Acute ingestion of resistant starch reduces food intake in healthy adults

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Resistant starch (RS), a non-viscous dietary fibre, may have postprandial effects on appetite regulation and metabolism, although the exact effects and mechanisms are unknown. An acute randomised, single-blind crossover study, aimed to determine the effects of consumption of 48 g RS on appetite compared to energy and available carbohydrate-matched placebo. Twenty young healthy adult males consumed either 48 g RS or the placebo divided equally between two mixed meals on two separate occasions. Effects on appetite were assessed, using an ad libitum test meal and 24-h diet diaries for energy intake, and using visual analogue scales for subjective measures. Changes to postprandial glucose, insulin and C-peptide were also assessed. There was a significantly lower energy intake following the RS supplement compared to the placebo supplement and 24-h diet diaries for energy intake, and using visual analogue scales for subjective measures. Changes to postprandial glucose, insulin and C-peptide were also assessed. There was a significantly lower energy intake following the RS supplement compared to the placebo supplement at both the ad libitum test meal (5241 (SEM 313) v. 5606 (SEM 345) kJ, P=0·033) and over the 24 h (12 603 (SEM 519) v. 13 949 (SEM 755) kJ, P=0·044). However, there was no associated effect on subjective appetite measures. Postprandial plasma glucose concentrations were not significantly different between supplements, but there was a significantly lower postprandial insulin response following the RS supplement (P=0·029). The corresponding C-peptide concentrations were not significantly different, although the ratio of C-peptide to insulin was higher following the RS supplement compared to placebo (P=0·059). These results suggest that consumption of 48 g RS, over a 24-h period, may be useful in the management of the metabolic syndrome and appetite. Further studies are required to determine the exact mechanisms.

Fibre: Appetite: Postprandial insulin

The incidence of obesity has increased over recent years and has reached an epidemic extent (1); this is also associated with other health issues such as an increased prevalence of insulin resistance and type 2 diabetes. Various strategies have been investigated to determine how this problem could be tackled and to prevent a further increase in body weight and the associated conditions. These strategies include investigations into individuals’ diets and specific dietary components that could be altered to subsequently impact on weight, insulin resistance and type 2 diabetes. One such component is dietary fibre. This diverse group of carbohydrates has been proposed to increase satiety (2) and therefore may have a beneficial role in weight management. Dietary fibre may increase satiety by several mechanisms and different fibres may exert their effects by different means although the specific processes are not known. Proposed mechanisms include the lower energy density of fibre foods, the impact of fibre on delaying gastric emptying, which subsequently could prolong the feeling of fullness, and fibre slowing glucose absorption, which may prolong satiety (3). There is also evidence to suggest that dietary fibres may influence insulin sensitivity (4) independently to any effects on appetite.

Resistant starch (RS), a fermentable carbohydrate, has been proposed to have properties similar to dietary fibre (5) and is not thought to affect gastric emptying or the absorption of nutrients (6). The effects on satiety, if indeed there are any, are unclear. Use of RS may be a more advantageous means of increasing fibre intake compared to traditional fibres, as it is easy to incorporate into everyday foods (7) without adverse alterations to either taste or texture (8).

There are few studies that have investigated the effects of including RS into the diet and the consequent effects on appetite. Animal studies show consistent positive effects on appetite regulation and insulin sensitivity; however, data in human studies are mixed (9). In particular, there are no current studies in human subjects that have explored the effects on appetite of including RS in mixed meals when compared to energy and carbohydrate-matched placebo. Of the four classifications of RS, the majority of studies looking at effects on appetite have used type 2, which are intrinsically resistant, and type 3, which are retrograded starches.

The postprandial effects of consumption of RS are also not known; however, as a non-viscous fibre, it would not be predicted to affect glucose absorption. Nevertheless, it has been shown in a recent study that short-term consumption of RS type 2 improves postprandial glucose metabolism in healthy individuals (6).

The present study therefore aimed to investigate the acute effects of including 48 g of RS type 2 on energy intake, subjective measures of appetite and changes to postprandial

Abbreviations: RDS, rapidly digestible starch; RS, resistant starch.

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glucose and insulin, compared to a placebo supplement in a randomised, single-blind balanced crossover, in which the 48 g were divided equally between breakfast and lunch (providing 24 g at each meal) and consumed as part of these mixed meals.

Experimental methods

Subjects

Twenty young, healthy, adult males, aged 19–31 years, with a mean BMI 23·2 (SEM 0·65) kg/m² (Table 1) participated in the study. Subjects had no history of gastrointestinal disease or endocrine disorders and were weight stable for at least the preceding 3 months. Highly restrained eaters, identified by the Dutch Eating Behaviour Questionnaire(10), were excluded from the study. The participants included in the study had a mean score of 2·1 (SEM 0·2) on the restraint scale, 1·98 (SEM 0·1) on the emotional scale and 3·1 (SEM 0·1) on the external eating scale. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Surrey’s Ethics Committee; written, informed consent was obtained from all subjects.

Study design

Subjects attended the investigation unit after an overnight fast, on two occasions, at least 1 week apart, when they consumed either the RS supplement or the placebo supplement within the test breakfast and lunch meals, which provided 24 g RS at breakfast and 24 g RS at lunch on the RS leg. Blood samples were taken, one 15 min before breakfast and the other just before lunch, which was served at time zero. The breakfast on both days was of a standardised portion size and consisted of a pasta-based meal which was made to a standard recipe with standard cooking times; however, while the energy content was identical on both visits, the energy and macronutrient intake was identical with only the presence of the RS differing. A similar study design has been used successfully in previous studies(12). The mean values for the amounts consumed at lunch are shown in Table 2.

At the end of the intervention (420 min) participants were placed in individual areas and provided with a large preweighed ad libitum homogeneous test meal, in excess of normal portion sizes (the whole dish provided: 9765kJ energy, 81·5 g protein; 339·1 g carbohydrate; 70·0 g fat; 15·9 g fibre). Subjects were instructed to consume freely until comfortably full and then the leftovers were weighed. The subjects were informed that they could take home any leftover test meal in order to prevent over-consumption. The ad libitum test meal was a pasta-based meal which was made to a standard recipe with standard cooking times; however, while the energy content was identical on both visits, the energy density per gram would have varied depending on the amount of water absorbed during cooking. Therefore, this was taken into consideration when the energy intake was calculated for each visit.

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were assessed by HOMA\textsuperscript{(13)} at the beginning of each study morning; while postprandial insulin sensitivity was assessed using the minimal model method described by Caumo \textit{et al.}\textsuperscript{(14)}.

Area under the curve was calculated for glucose, insulin and C-peptide, using the trapezoid method. The ratio of C-peptide to insulin was calculated using the area under the curve for 2 h after each meal for both measures and used as a marker of hepatic insulin clearance. Time course data were analysed by repeated measures ANOVA. The data were normally distributed and paired \textit{t} tests were used to compare between the groups. Statistical analyses were carried out using SPSS version 12.0.1 for Windows (SPSS, Inc., Chicago, IL, USA), with significance assumed as \(P<0.05\). All the results are means with their standard errors.

### Results

Both the supplements were well tolerated by the subjects with no adverse gastrointestinal effects reported on the day of the study or the following day.

The fasting insulin sensitivity and \(\beta\)-cell function (assessed by the homeostatic model assessment\textsuperscript{(13)}) were not significantly different at the start of each of the study days (Table 3), which confirms that the subjects were in a similar metabolic state at the start of each study day.

#### Energy and macronutrient intake

Supplementation with 48 g RS over two meals resulted in a significantly lower energy intake, 5241 (SEM 313 kJ) at the offered \textit{ad libitum} test meal compared to the energy intake seen with the placebo, 5606 (SEM 345 kJ; \(P=0.033\)).

Over the whole 24-h period there was also a significantly lower energy intake following the 48 g RS supplement compared to the placebo supplement, from 12603 to 13949 kJ (\(P=0.044\); Table 4). With the RS leg the mean energy intake was 104\% of calculated habitual energy requirements (calculated with the Schofield equation\textsuperscript{(15)} and a moderate activity level of 1.6 for all subjects) compared to 116\% for the placebo leg.

The lower 24-h energy intake appeared to be mainly due to a significantly lower fat intake, 13.3 g lower, following the RS supplement compared to the placebo (Table 4). The dietary fibre intake during this 24-h period was significantly different

### Table 2. Nutritional composition of the breakfast and lunch meals consumed on both study days

(\textit{Mean values with their standard errors for twenty subjects})

<table>
<thead>
<tr>
<th></th>
<th>Breakfast</th>
<th>Lunch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>1595</td>
<td>3865</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>9.6</td>
<td>35.6</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>67.2</td>
<td>111.9</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>7.9</td>
<td>36.8</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>0.5 (24.5\textsuperscript{*})</td>
<td>5.0 (29.0\textsuperscript{*})</td>
</tr>
</tbody>
</table>

\* Resistant starch meal only.

Overall 24-h intake on the study days was assessed from weighed intakes from the breakfast, lunch and \textit{ad libitum} dinner provided and from diet diaries completed during the late evening by subjects after they had left the unit. All dietary analysis was performed using WinDiets Professional Version program (Robert Gordon University, Aberdeen, UK). Bowel habit diaries were completed on the day of the study and the following day for assessment of gastrointestinal tolerance.

### Biochemistry

Whole blood for glucose analysis was collected into sodium oxalate tubes, for insulin analysis into potassium EDTA tubes and for C-peptide analysis into potassium EDTA tubes with 200 kallikrein inhibiting units (KIU) aprotinin per ml of whole blood (Trasylol; Bayer, Newbury, UK). All samples were centrifuged for 10 min at 3000 rpm and then plasma aliquots were stored at \(-20^\circ\text{C}\) until batch analysis to reduce inter-assay variation. Plasma glucose was measured enzymatically using a commercially available kit (Instrumentation Laboratory, Warrington, UK) for the ILab650 (Instrumentation Laboratory) and the inter-assay variation was <2\%. Concentrations of plasma insulin and C-peptide were measured by RIA with commercially available kits (Millipore; Watford, UK). The sensitivity of the insulin assay was 12 pmol/l (2 \(\mu\text{U} / \text{ml}\)) and for the C-peptide assay was 0.1 ng/ml, with an inter- and intra-assay variation of <10\%.

### Calculations and statistical analysis

Fasted insulin sensitivity and \(\beta\)-cell function (homeostasis model assessment (HOMA) % S and HOMA % B, respectively) were assessed by HOMA\textsuperscript{(13)} at the beginning of each study morning; while postprandial insulin sensitivity was assessed using the minimal model method described by Caumo \textit{et al.}\textsuperscript{(14)}.

#### Table 3. Indices of insulin sensitivity following consumption of 48 g resistant starch (RS) or placebo

(\textit{Mean values with their standard errors in twenty healthy, young adult males})

<table>
<thead>
<tr>
<th></th>
<th>RS</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA % S</td>
<td>95.9</td>
<td>97.0</td>
</tr>
<tr>
<td>HOMA % B</td>
<td>105.9</td>
<td>111.4</td>
</tr>
<tr>
<td>C-peptide:insulin AUC 0\textendash;300 min</td>
<td>6.9</td>
<td>6.13</td>
</tr>
<tr>
<td>Oral (S_\text{b}) breakfast (dl/kg/min per (\mu\text{U}/\text{ml})</td>
<td>(3.36 \times 10^{-3})</td>
<td>(5.58 \times 10^{-3})</td>
</tr>
<tr>
<td>Oral (S_\text{l}) lunch (dl/kg/min per (\mu\text{U}/\text{ml})</td>
<td>(5.65 \times 10^{-3})</td>
<td>(4.43 \times 10^{-3})</td>
</tr>
</tbody>
</table>

\* Comparisons were made with a paired samples \textit{t} test.
due to the supplementation with 48 g RS, and there did not appear to be a difference in fibre intake by the subjects once they left the investigation unit at the end of the postprandial study period.

Subjective appetite measures
There was no difference in the subjective appetite scores, measured by the visual analogue scales, for hunger (Fig. 1), fullness, prospective food consumption, thirst or desire for different foods (sweet, salty, savoury or fatty foods) between the two supplements.

Postprandial metabolites
The postprandial glucose concentrations were not significantly different between the RS supplement and the placebo supplement (Fig. 2(a)).

There was a significantly lower postprandial insulin response following the RS supplementation compared to the placebo supplement over the whole acute study period ($P=0.029$; Fig. 2(b)). However, the corresponding C-peptide concentrations were not significantly different between the two supplements. Consequently, there was an increase in molar ratio of C-peptide to insulin as a surrogate marker for hepatic insulin clearance (Table 3); however, there was no significant difference in postprandial oral insulin sensitivity between the two supplements at either meal (Table 3).

Discussion
The present study found that after consumption of 48 g RS (split equally over the test breakfast and lunch meals), there was a lower energy intake at both the ad libitum test meal and over the whole 24-h period, without an associated effect on subjective appetite ratings; the study also found a significant effect of the RS supplement on lowering the postprandial insulin response. To our knowledge this is the first study where RS has been provided to participants as part of a mixed meal and compared to a placebo, where available carbohydrate and energy load have been matched.

Previous studies investigating the effects of RS on appetite have replaced proportions of carbohydrate with RS and therefore the amount of glycaemic carbohydrate provided has varied between the supplements which would confound the interpretation of the results. Indeed, in one study consumption of RS appeared to cause a reduction in subjective feelings of satiety(16); however, a limitation of the study was that the RS and the corresponding 100 % digestible starch were mixed into fruit syrup drinks as a means of delivering the starches, which resulted in the two supplement drinks having a different texture, one liquid and the other semi-solid, with liquids being known to be less satiating than solid foods(17); this study also matched the supplements by weight of starch and therefore the supplements differed in energy and available carbohydrate content. In another study by de Roos et al. (18) two types of RS (RS type 2 and RS type 3) were compared to glucose and, while the supplements were matched for total carbohydrate content, the proportion of available carbohydrate was lower in both of the RS supplements. The study also found that after consumption of each product for 1 week, there was little effect of the RS on appetite. Other studies have investigated the effects on appetite of varying the amylose to amylopectin ratios. One of these studies found that immediately after the meal the high level of amylase was more satisfying than the low amylase but that

Table 4. Total 24-h intake following supplementation with 48 g resistant starch (RS) or placebo, measured from 24-h diet diaries (*).

<table>
<thead>
<tr>
<th></th>
<th>RS</th>
<th>Placebo</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>12 603</td>
<td>13 949</td>
<td>519</td>
<td>755</td>
<td>5 63</td>
<td>6 87</td>
<td>NS</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>104 9</td>
<td>115 3</td>
<td>5 63</td>
<td>6 87</td>
<td>4 66</td>
<td>4 79</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>424 4</td>
<td>452 6</td>
<td>18 40</td>
<td>23 00</td>
<td>21 86</td>
<td>21 96</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>96 7</td>
<td>110 0</td>
<td>4 56</td>
<td>4 79</td>
<td>4 52</td>
<td>4 79</td>
<td>NS</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>39 2</td>
<td>45 2</td>
<td>2 18</td>
<td>2 37</td>
<td>2 18</td>
<td>2 37</td>
<td>NS</td>
</tr>
<tr>
<td>Dietary fibre (g)</td>
<td>65 1</td>
<td>16 7</td>
<td>0 95</td>
<td>0 84</td>
<td>0 84</td>
<td>0 84</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Comparisons were made with a paired samples t test.
The lower energy intake seen in the present study over 24 h appeared to be mainly explained by a significantly lower fat intake following the RS supplement. However, the reason for this lower fat intake is unclear, as the participants did not report a difference in desire for fatty foods on the subjective appetite ratings during the postprandial study. This difference in macronutrient choice following RS consumption would require further investigation to determine whether it was an incidental finding or a true effect. There also did not appear to be an influence of the high RS intake on fibre intake later in the day; however, there may have been an effect on the following day’s fibre intake that was not monitored in the present study.

Although in the present study a reduced energy intake was found following consumption of the RS supplement, it is not possible from the design of the present study to determine exactly what the mechanisms are for the observed effect on food intake. However, as the main effect on food intake appeared to occur at the ad libitum dinner and then later in the day once the participants had left the unit, a possible mechanism could be the fermentation in the colon by colonic microflora and the subsequent effects of SCFA, which has been hypothesised as a mechanism for the effects of fibre on appetite.

While the metabolism of SCFA may result in potential additional energy being provided to the body, this was not accounted for in the present study and no value was assigned for the fermentation of the RS. The actual amount delivered to each participant is likely to vary, dependant on the extent of fermentation and metabolism within the colon and variations in individuals’ gut transit time, as well as a loss to the faeces of a proportion of the ingested RS and the SCFA produced. Published works have stated an energy value of 8 kJ/g for Hi-Maize accounting for SCFA metabolism compared to the 16 kJ/g for the placebo. In the present study, we matched the supplements by glycaemic load as the largest confounder to the glycaemic response. When taking into consideration the amounts of each supplement given this would have resulted in maximal energy doses of 640 kJ for the RS and 512 kJ for the placebo, with a difference of only 128 kJ. This therefore does not account for the full difference in energy intake observed in the study over the 24-h period which was 1346 kJ.

It is possible that the increase in production of SCFA may consequently increase production of anorexigenic hormones from the colon, such as peptide YY and glucagon-like peptide, has been shown in rodent studies, which are, in terms of the gastrointestinal tract, anatomically different to human subjects. Human studies which have measured gastrointestinal peptides have not reported a difference in desire for fatty foods on the RS intake following the RS supplement. However, the reason for this lower fat intake is unclear, as the participants did not report a difference in desire for fatty foods on the subjective appetite ratings during the postprandial study. This difference in macronutrient choice following RS consumption would require further investigation to determine whether it was an incidental finding or a true effect. There also did not appear to be an influence of the high RS intake on fibre intake later in the day; however, there may have been an effect on the following day’s fibre intake that was not monitored in the present study.

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It is possible that the increase in production of SCFA may consequently increase production of anorexigenic hormones from the colon, such as peptide YY. However, so far the only evidence of an effect of RS on these hormones, particularly peptide YY and glucagon-like peptide, has been shown in rodent studies, which are, in terms of the gastrointestinal tract, anatomically different to human subjects. Human studies which have measured gastrointestinal peptides have not found no effect of fibre feeding.

As insulin and C-peptide are co-secreted, the lower postprandial insulin concentration detected was likely to be due to increased hepatic insulin clearance, as there was a trend towards significance between the two supplements for the molar ratio of C-peptide to insulin. An increase in hepatic insulin clearance has previously been reported following RS intake over 24 h. It has been proposed that the increase in
production of SCFA and their exposure to the liver may ultimately be responsible for the increase in insulin clearance.

The lower energy intake seen in the present study following RS consumption as part of a mixed meal could have beneficial implications in weight management and, potentially, weight loss; however, further studies are required to confirm whether a similar finding is seen in other population groups such as the overweight or obese, and to determine the actual mechanisms for the effect. Although the dose in the present study was well tolerated over the one day of the study and a lower energy intake was observed, further investigations are needed to establish whether the dose has similar effects when ingested chronically. A lower postprandial insulin response was also observed which could be explained by an increase in hepatic insulin clearance. Increased intakes of RS in the diet may therefore have beneficial implications in weight management.

Acknowledgements

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References