Jarvi et al., 1995; Juntunen et al., 2003). The rate of digestion may also indirectly be affected by the overall diet content of lipids and proteins. When present as degradation products in the gut, these components stimulate the release of cholecystokinin and reduce the rate of gastric emptying (Furness, Rivera, Cho, Bravo, & Callaghan, 2013; Yamagishi & Debas, 1978). The content of soluble dietary fibre increases the viscosity of bolus resulting in reduced gastric emptying as well as limited enzymatic access and reduced diffusion of released glucose units (Englyst, Kingman, & Cummings, 1992; Hagander et al., 1987; Lund, Gee, Brown, Wood, & Johnson, 1989).

In the current study we evaluated the digestion kinetics of three diets; a western style diet (WSD) high in sugars, fat and refined wheat flour and two diets rich in soluble dietary fibre (DF) in the form of arabinoxylan (AX) from rye and enzyme-treated wheat bran and resistant starch (RS) from potato and high-amylose maize, respectively. The structural conformation of wheat and rye starch granules is similar with both exhibiting a type A polymorphic structure and comparable degree of crystallinity and amylose content (Tester, Karkalas, & Qi, 2004; Zobel, 1988). However the surrounding matrix of glutenin and gliadin differs, making rye granules more vulnerable to enzymatic attack (Hamaker & Bugusu, 2003; Shewry & Tatham, 1990). High amylose maize (HAM) starch comprises a high amylose:amylopectin ratio, supporting the formation of RS (Englyst & Englyst, 2005; Englyst et al., 2003; Gallant, Bouchet, Buleon, & Perez, 1992). In addition it exhibits type B polymorphic structure unlike native maize. The crystalline transition from type A to B is thought to depend on the amylose content (Cheetham & Tao, 1998). Potato starch (PS) granules differ in structure from cereal starches by displaying a distinct crystalline structure (type B), a smoother surface limiting enzymatic access, and larger granules (Oates, 1997; Singh et al., 2010; Zobel, 1988).

For classification of nutritionally important starch fractions a two-hour *in vitro* assay suggested by Englyst et al. (1992) is commonly used. This assay determines rapidly digestible, slowly digestible, and RS and can thus be used to evaluate carbohydrate-containing foods (Englyst & Englyst, 2005; Englyst et al., 2003). In the present study the assay was applied with significant modifications regarding the number of time points analysed and total time of incubation. Until 60 min, glucose was measured with 20 min intervals, followed by 2-hour intervals until 360 min. 20 min intervals were chosen, as this was the shortest practically feasible interval. The incubation time was extended from 120 min to 360 min in order to mimic human intestinal transit time (Read, Al-Janabi, Holgate, Barber, & Edwards, 1986; Worsøe et al., 2011). Measurements were chosen on the basis of previous studies (Muir & O'Dea, 1993; van Kempen, Regmi, Matte, & Zijlstra, 2010). The main objective of this study was to investigate the *in vitro* digestion kinetics of diets varying in RS and AX. In order to evaluate the reliability of the *in vitro* assay as a predictor of *in vivo* events, *in vivo* data from a study with portal vein catheterised pigs (Ingerslev, Theil, Hedemann, Larke, & Bach Knudsen, 2014) fed the actual diets were used for comparison and discussion.

2. Materials and methods

2.1. Diets

The classification was performed on three diets previously fed to pigs in an *in vivo* study (Ingerslev et al., 2014). The diets consisted of a low-DF western style diet (WSD) based on white wheat flour as the main cereal and two high-DF diets, a resistant starch diet (RSD) and an arabinoxylan diet (AXD). The WSD was intended to mimic a diet typically consumed in affluent societies, being high in saturated fat and refined carbohydrates. The high DF RSD was obtained by replacing a fraction of white wheat flour with raw PS and HAM increasing the type 2 RS (RS_2) content according to Englyst et al. (1992). The high DF AXD was based on rye flakes and enzyme treated wheat bran increasing the AX content. Before incorporating the wheat bran into the AXD it was treated with xylanases and cellulases to increase the content of AX oligosaccharides. Diets were balanced with regard to protein, fat, and gross energy, but varied in DFs and structural characteristics (Table 1). In the *in vivo* study the AXD containing rye flakes was ground through a hammer mill fitted with a 3.5 mm screen to ensure similar particle size across diets. Overall the particle size distribution (Retsch AS 200 control, Retsch GmbH, Haan, Germany) across diets was 93.8, 92.2, 86,5% <1 mm and 5.1, 6.8, and 13.1% >1 mm in WSD, RSD, and AXD respectively (Table 1). In this *in vitro* study the original diets were freeze-dried and ground to pass a 0.5 mm screen. PS and HAM were analysed as raw starches.

Potato starch was obtained from KMC amba (Brande Denmark), HAM (HiMaize®) from Ingredion Incorporated (Westchester, Illinois), rye flakes from Lantmännen Cerealia (Stockholm, Sweden), and DuPont (Brabrand, Denmark) produced the enzyme-treated wheat bran.

2.2. Chemical analyses

All chemical analyses o diets were performed in duplicate on freeze-dried material. The dry matter (DM) content was determined by drying the samples at 103 °C to constant weight and ash was analysed according to the AOAC method (923.03; AOAC) (Association of Official Analytical Chemists, 1990). Nitrogen was measured by Dumas (Hansen, 1989) and protein calculated as N × 6.25, fat determined using the Stoldt procedure (Stoldt, 1952), and the dietary contents of sugars (glucose, fructose and sucrose) and fructans were analysed as described by Larsson and Bengtsson (1983). Starch, in the following

Table 1
Chemical composition of the western-style diet (WSD), the resistant starch diet (RSD) and the arabinoxylan diet (AXD).

	Diet		
	WSD	RSD	AXD
Chemical composition (g/kg DM)			
DM (g/kg, as-fed basis)	915	903	891
Protein (N × 6.25)	207	191	154
Fat	152	150	135
Ash	37	34	51
Digestible carbohydrates			
Available sugars	113	3	22
Available starch[a]	422	470	420
Total starch[b]	470	554	440
Non-digestible carbohydrates			
Total NSP (soluble NSP)	58 (11)	55 (8)	144 (33)
Cellulose	29	34	37
β-glucan (soluble β-glucan)	1 (<1)	1 (<1)	16 (3)
AX (soluble AX)	18 (6)	15 (4)	72 (22)
RS	6	113	8
Fructans	0	3	22
AXOS	2	2	7
Total non-digestible CHO[c]	64	173	181
Klason lignin	6	13	15
Total dietary fibre[d]	72	186	196
Particle size distribution (%)[e]			
>2 mm	1.5	1.0	0.0
>1 mm	5.1	6.8	13.1
<1 mm	93.8	92.2	86.5

[a] Estimated by the method of Bach Knudsen, (1997).
[b] Estimated as total starch in the method modified procedure of Englyst et al., (1992).
[c] Calculated as: Total non-starch polysaccharide (NSP) + fructans + RS + arabinoxylan oligosaccharides (AXOS).
[d] Calculated as: Total NSP + fructans + RS + arabinoxylan oligosaccharides (AXOS) + lignin.
[e] 50 g of sample size for each diet.