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**The effect of carbohydrate digestibility on appetite and its relationship to postprandial blood glucose and insulin levels**

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Running title: carbohydrate digestibility and appetite

## Abstract

**Background:** ‘Slowly digestible’ carbohydrates have been claimed to reduce appetite via effects on postprandial glucose and insulin levels, but literature is inconsistent. The inconsistencies between studies might be explained by factors other than glycemic effects *per se*, e.g., nutritional or physical properties. We tested this possibility by examining postprandial glucose, insulin and appetite responses to drinks differing only in rate and extent of digestibility of carbohydrates. This was accomplished by comparing different glucose polymers: maltodextrin (rapidly digestible) versus medium-chain pullulan (slowly but completely digestible) versus long-chain pullulan (indigestible).

**Subjects and Methods:** In a randomized double-blind balanced crossover design, 35 subjects received drinks with 15g test carbohydrate polymers. Key outcome measures were appetite scores, digestibility (*in vitro* test and breath hydrogen), and (in a subset) glucose and insulin levels.

**Results:** Digestibility, glucose and insulin data confirmed the rapid, slow, and non-digestible nature of the test carbohydrates. Despite its low digestibility, only long-chain pullulan reduced appetite versus the maltodextrin control, while the medium-chain pullulan did not.

**Conclusions:** We conclude that glycemic responses *per se* have minimal effects on appetite, when tested in products only differing in carbohydrate digestibility rate and extent.

**Keywords:** appetite, glycemic index, “slow carbs”, fibre, plasma glucose, blood insulin, carbohydrate digestibility

## Introduction

Slowly digestible carbohydrates (or “slow carbs”) are defined as ‘carbohydrates that are likely to be completely digested in the small intestine but at a slower rate’ (Englyst H.N. et al., 1992). The use of slowly digestible carbohydrates, resulting in a lower or sustained postprandial glycemia, has been claimed to reduce appetite (Bornet et al., 2007; Thomas et al., 2007). However, others find that the evidence that lowering of postprandial glycemia reduces appetite is inconclusive (Pi-Sunyer, 2002; van Dam & Seidell, 2007), that postprandial glycemia and appetite are not related (Niwano et al., 2009; Raben, 2002), or that blood insulin, but not glucose, is associated with increased short-term appetite reduction (Flint et al., 2007).

The inconsistencies between studies might be explained by a lower than expected energy availability of the carbohydrates tested or by other properties (nutritional, physical) differing amongst test products (Alfenas & Mattes, 2005). For example, many of these studies have used different dietary fibres, but it is generally not possible to distinguish effects of lowering postprandial glycaemia *per se* from other known effects of the fibres (Bornet et al., 2007; Hlebowicz, 2009; van Dam & Seidell, 2007). Indeed, recently it was concluded that the difference in appetite sometimes seen after low-glycemic foods is due to factors other than the difference in blood glucose (Wolever et al., 2009).

One way to test this is to compare test foods differing only in the digestibility of carbohydrates, but with identical chemical makeup and physical properties, and evaluate effects on the postprandial glucose, insulin and appetite responses.

The comparison of maltodextrin to differing chain lengths of the glucose-polymer pullulan potentially provides a test of rapidly- to slowly- to non-digestible carbohydrate materials, differing only in digestibility. Pullulan is indigestible in its native (long-chain) state. We hypothesized that the use of smaller (thus slowly but fully digestible) pullulan fragments would result in a more sustained postprandial blood glucose and plasma insulin response, leading to a reduction in appetite as compared to the rapidly digestible control.

## **Subjects and Methods**

### **Subjects**

Subjects were recruited from local area of the research centre (Vlaardingen, Netherlands). Selection criteria were: age 18-60 years, Body Mass Index (BMI,  $\text{kg/m}^2$ )  $\geq 21$  and  $\leq 32.0$ , apparently healthy, and not using medicines judged likely to influence the study results. Subjects with high dietary restraint scores (Van Strien et al., 1986) and with tendencies toward diagnosable eating disorders (Morgan et al., 1999) were excluded.

Thirty-five subjects (27 women) completed the study (there was one drop-out), with a mean age 47.5 (range 20-59) years and mean BMI 22.9 (range 20.0-25.3)  $\text{kg/m}^2$ .

### **Study design**

In order to test carbohydrates that differed only in digestibility we used sources of the glucose-polymer pullulan differing in chain length only (see below), based on the recognition that the rate and extent of carbohydrate hydrolysis could be controlled by varying these chain lengths. In this case, the long-chain would be largely indigestible; the medium-chain would be almost completely digestible, but at a slower rate (e.g., the “slow-carb”); and the maltodextrin glucose-polymer would be quickly and completely digestible. The use of maltodextrin as control versus pullulan or pullulan fragments ensured that all carbohydrates tested were solely based on glucose and only digestibility differed.

The study protocol was approved by the Wageningen Ethical Committee, The Netherlands. The study was designed according to a balanced cross-over design. Over three weeks each subject visited the test facility 1 day per week, on the same weekday. During these test days subjects consumed a meal replacement drink (either control or one of the alternatives, see below) at 08.00 hours. Appetite scores (6 line scales), digestibility (breath hydrogen), gastro-intestinal (GI) symptoms and (in an n=12 subset) blood glucose and plasma insulin levels were measured for the subsequent 5 hours.

### **Test foods**

Three products (all 325 ml) were evaluated (see Table 1): regular meal replacement drink (Slim·Fast Optima Chocolate ready-to-drink, Slim·Fast, Unilever, Covington, TN) in which the regular carbohydrates sucrose and lactose were replaced by 15g of either:

- 1) maltodextrin (“MDX”);
- 2) long-chain pullulan (“LCP”, molecular weight 200 kDa); or
- 3) medium-chain pullulan (“MCP”, 22.6 kDa).

### **Preparation and measurement of pullulan fragments**

Pullulan is a fermentation product of the yeast *Aerobasidium pullulans*. It is a water soluble, viscous polysaccharide consisting of basic unit of three  $\alpha$ -1,4 linked glucose molecules that is repeatedly polymerized by an  $\alpha$ -1,6 linkage on the terminal glucose (Kimoto et al., 1997; Nakamura, 1984). Unhydrolyzed (native) pullulan has a molecular weight up to 500 kDa, is resistant to enzymatic

hydrolysis by human intestinal enzymes and reaches the colon largely undigested (Nakamura, 1984). To obtain slowly, but completely digestible carbohydrates, LCP (pullulan from Hayashibara Co, Ltd, Okayama, Japan) was splitted into smaller fragments (see Supplemental information for the preparation of the pullulan batches and the determination of their *in vitro* digestibility). As hypothesized, MCP showed slow and almost complete hydrolysis, while LCP was hardly hydrolyzed and MDX rapidly hydrolyzed. Viscosities of the products were similar (see Supplemental information).

### **Subjective feelings of appetite**

Subjects arrived at the test facility at 07.30 and at 08.00 hours they received the test products which had to be eaten within 15 minutes. By means of a mark on 60-mm line scales using an Electronic Visual Analogue Scale (EVAS)(Stratton et al., 1998), subjects rated their appetite feelings ('prospective food consumption', 'desire for a snack', 'hunger', 'desire for a meal', 'satiety', 'fullness') just before consumption and every half hour from 08.00 hours till 13.00 hours. These 6 scales were anchored at the low end with the most negative feelings (e.g., "not at all"), and with opposing terms at the high end (e.g., "very high") as described by Flint *et al.* (Flint et al., 2000).

During each test day subjects were allowed to drink water or non-caloric drinks each hour (max 150 ml each hour) immediately after filling in the appetite questionnaires. Drinks consumed during the test session on the first day were recorded and repeated on the other test days.

### **Gastrointestinal disturbance & carbohydrate malabsorption**

GI symptoms were scored every 2 hours from 08.00 hours until 13.00 hours on 4-point scale (0=not, 1=mild, 2=moderate, 3=severe) using EVAS. Breath hydrogen method was used to assess carbohydrate malabsorption (Bond & Levitt, 1977; Strocchi et al., 1993). Ten minutes before ingestion of the test meal and at 30-min intervals after ingestion for 5 hours, subjects provided a breath sample into a 20 ml syringe. The H<sub>2</sub>-enrichment of the breath samples, due to the colonic fermentation of the unhydrolyzed carbohydrates, was determined by injecting the end-expiratory breath sample into a H<sub>2</sub> breath test unit (Lactoscreen, Hoekloos, The Netherlands).

### **Blood glucose and plasma insulin**

Because of concerns that blood draws could potentially affect the appetite measures, blood was obtained from a randomly selected subset of 12 subjects. For blood sampling a flexible 1.0 mm intravenous cannula was inserted into an antecubital vein of one arm for blood sampling. Blood (5 ml) was collected before ingestion of the test drink and at 15-min intervals during the first hour, at 30-min intervals during the second, third and fourth hour, and at the end of the fifth hour after ingestion. The blood was collected into ice-chilled tubes (SST-BD Vacutainer; BD Diagnostics, Oxford, UK) containing EDTA. Immediately after sampling, blood glucose was measured by a hexokinase method (Roche Diagnostics, Basel, Switzerland). After the last sample, the tubes were centrifuged (3 000 rpm, 15 min at 4 °C). The supernatant plasma was stored at –20 °C until analyzed for insulin using an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden).

### **Background diet, physical activities and other measurements**

The subjects were asked to avoid alcohol intake and sports activities on the evening before each test day. They could freely select their own evening meal, but this same meal was then consumed in the evening prior to every subsequent test day. Eating after 22.00 hour in that evening was not allowed and only non-caloric drinks were permitted. Subjects were asked not to change their life style during the study. These steps were taken to ensure roughly similar states of hunger and glycogen stores every test day (Flint et al., 2000). Sleeping time, mode of transportation, menstrual cycle and incidental lifestyle changes were recorded. Body weight was assessed during each visit before the test meal was given.

### **Statistical analyses**

Feelings of appetite scores were transformed to 100 mm for analysis and simplicity of presentation. In other studies we regularly observed a within-subject variance of about 85 mm·min for Area-Under-the-Curve (AUC) for appetite. Power calculation based on this variance, using a power of 0.8 and a confidence limit of 0.05, showed that 18 subjects were required for this experiment. In case of dropouts 24 subjects were selected. As the blood draws could disrupt the appetite measures, an additional group of 12 subjects was tested, both for blood measurements and appetite. As analysis (ANOVA) showed that the characteristics of self-report scores of the 12 subjects who had blood drawn did not differ from the total group of subjects, all subjects were combined for the analyses of the self-report data.

GI disturbance was analyzed using frequency of severity per time point and a standard ANOVA with the personal scores corrected for each subject's overall mean score.

We hypothesized that the use of smaller pullulan fragments would result in a more sustained postprandial blood glucose and plasma insulin response, thus behaving as a "slow carb", and would lead to a reduction in appetite as compared to a rapidly digestible control. The drinks differed only in the rate and extent of digestibility of the carbohydrates. The data were analyzed using analysis of covariance (ANCOVA) with baseline values as covariate, subjects as blocks and period and group (treatments and control) as factor using SAS version 8.2 (SAS Institute, Cary, NC). A criterion of  $p \leq 0.05$  was used for statistical significance. Given the specific test hypothesis, and to avoid inflation of experiment-wide error rate, we compared MCP and LCP only to MDX, and not to each other. The difference of each treatment versus MDX was estimated using the Dunnett multiple comparison test (2-sided). The primary analysis was focused on AUC, but to assess the significance of timeframes where differences were observed between treatments and control, further analysis was also done per time point. The incremental cumulative AUC was calculated using the values at  $t=0$  min (just before consumption of the test drinks) as a covariate and using the trapezoid rule.

Correlations between appetite scores AUC and either plasma insulin or blood glucose responses were also calculated using conventional linear regression analysis.

## Results

### Blood glucose and plasma insulin

LCP produced the lowest and MDX the highest blood glucose excursions, while MCP showed an intermediate and more sustained pattern (Figure 1). AUC for blood glucose was significantly higher for LCP and MCP compared to MDX for the period  $t=0-150$  min (4.78, 4.85, and 4.57 mmol·min for LCP, MCP and MDX, respectively). Blood glucose level dropped below 3.9 mmol/l (chosen as an arbitrary threshold for hypoglycaemia (Childs et al., 2005)) in 10, 6 and 4 out of the 12 subjects during MDX, MCP and LCP, respectively.

MDX not only generated the most pronounced changes in blood glucose but also in blood insulin (Figure 2). The biggest rise in blood insulin was seen with MDX, while the smallest increase was observed for LCP. The relatively steep increases in insulin for MDX and MCP probably also explain the relatively steep decline in blood glucose 30 to 60 minutes after consumption of these drinks.

AUC for insulin was significantly lower for LCP compared to MDX for the period  $t=0-150$  min (11.32, 15.73 and 17.90 mmol·min for LCP, MCP and MDX, respectively). Although MCP showed a smaller insulin excursion than MDX, this was not significantly different.

In addition, there were no significant relationships between appetite scores AUC and either plasma insulin or blood glucose responses in the subset of subjects where these were measured (all explained variance was generally below 25%;  $P>0.05$ ).

### **Subjective feelings of appetite**

Results for 'prospective food consumption' and 'fullness' are shown in Figures 3 and 4 (other appetite curves show comparable changes, data not shown). All self-report scores were significantly indicative of reduced appetite after LCP compared to MDX, and this is apparent from several different specific time points as well as a significantly different AUC for the time period t=0 to t=150 min. All significant differences were apparent during the first 150 min. MCP differed from MDX at some isolated time points, but not for AUC.

### **Carbohydrate malabsorption and gastro-intestinal symptoms**

Breath hydrogen excretion after 4 and 5 hours was highest for LCP and lowest for MDX (Figure 5). The hydrogen excretion after MCP was significantly higher than after MDX. The number of subjects reaching 10 ppm or higher (a value considered indicative of true malabsorption and colonic fermentation (Bond & Levitt, 1977; Strocchi et al., 1993)) for MDX, MCP and LCP, respectively, were 2, 14 and 18 out of 35 subjects.

Although there were occasional reports of GI complaints, most of them were rated "mild" and a few "moderate" and they did not differ significantly from MDX (data not shown).

## Discussion

These results cast further doubt on the notion that slowly digested carbohydrates may influence appetite via their effects on glycemic responses. Differences in glycemic responses were unrelated to appetite scores following consumption of test products controlled in composition and differing only in carbohydrate digestibility rate and extent. Despite differing effects on plasma glucose levels following consumption of drinks containing rapidly or slowly digestible glucose polymers, appetite responses to these were not different. In contrast, a largely indigestible glucose polymer led to minimal changes in blood glucose, yet produced the largest reduction in appetite scores.

Literature on the effect of reduced or sustained blood glucose rises on appetite is inconsistent (Bornet et al., 2007; Niwano et al., 2009; Pi-Sunyer, 2002; Raben, 2002; Thomas et al., 2007; van Dam & Seidell, 2007). These inconsistencies might be explained by a lower than expected energy availability of some of the carbohydrates tested or by other parameters that differed between the test products like taste, physical properties, structure, fibre type or content (Bornet et al., 2007; Hlebowicz, 2009; van Dam & Seidell, 2007; Wolever et al., 2009). Thus, this literature does not provide consistent and direct evidence for the effects of glycemic responses *per se* on appetite. For these reasons we prepared a slowly digestible carbohydrate by splitting the fibre pullulan (consisting of glucose only) into smaller glucose-polymer fragments. By doing this we prepared test materials that differed only in the rate and extent of carbohydrate hydrolysis. Both the *in vitro* and *in vivo* tests (glucose and

hydrogen) confirmed that controlling the chain length of pullulan also controlled the rate and extent of hydrolysis.

MCP produced a more sustained plasma glucose profile (and also a higher AUC and smaller peak, although interpretation of the blood glucose results is hampered by the blood glucose-suppressive effects of insulin). As such we believe it fulfils the criteria for “slow carbs” as defined by Englyst *et al.* (Englyst H.N. *et al.*, 1992), namely ‘completely digested in the small intestine but at a slower rate’. In contrast, LCP exhibited a more fibre-like behaviour as it was only about 23% digested. As colonic delivery and fermentation of fibre takes many hours (Klein *et al.*, 2005; Saito *et al.*, 1991), LCP would be expected to provide only negligible amounts of available energy value during the first 4 to 5 hours after ingestion. One would therefore presume that the ‘slow carbs’ (MCP) provided more available energy in the 5 hour test period than the largely indigestible long-chain pullulan (LCP), yet the latter had the most robust effects on appetite.

One of the criticisms of previous studies testing the effect of low glycemic index products or ‘slow-carbs’ on appetite is that most studies compared products differing in ways other than just the carbohydrate composition, and thus included confounding variables affecting the interpretations with regard to blood glucose and/or appetite (Alfenas & Mattes, 2005; Raben, 2002). Indeed, in related research on materials differing in glycemic index, no effect of glycemic index on appetite responses may be observed when controlled for nutritional composition (Alfenas & Mattes, 2005).

LCP improved appetite control on all six scales when compared to MDX. MCP was better than MDX at some time points in a two scales, but this was not statistically significant for the summary measure of AUC. Contrary to expectations, all differences were apparent in the period up to 150 minutes, suggesting that it was not (or only partially) a 'slow-carb' effect. We expected that a slow-carb like MCP might have reduced appetite more than LCP, because the energy provided by the latter in the post-meal period should be small (most escaped to the colon). As the physical properties of all treatments were similar, other mechanisms could play a role here, e.g., effects on brush border enzymes (Ueda et al., 1993; Wakabayashi et al., 1999).

Gastric distension and gut hormones like GLP-1 have been mentioned as important determinants of appetite (Naslund et al., 1998; Peters & Mela, 2008). It is, however, unclear how either of these can explain the difference in appetite responses between LCP and MCP. LCP was hardly digestible and thus did not produce many glucose molecules that could have affected the release of gut hormones like GLP-1. It is possible that LCP might have been fermented in the colon and that the glucose molecules induced GLP-1 release there, but in that case any effects on GLP-1 and appetite should only become visible several hours after ingestion. Furthermore, if GLP-1 would explain the appetite-reducing effects then the MDX, by producing the fastest glucose availability and likely the most immediate rise in GLP-1, should have reduced appetite most. The immediate effect of ingesting the liquids will increase gastric distension and affect appetite, but we do not see how this would be different between the drinks. The effect of LCP on appetite is therefore intriguing but unexplained, and further research could be focused on this observation, including use of pullulan

fragments of intermediate molecular mass (between LCP and MCP versions used here).

The immediate increase in postprandial glycemia was relatively modest compared with studies where much larger loads were used (50g) in drinks containing only carbohydrates and water. We used 15 g of the test carbohydrates in a drink containing also fat and protein. These are more realistic levels, especially for indigestible carbohydrates, in more realistic food formats. Higher levels would have affected palatability and especially viscosity, while it was our intention to make products only differing in carbohydrate digestibility. The insulin levels, actually, were more similar to other studies using higher carbohydrate levels. Apparently the protein levels in the drink increased insulin (explaining the insulin increase after ingestion of the long-chain pullulan drink) and this might have dampened the blood glucose response.

It has been proposed that solid meals generally produce more satiety than liquid meals, although there is currently no consensus on this (Almiron-Roig et al., 2003). Solids meals produce different blood glucose effects than liquid meals (e.g. (Brynes et al., 1998; Leidy et al., 2010), while the effect of food form on gut hormones like CCK or GLP-1 is equivocal. Both a smaller and an equal increase of GLP-1 have been found after a solid versus a liquid meal (Brynes et al., 1998; Leidy et al., 2010). Effects of a slowly digestible carbohydrate on appetite after a solid meal might therefore be different from the liquid meals used here, although the mechanism for this is unclear.

Based on the work of Wolf *et al.* (Wolf et al., 2003), who found GI symptoms and malabsorption after consumption of a long-chain pullulan, we expected LCP to cause measurable GI disturbance and malabsorption (as it would be only partly

hydrolyzed), while MCP (that would be hydrolyzed completely) would not. The poor digestibility of LCP is indicated by our *in vitro* results and hydrogen excretion after 4 and 5 hours, and also in the number of subjects excreting 10 ppm hydrogen or more. However, absolute levels of hydrogen excretion were relatively low, and GI disturbances were also low and not different from MDX. In their research, Wolf *et al.* (Wolf et al., 2003) used 50 g of pullulan, much higher than the 15 g used here. There is perhaps a threshold for occurrence of GI symptoms after long-chain pullulan, somewhere in between 15 and 50 g, although that might also be dependent on the product matrix.

In conclusion, our results in a study population of mostly women suggest that slowly (relative to rapidly) digestible carbohydrates will not have meaningful effects on acute appetite control when applied in food products at the levels tested here. A higher dose or a longer exposure may reveal the putative appetite effects of slow-carbs; however, this needs to be balanced against feasibility issues (especially product quality and GI complaints) to ensure that the results are relevant and practicable. While the slowly digestible carbohydrate did not meaningfully decrease appetite, the indigestible long-chain pullulan did and further research should be focused on this observation.

Supplementary information is available at *The European Journal of Clinical Nutrition's* website.

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## **Conflict of interest**

None of the authors had any conflict of interest (all authors are employed by a commercial manufacturer of meal replacement products)

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Table 1. Composition of the test drinks containing either maltodextrin (MDX), or medium-chain (MCP) or long-chain (LCP) pullulan.

	<b>MDX</b>	<b>LCP</b>	<b>MCP</b>
	<b>(g)</b>	<b>(g)</b>	<b>(g)</b>
fructose	2.5	2.5	2.5
maltodextrin	15.0		
pullulan		15.0	15.0
total sugars	17.5	2.5	17.5
other soluble fibre	2.1	1.8	2.0
insoluble fibre	3.1	1.0	2.7
"total carbs"	24.3	21.9	24.0
protein	10.0	10.0	10.0
fat	5.1	5.1	5.1
energy (kJ)*	766	728	758

\* based on 17 kJ/g for fibres (including LCP)

## Legends to Figures

Figure 1. Blood glucose (LS means  $\pm$  SE; n=12) after consumption of a meal replacement drink containing either maltodextrin (MDX), medium-chain (MCP) or long-chain (LCP) pullulan. Area-under-the curve is shown as an insert.

<sup>a</sup> significant difference (P<0.05) at that time point between LCP and MDX

<sup>b</sup> significant difference (P<0.05) at that time point between MCP and MDX

AUC 0-150 min for LCP and MCP significantly higher (P<0.05) than for MDX

Figure 2. Plasma insulin (LS means  $\pm$  SE; n=12) after consumption of a meal replacement drink containing either maltodextrin (MDX), medium-chain (MCP) or long-chain (LCP) pullulan. Area-under-the curve is shown as an insert.

<sup>a</sup> significant difference (P<0.05) at that time point between LCP and MDX

<sup>b</sup> significant difference (P<0.05) at that time point between MCP and MDX

AUC 0-150 min for LCP significantly lower (P<0.05) than for MDX

Figure 3. Prospective food consumption scores (LS means  $\pm$  SE; n=35) after consumption of a meal replacement drink containing either maltodextrin (MDX), medium-chain (MCP) or long-chain (LCP) pullulan. Area-under-the curve is shown as an insert.

<sup>a</sup> significant difference (P<0.05) at that time point between LCP and MDX

<sup>b</sup> significant difference (P<0.05) at that time point between MCP and MDX

AUC 0-150 min for LCP significantly lower (P<0.05) than for MDX

Figure 4. Fullness scores (LS means  $\pm$  SE; n=35) after consumption of a meal replacement drink containing either maltodextrin (MDX), medium-chain (MCP) or long-chain (LCP) pullulan. Area-under-the curve is shown as an insert.

<sup>a</sup> significant difference (P<0.05) at that time point between LCP and MDX

<sup>b</sup> significant difference (P<0.05) at that time point between MCP and MDX

AUC 0-150 min for LCP significantly higher (P<0.05) than for MDX

Figure 5. Breath hydrogen (LS means  $\pm$  SE; n=35) excretion 4 and 5 hours after consumption of a meal replacement drink containing either maltodextrin (MDX), medium-chain (MCP) or long-chain (LCP) pullulan.

<sup>a</sup> significant difference versus MDX (P<0.05).









