Retrograded (RS₂) but not uncooked (RS₃) resistant starch lowers fecal ammonia concentrations in healthy men

Dear Sir:

Birkett et al (1) reported that consumption of 39 g resistant starch (RS) d⁻¹ for 3 wk, lowered fecal concentrations of ammonia in healthy subjects. The RS used was a mixture of the three major types that occur naturally in the human diet, namely, physically entrapped starch (RS₁), uncooked starch granules (RS₂), and retrograded starch (RS₃). We wish to extend the interesting findings of Birkett et al (1) by reporting here for the first time the results of an experiment that compared RS₂ and RS₃ as derived from well-defined corn starches.

Healthy men consumed a supplement each day in addition to their habitual diet in a single-blind, randomized 3 × 3 Latin-square experiment (3). During the first week (run-in period) all subjects consumed the control supplement containing glucose. Subsequently, each subject consumed for 1 wk a supplement with RS₂ (Hylon VII; ie, uncooked high-amylose corn starch), RS₃ (extruded, retrograded Hylon VII), and glucose. The 24 subjects were randomly divided into six groups before the start of the run-in period. Each group consumed the supplements in one of the six possible sequences so as to eliminate variation due to residual effects of the previous supplement or to drift of variables over time. The daily supplements provided 2 MJ and consisted of a mixture of skim yogurt, skim milk, mashed canned fruit, citrus, and either glucose, RS₃, or RS₃ (3). The dietary variables were added to the supplements as identical amounts of glucose units (101 g glucose units/d). Radioopaque, barium-sulfate impregnated, polyethylene rings were swallowed with each supplement portion to serve as a marker for feces collection. The RS₂ and RS₃-containing supplements each provided 32 g RS/d and the supplement with glucose contained 4 g RS/d as determined in vitro by the Englyst method (2). Compliance, as measured by urinary recovery of lithium, was satisfactory and comparable in the three supplementation periods (3). Weekly 24-h food consumption recalls showed that the amount and composition of the background diet were similar for all dietary periods (3). Body weight remained constant throughout the study. On the last 2 d of each period, the subjects collected 24-h urine. Urinary urea and creatinine were measured by using commercial test combinations (no. 1688-05 and no. 1694-06; Abbott Laboratories, Irving, TX). During the last 3 d of each period, the subjects defecated twice at the Department of Human Nutrition. The feces were weighed and frozen immediately at −20 °C. Ammonia was extracted from homogenized feces with perchloric acid and measured with the use

<table>
<thead>
<tr>
<th>Dietary supplement</th>
<th>Glucose</th>
<th>RS₂</th>
<th>RS₃</th>
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</thead>
<tbody>
<tr>
<td>Feces</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet weight (g/d)</td>
<td>232 ± 19*</td>
<td>277 ± 20b</td>
<td>301 ± 29b</td>
</tr>
<tr>
<td>Ammonia (µg/g of feces)</td>
<td>648 ± 41b</td>
<td>595 ± 39b</td>
<td>481 ± 36*</td>
</tr>
<tr>
<td>(mg/d)</td>
<td>143 ± 10</td>
<td>157 ± 10</td>
<td>134 ± 11</td>
</tr>
<tr>
<td>Urinary urea (g/d)</td>
<td>26.2 ± 1.1</td>
<td>27.4 ± 1.1</td>
<td>24.9 ± 1.1</td>
</tr>
<tr>
<td>(g/g creatinine)</td>
<td>15.7 ± 0.7</td>
<td>15.7 ± 0.5</td>
<td>15.3 ± 0.6</td>
</tr>
</tbody>
</table>

* ± SEM; values in the same row with different superscript letters are significantly different, P < 0.05, as assessed by ANOVA with “subject” as the random factor and “supplement” as the fixed factor, followed by Tukey’s studentized range test.
of a commercial test combination (Ammonia UV-method, cat no. 1112732; Boehringer Mannheim GmbH, Mannheim, Germany).

One subject took antibiotics during the experiment; his data were excluded from statistical analysis. Glucose and RS2 consumption produced similar fecal ammonia concentrations, whereas RS3 had a significant lowering effect (Table 1). Thus, the finding of Birkett et al (1) that a diet rich in a mixture of RS types lowered fecal ammonia concentrations may have been caused specifically by the RS3 component. Birkett et al (1, 4) and ourselves (Table 1) found that consumption of RS increased fecal output, which explains the decrease in fecal ammonia concentration because the absolute ammonia excretion was only slightly affected (Table 1).

It is difficult to see that RS2 lowers fecal ammonia concentrations by a specific mechanism rather than by raising the bulk of feces. Birkett et al (1) suggested that RS fermentation in the colon stimulates bacterial growth and thereby ammonia incorporation into bacterial protein. This mechanism should be associated with less absolute excretion of fecal ammonia, and with less urinary urea excretion, which was not observed either by Birkett et al (1) or by ourselves (Table 1). It would also imply that RS3 is more fermentable than RS2, for which there is no evidence (5–7).

This study shows that RS3, but not RS2, significantly lowers fecal ammonia concentrations in healthy men, which might be advantageous in the protection against colon cancer. In further research on RS in health and disease, discrimination between the various types of RS would appear to be relevant.

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REFERENCES

Reply to M-LA Heijnen et al

Dear Sir:

Thank you for the opportunity to respond to the comments of Heijnen et al.

The study by Heijnen et al (1) confirms our observation that resistant starch (RS) lowers fecal concentrations of ammonia in humans. Their study also shows that this effect was specific to RS3 (from extruded, retrograded high-amylose corn starch) and not RS2 (from uncooked high-amylose corn starch). On the basis of this observation, Heijnen et al suggest that the results obtained in our study may also be due to the RS3 component of our mixed-RS experimental diet.

We agree that it is possible that our results may be due, in part at least, to the RS3 fraction of our mixed-RS experimental diet. Note, however, that our high-RS diet also contained RS1 from uncooked, coarsely milled wheat seeds. It is possible that this form of RS may also be effective at lowering fecal concentrations of ammonia. This form of RS was not tested in the study of Heijnen et al. We believe that forms of RS that will be effective at producing beneficial changes in the colon will be those types that are fermented slowly along the entire length of the colon. The results of Heijnen et al support this idea. Therefore, it is possible that RS2 could be effective if it is slowly fermented, for example, as in physically trapped intact granules found in partly milled grains.

Both studies found that RS significantly lowered the fecal concentration of ammonia but not net excretion over the day. Neither showed significant changes in total urea excretion. Heijnen et al suggest that the mechanism by which RS lowers fecal concentration of ammonia is due to dilution, as a result of the increased fecal bulk, and not redirection of the ammonia into bacterial protein synthesis.

It is difficult to make conclusive statements about the mechanism by which RS lowers ammonia concentration from the results of these two studies alone. The luminal environment is very complex with rapid changes in production, absorption, and utilization of the many metabolites generated. We maintain that although dilution is an important factor, our data are not inconsistent with the possibility that some ammonia was incorporated into bacterial protein during the high-RS diet. For example, we showed that the high-RS diet lowered fecal pH.