Dose-Dependent Inhibition of the Post-Prandial Glycaemic Response to a Standard Carbohydrate Meal following Incorporation of Alpha-Cyclodextrin

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Key Words
Glycaemic index  ·  Cyclodextrin  ·  Satiety  ·  Glucose  ·  Insulin  ·  Palatability

Abstract
Background: This study evaluated the dose-response effects of α-cyclodextrin, a cyclic oligosaccharide, on the glycaemic and insulinemic responses to the consumption of a standard carbohydrate meal. Methods: In a double-blind, randomised, cross-over design, 10 healthy subjects consumed boiled white rice containing 50 g of digestible carbohydrate to which 0 (control), 2, 5 or 10 g of α-cyclodextrin was added. Plasma glucose and insulin concentrations were determined prior to and for 2 h after consumption of each meal. Results: The area under the plasma glucose curve was negatively related to the dose of α-cyclodextrin ($r^2 = 0.97$, $p = 0.02$), with the areas being significantly reduced at the 5- and 10-gram doses compared with the control ($p < 0.05$). α-Cyclodextrin did not affect the area under the plasma insulin curve ($p = 0.39$). Higher doses of α-cyclodextrin resulted in greater satiety, but were associated with reduced palatability and an increased incidence of minor gastrointestinal complaints (stomach ache, nausea, bloating). Conclusion: α-Cyclodextrin reduces the glycaemic response to a standard carbohydrate meal in a dose-dependent manner and may be useful as an ingredient for reducing the glycaemic impact of such foods.

Introduction
Recent studies have indicated that post-prandial elevations in blood glucose concentrations are a significant risk factor for the development of diabetes, cardiovascular disease and certain cancers [1–5]. In contrast, diets based on foods which produce lower post-prandial blood glucose responses (i.e. low glycaemic index (GI) foods) reduce the risk of developing these diseases, and improve insulin sensitivity, blood glucose control and blood lipid profiles [6–10]. There is also recent evidence from studies in both humans [11, 12] and animals [13] that low GI diets reduce body fat deposition. Taken together, these studies suggest that foods which elicit a lower post-prandial glycaemic response may be useful as part of an overall strategy for combating obesity, and its associated burden of chronic disease.

The relationship between elevations in post-prandial glycaemia and disease risk has resulted in efforts on the part of the food industry to develop foods and/or food...
The potential for CDs to inhibit the hydrolysis of complex carbohydrates led Raben et al. [20] to investigate the effect of β-CD on the glycaemic response to consuming carbohydrate. Raben et al. [20] found that the addition of 1 g of β-CD to 50 g of potato starch resulted in a flattening of the post-prandial glucose response compared with an unmodified potato starch control, but there was no difference in the area under the curve (AUC) for blood glucose. In a subsequent study Diamantis and Bar [21] reported that the consumption of 10 g of α-CD with 100 g of white bread, containing 50 g of digestible carbohydrate, significantly reduced the AUC for blood glucose. This study was quoted in support of the recent safety assessment of α-CD as a food ingredient by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [22] and in its approval as a novel food by Food Standards Australia and New Zealand [23]. α-CD has also been approved for use as a food in Japan, and Generally Recognised as Safe notification has been lodged with the FDA in the USA. The purpose of the present study was to evaluate the dose-response effects of α-CD on the glycaemic and insulin-aemic responses to the consumption of a standard carbohydrate meal.

### Subjects and Methods

#### Subjects and Experimental Conditions

Ten healthy, non-smoking adults (5 male, 5 female) completed the study. Subject characteristics are shown in table 1. The study was approved by the Human Research Ethics Committee of the University of South Australia, and all experiments were conducted in the Nutritional Physiology Research Centre at the university.

**Table 1. Characteristics of subjects**

| Age, years | 24 ± 4 |
| Height, cm | 169 ± 9 |
| Mass, kg | 70 ± 11 |
| BMI, kg/m² | 24.5 ± 3.4 |

BMI = Body mass index. Values are mean ± SD.

#### Study Design

The study used a double-blind, randomised, cross-over design. Subjects attended the laboratory on 4 separate occasions in the morning, after an overnight fast, with a wash-out period of 48 h between consecutive visits. Subjects were instructed to maintain similar patterns of food intake and physical activity during the 24 h prior to each visit.

At their first visit to the laboratory subjects' height and body mass were measured. At every visit a venous blood sample was taken for determination of baseline plasma glucose and insulin concentrations, after which the subjects consumed a serving of boiled white rice containing 50 g of digestible starch combined with 0 (control), 5 or 10 g of α-CD (Cavamax, Wacker-Chemie GmbH, Munich, Germany). Blood samples were taken at selected time points post-prandially in order to determine the plasma glucose and insulin responses to the meal. The amount of α-CD added to the rice (i.e. 0 g, 5 g, 10 g) was randomised for each of the initial 3 visits. A 4th visit was planned to allow for the addition of another dose of α-CD which would help to better define the dose-response relationship, based on an interim analysis of the responses to the initial randomised doses. This design was used in preference to a 4-dose randomised design because of uncertainty as to the effective dose range and to limit the number of subject visits and doses of α-CD that would be administered to each individual. As the 5-gram dose appeared to be effective, a 2-gram dose of α-CD was chosen for the final visit. The randomisation of dosing was carried out using a Latin square by a member of the research team (P.R.C.H.) who did not have any direct contact with the subjects or the researchers administering the interventions (A.J.T., K.J.M.) during the data collection period to ensure double-blinding. P.R.C.H. was also responsible for determining the dose (i.e. 2 g) to be administered during the final visit, but did not reveal the dose to the other researchers or the subjects to ensure blinding.

#### Height and Body Mass

Body mass was measured to the nearest 0.2 kg, using electronic digital scales (Tanita, Model 2204, Tokyo, Japan). Stature was measured to the nearest 0.1 cm using a wall-mounted stadiometer (SECA, Model 220, Hamburg, Germany) with the subjects barefoot in the free-standing position.
Blood Sampling
To obtain blood samples a catheter (Terumo, 21G x ¾”, New Jersey, N.J., USA) was inserted into a forearm vein under local anaesthesia (lignocaine hydrochloride, 2%) at least 30 min prior to the first blood sample being taken. Blood samples (2 ml) were collected immediately prior to the consumption of the rice meal, and 15, 30, 45, 60, 90, and 120 min after the subjects began consuming the meal. To maintain blood volume and maintain the patency of the catheters, each sample was immediately replaced with an equivalent volume of normal saline.

Each blood sample was placed into a plastic tube containing sodium fluoride/potassium oxalate (Vacuette, Greiner Bio-one, Kremsmünster, Austria), inverted several times, and then placed on ice until centrifuged at 4,000 rpm for 10 min at 4°C (Universal 32R, Hettich, Tuttingen, Germany). The plasma was then drawn off and stored at –20°C prior to glucose and insulin analyses which were conducted within 4 weeks.

Plasma Glucose and Insulin Analysis
Plasma glucose concentrations were measured on a Cobas-Bio centrifugal analyser (Roche Diagnostics, Basel, Switzerland) using a commercially available enzymatic kit (Roche Diagnostics) and control sera. The intra-assay coefficient of variation for this method is 3.3%.

Plasma insulin concentrations were determined using a commercially available ELISA kit (Mercodia, American Laboratory Products Co., Salem, N.H., USA). The intra-assay coefficient of variation for this method is <4% and cross-reactivity with pro-insulin is <0.01%.

Meal Preparation
At each visit to the laboratory the subjects consumed a meal of white medium grain Calrose rice (Sunrice® , Rice Growers Cooperative, Ltd, Leeton, Australia) to which either 0, 2, 5 or 10 g of α-CD had been added.

The meals were prepared by soaking 64 g of rice, containing 50 g of digestible carbohydrate (according to the manufacturers’ data) overnight at 4°C in 160 ml of water to which the appropriate amount of α-CD had been added. The mixture was then cooked immediately prior to serving using a microwave oven, with particular care being taken to ensure that all of the water had been absorbed into the rice. The rice meal was served to each subject together with a 250-ml glass of plain water, and subjects were required to consume the meal within a 5-min period. The meals were prepared by a member of the research team (K.J.M.) who did not have direct contact with the subjects during the intervention period to ensure blinding.

Calculation of Glycaemic and Insulinaemic Responses
Calculation of the AUC for each dose of α-CD was carried out to provide an index of the overall glycaemic and insulinaemic responses to the test meals. The AUCs were calculated from the concentrations of glucose and insulin measured in the blood samples which were taken immediately prior to the consumption of the rice meal, and 15, 30, 45, 60, 90 and 120 min after the subjects began consuming the meal, using the incremental AUC (iAUC) method [24]. The blood-sampling schedule used to determine the iAUC and the use of the iAUC itself have both recently been reported to provide the most valid and precise values for calculating the GI of foods [24].

Incremental Area under the Curve
The mean iAUC for glucose was negatively related to the dose of α-CD (r² = 0.97, p = 0.02), with the iAUC being significantly lower than the control dose (i.e. lower than 0 g) for the 5- (p = 0.03) and 10-gram (p = 0.001) doses (fig. 2). The reductions in iAUC at the 2-, 5- and 10-gram doses of α-CD represented reductions of −1.7 ± 17.2, −20.4 ± 15.4 and −49.6 ± 9.9%, respectively, compared with the control (i.e. 0 g). There were no significant differences in the iAUC for insulin between the different doses of α-CD (p = 0.39) (fig. 3), and there was no relationship between the mean iAUC for insulin and the dose of α-CD (r² = 0.51, p = 0.29).

Organoleptic Assessment and Adverse Effects
After consuming the meal subjects were asked to qualitatively rate the palatability of the meal by responding yes or no when asked whether they liked the taste of the rice or not. Satiety was also rated once subjects had finished consuming the meal by asking them to indicate whether they felt hungry, satisfied or overfull/unwell. The occurrence of any adverse effects, in particular gastrointestinal effects, were also monitored during the study visit, and the 48-hour wash-out periods. In order to monitor adverse effects during the 48-hour wash-out periods between visits, subjects were interviewed when returning to the laboratory for a subsequent visit or, in the case of the final visit, were interviewed 48 h after completing their final laboratory visit.

Statistical Analysis
To determine the effects of the treatment on the dependent measures, ANOVA with repeated measures was used (Statistica v5.1, StatSoft Inc., Tulsa, Okla., USA). Where ANOVA showed a statistically significant main effect, pair-wise comparisons of means were made post-hoc using a test of least significant differences. Relationships between variables were determined by linear regression. Data shown in the tables and figures represent mean ± SE unless otherwise stated. An α level of p < 0.05 was taken as indicating statistical significance.

Results
Plasma Glucose and Insulin Concentrations
Plasma glucose concentrations had increased significantly by 30 min after beginning to consume the rice meal (p < 0.05), regardless of the dose of α-CD that had been incorporated (fig. 1a), and were still significantly higher than baseline values by 120 min. Plasma insulin concentrations had increased significantly by 15 min after beginning to consume the meal (p < 0.05) and remained elevated for the remainder of the 2-hour sampling period (fig. 1b).
**Organoleptic Assessment and Adverse Events**

In terms of the satiating effects of the meals, 2 subjects indicated that they were still hungry after consuming the rice meals containing 0 and 5 g of α-CD, while 3 subjects indicated that they were still hungry after consuming the meal containing 2 g of α-CD. No subjects reported being hungry after consuming the meal containing 10 g of α-CD.

With regard to the taste of the meals, only 1 subject reported not liking the taste of the meal containing no α-CD (i.e. 0 g), but the number of subjects indicating a dislike for the taste increased as the dose of α-CD was increased. Three subjects indicated a dislike for the taste when the rice was combined with 2 g of α-CD, 4 disliked the taste with the 5-gram dose, and 5 disliked the taste with the 10-gram dose.

The incidence of minor gastrointestinal complaints also increased with increasing doses of α-CD. No subjects reported any gastrointestinal symptoms following the meal containing no α-CD, but 1 subject reported a gas-

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*Fig. 1.* Plasma glucose (a) and insulin (b) concentrations immediately prior to and following the consumption of a standard meal of white rice containing 50 g of digestible carbohydrate and either 0, 2, 5 or 10 g of α-cyclodextrin. * Significantly different from time 0: p < 0.05.
intestinal upset (stomach ache) after consuming the meal containing 2 g of α-CD, 2 reported gastrointestinal upsets (stomach ache, abdominal cramps) with the 5-gram dose, and 3 reported gastrointestinal upsets with the 10-gram dose (nausea, bloating, diarrhoea).

**Discussion**

The principal finding of the present study was that the addition of α-CD to a standard serving of white rice reduced the post-prandial glycaemic response in a dose-dependent manner, with the addition of both 5 and 10 g of α-CD resulting in statistically significant reductions in the iAUC for plasma glucose. Given that attenuation of post-prandial glycaemia can reduce body fat deposition [11–13] and the risk of developing certain chronic diseases [6–13], the addition of α-CD to foods containing complex carbohydrates may form a useful part of an overall strategy for combating the increasing incidence of obesity and its associated disease burden.

The finding of a reduction in post-prandial glycaemia in the present study is in part in agreement with a previous finding of Raben et al. [20], who reported that the addition of 1 g of β-CD to 50 g of potato starch resulted in a flatter post-prandial glucose response, but no difference in the AUC. In the present study the addition of 2 g of α-CD also flattened the post-prandial glucose response (not significant), with no significant reduction in the iAUC. This suggests that doses of 1 g of β-CD or 2 g of α-CD may be insufficient to elicit any significant effect on the post-prandial glycaemic response. At the higher doses of α-CD (i.e. 5 and 10 g) the iAUC was reduced by almost 50% (at the 10-gram dose), which is similar to the 57% reduction reported by Diamantis and Bär [21] following the addition of 10 g of α-CD to a serving of white bread containing 50 g of digestible carbohydrate. These reductions in iAUC support our hypothesis that the inhibitory effect of α-CD on pancreatic amylase would reduce starch hydrolysis in the small intestine and attenuate the post-prandial glycaemic response, but some of the effect of α-CD may also be related to a reduction in intestinal transit time. α-CD is a hydrophilic molecule [14, 15], and as such, would exert osmotic pressure drawing fluid into the small intestine. At the same time the inhibitory effect on amylase would provide a greater residual starch volume in the small intestine, which would also contribute to the overall osmotic pressure and act to hold fluid in the intestinal lumen. Increasing the fluid volume in the small intestine would reduce intestinal transit time, and the time available for glucose absorption [25], thereby also possibly contributing to the observed attenuation of post-prandial glycaemia.

Regardless of the mechanism(s), the dose-dependent reduction in the iAUC for glucose indicates that the addition of α-CD effectively reduced the GI of the rice meal. Given the accumulating data which indicate that the consumption of a low GI diet can have a number of health benefits, the addition of α-CD to foods high in complex carbohydrates may form a useful part of a dietary strategy for combating the increasing incidence of obesity and its associated disease burden.
benefits [6–12], the addition of α-CD to foods in order to reduce their glycaemic impact may be desirable, particularly since modification of the glycaemic effects of existing foods may be a more effective strategy than trying to motivate consumers to change their eating habits and select foods with a lower GI.

In addition to reducing the iAUC for glucose, the higher doses of α-CD also achieved greater satiety, an effect that was also reported by Raben et al. [20] following the addition of β-CD to potato starch. The satiating effect of low GI foods is well recognised and may contribute to the weight-loss associated with their consumption by reducing food intake [11, 26]. However, while the higher doses of α-CD provided greater satiety, they also tended to be associated with an increasing incidence of adverse sensory effects. As the dose of α-CD was increased the proportion of subjects who reported not liking the taste also increased, which may have implications for the consumption of foods containing α-CD since palatability is a major determinant of food choice. Strategies for improving the taste of foods containing α-CD should be investigated. The incidence of minor gastrointestinal complaints also tended to increase with increasing doses of α-CD. Studies in rats have shown that α-CD is almost completely fermented by microflora in the large intestine [16, 17], and the JECFA [22], in its recent evaluation of the safety of α-CD as a food ingredient, concluded that the abdominal discomfort associated with the consumption of α-CD is due partly to the production of fermentation by-products, and partly because of the osmotic effect of α-CD drawing water into the small intestine to maintain isotonicity. It therefore follows that intestinal tolerance would not only be determined by the dose of α-CD, but also by the dose of co-ingested starch and the magnitude of the amylase-inhibitory effect of α-CD, which in turn may be modulated by a number of other factors, including the characteristics of the starch, the pH of the small intestine and the presence of other nutrients. This latter point is of particular relevance to the present study where the gastrointestinal symptoms may have been exacerbated by the fact that the subjects had fasted overnight prior to consuming the meal. While the gastrointestinal symptoms were only minor, further investigations should attempt to determine means of reducing these symptoms.

In conclusion, the present study provides evidence that incorporation of as little as 5 g of α-CD into a standard meal of white rice containing 50 g of digestible carbohydrate can significantly reduce the post-prandial glycaemic response. Consumption of the efficacious 5- and 10-gram doses in 3 meals/day is within the intake range assessed by Food Standards Australia and New Zealand (i.e. up to 38.6 g·day⁻¹) in its recent safety evaluation of this ingredient, although its organoleptic acceptability in various food products still needs to be established. Further research is now warranted to determine the health benefits which might arise from regular consumption of foods containing α-CD, particularly in populations with risk factors for diseases such as diabetes and cardiovascular disease, which might be modifiable by consuming foods which elicit a lower glycaemic response.

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