Cereal Alkylresorcinols Are Absorbed by Humans

ABSTRACT Currently there is no biomarker to link consumption of whole grain cereals and their observed health benefits. A candidate for a biomarker of whole grain wheat and rye intake is a class of phenolic lipids, the alkylresorcinols (AR). Studies to determine the uptake of AR in humans were carried out with a low fiber diet based on white wheat bread (AR free) and a high fiber diet based on rye bran–enriched bread (AR rich). For each diet, two meal frequencies were used: nibbling (7 small meals/d) and ordinary (3 large meals/d). Ten human ileostomy-operated subjects started with the AR-free diet for 2 wk, wk 1 on either nibbling or ordinary and wk 2 on the other meal frequency in a crossover design, followed by a 1-wk washout period, before the AR-rich diet performed as the AR-free diet. Food and ileostomy samples were analyzed for AR. Approximately 40% of AR were recovered in effluent from the small intestine, indicating that 60% of AR are taken up from or converted in the small intestine (ileal digestibility) with no difference between nibbling and ordinary meal frequencies. AR absorbed by humans may be of importance as bioactive compounds, or as a biomarker of whole grain wheat and rye intake. J. Nutr. 133: 2222–2224, 2003.

KEY WORDS: alkylresorcinols • biomarker • human ileostomists • rye bread • whole grain cereals

Many epidemiological studies have linked the consumption of diets rich in whole grain cereals to a decreased incidence of several degenerative “Western” diseases (1–3). The FDA has approved health claims stating that whole grain cereals may protect against coronary heart disease and some cancers (4). A direct link between consumption of whole grain cereals and the proposed health effects has not been established, in part because of the lack of a suitable biomarker of whole grain cereal intake (5).

Whole grain cereals are high in dietary fiber, vitamins and minerals, and many phenolic antioxidants thought to be beneficial to health (6). These are largely lost on removing the outer layers of cereals when milling (7). Alkylresorcinols (AR) are phenolic lipids located in the outer part of some whole grain cereals, and found in particularly high amounts in wheat, triticale and rye grains (8). AR are 1,3-dihydroxybenzene compounds with an alkyl chain substituted at position 5 of the phenolic ring. Nomenclature refers to the length of the carbon chain; for example, nonadecylresorcinol, an AR with a saturated 19-carbon chain, is referred to as C19:0. Concentrations in whole grain rye and wheat have been found to vary between 360 and 3200 µg/g and 317 and 1429 µg/g, respectively (9 and references therein). AR are also found in some other plants, bacteria and fungi (10), although not in significant levels in any food plants other than wheat and rye (9).

Unsaturated and oxygenated analogs of AR have also been reported in rye (11), although AR are the predominant phenolic lipids in rye (~80–85%) and wheat (~95%) (9,12). Processing, such as baking or heating does not appear to affect the levels of AR in food products (9). A person eating a diet rich in whole grain wheat and rye is estimated to have an intake of 100–200 mg/d (Ross, A. B. et al., unpublished results).

AR are reported to be antioxidants (10), although their activity is negligible in in vitro model systems when compared with α-tocopherol (13). AR have also been shown to have anticancer activity, to modify the structure and function of phospholipid membranes in vitro and to inhibit some enzymes, as comprehensively reviewed by Kozubek and Tyman (10). Despite extensive research on the biochemical effects of AR, their uptake and metabolism in animals and humans are essentially unknown (10). AR have been proposed as antinutritional factors in rye (14), although rat studies are inconclusive (15,16). The soluble fiber fraction of rye is now considered to cause the antinutritional effect originally attributed to AR (17).

Ileal digestibility of AR in pigs was found to be between 60 and 79%, and rats fed radiolabeled AR excreted ~34% of the radioactivity in the urine, showing that significant amounts of AR had been absorbed and metabolized (Ross, A. B. et al., unpublished results). AR were also found in intact form in human plasma (18), indicating that they are absorbed, although to what extent is not known. In this study, we used an ileostomy model to demonstrate that AR disappear from the small intestine of humans, suggesting that AR are absorbed.
SUBJECTS AND METHODS

Subjects. Ten subjects volunteered to participate in the study (2 females, age 34 and 51 y; 8 males, mean age 54.4 y, range 43–65 y). All subjects had undergone proctocolectomy 8–29 y earlier as a result of ulcerative colitis and had conventional ileostomies that functioned well with no sign of inflammation. All subjects were in good general health on the basis of physical examination and blood tests before the experiment. The study was approved by the Ethical Committee of the Umeå University Hospital. For further details regarding the subjects, diets and study design see Lundin et al. (19).

Study design and diets. The subjects were studied for a total of 5 wk (consecutive) on an outpatient basis. Subjects were randomly arranged into two experimental groups, with five in each group. Two crossover studies were carried out, with two diets, and two meal frequencies (nibbling and ordinary) per diet. All subjects ate both diets, with both meal frequencies. Both of the experimental groups started a low fiber diet (LFD, AR free) for 2 wk in which one group was given the nibbling meal frequency for 1 wk and the other group the ordinary meal frequency for 1 wk. In the following week, those on the nibbling meal frequency switched to the ordinary meal frequency and vice versa. After 2 wk, a 1-wk washout period was used when the subjects ate their ordinary diet. This was followed by a high fiber diet (HFD, AR rich) period for 2 wk with the same crossover design of ordinary and nibbling meal frequencies as for the LFD. During the nibbling meal frequency, the subjects ate seven identical meals/d, whereas during the ordinary meal frequency they consumed the same diet divided into three meals/d; one seventh of the daily diet for breakfast, two sevenths for lunch and four sevenths for dinner. Based on a 3-d dietary recall according to the recommendation of FAO/WHO/UNU (20), the subjects were arranged in three energy levels corresponding to 1.0, 1.25 or 1.5 portions of the diets.

During the LFD period a 1.0 energy level dietary portion of the breads consisted of white wheat flour soft bread (142.8 g/24 h) and white wheat flour crispbread (Wasavete, Wasabrod AB, Filipstad, Sweden; 92.4 g/24 h), whereas during the HFD period, the breads consisted of rye bran-enriched soft bread (180.6 g/24 h) and whole grain rye crisp bread (Husman, Wasabrod AB, 91.0 g/24 h). One piece of wheat flour soft bread contained 37 g of wheat flour, 4 g margarine, 4 g sugar, 4 g low fat milk powder and 2 g yeast. One piece of rye bran soft bread contained 15 g wheat flour, 18 g rye bran, 3 g margarine, 3 g sugar, 2 g low fat milk powder and 2 g yeast. The amount of dietary fiber per piece of bread was 1.5 g in white wheat flour–based soft bread (61.1 g), 0.5 g in Wasavete (13.2 g), 5.4 g in rye bran–based soft bread (51.5 g) and 1.7 g dietary fiber in Husman (13.0 g). The rye breads used in the study were the only source of AR in the diet. The Swedish Food Tables (21) were used to calculate energy and nutrient contents of the diets.

On d 3 in each dietary period, 24-h ileal effluents were collected. On that day the subjects were admitted to the research ward and stayed in a nearby hotel overnight. The ileostomy efflux bags were changed every 2 h from 0700 to 2100 h on the sampling day and at 0700 h on the next day (19). The bags were immediately frozen, and the effluents were freeze-dried to constant weight, mixed, homogenized and stored at −70°C until analysis. Two portions of the bread were collected on the sampling day and treated the same way as the ileostomy effluents.

Analysis. Before analysis, samples were ground in a Cyclotec sample mill (Tecator AB, Höganäs, Sweden) to pass a 0.5-mm screen. Dry matter content was determined by oven drying at 105°C for 16 h. Bread samples were analyzed for AR according to Ross et al. (9). Full-recovery AR in ileostomy samples was possible by use of the same extraction method as for whole grain rye (12), the complexes formed during baking being broken down in the gastrointestinal tract. Quantification of total and individual AR homologs was performed by gas chromatography (12). The presence of AR in rye ileostomy extracts was confirmed by use of gas chromatography–mass spectrometry according to Ross and colleagues (Ross, A. B. et al., unpublished results) (Fig. 1). Results are reported on a dry matter basis and as an average of triplicate analyses (any sample with a CV > 5% was repeated).

Ileal disappearance of AR between the two meal frequencies was compared by use of the general linear model; differences between uptake of AR homologs and uptake attributed to intake were assessed by one-way ANOVA adjusted with Tukey’s pairwise comparisons. Differences were considered significant at P < 0.05. Statistical calculations were performed by use of Minitab statistical software (version 11) for Windows.

RESULTS

AR were not detected in the food samples, except for the two breads containing rye consumed as part of the HFD. Similar, AR were recovered only in the ileostomy samples from the HFD period (AR rich), and not detected in the ileostomy effluents of subjects when eating the LFD (AR free) (Fig. 1). One portion of the rye breads (180 g dry matter) contained 147 mg of AR. The concentration of AR in the ileal excreta of a person eating one portion of HFD was ~600 μg AR/g dry matter.

The total recoveries of AR from the small intestine during the HFD dietary period were 42%, and 43% for the nibbling and ordinary dietary meal frequencies, respectively, indicating an ileal digestibility of ~60% (Table 1). In this study, no difference in ileal disappearance of AR was observed between subjects eating 1 portion (147 mg AR/d) or 1.5 portions of experimental diet (220 mg AR/d). There was large individual variation for ileal digestibility (range 45–71%).

The relative distribution of the different AR homologs in the ileal excreta was similar for both the nibbling and ordinary meal frequencies. Shorter-chain homologs C17:0, C19:0 and C21:0 disappeared to the same extent (~59% ileal digestibility), but the ileal digestibility of C23:0 and C25:0 was signif-
acids (23), and this may also be the case for AR. Fatty acids being taken up at a greater rate than long-chain fatty acids is consistent with the observation that there was a slight difference in the uptake of nutrients across different meal frequencies (Ross, A. B. et al., unpublished results; 22). In this study, meal frequency had no apparent impact on AR absorption, with decreased uptake at higher levels of meal frequency (Table 1).

The amount of dietary AR has been suggested to affect the postileal stage and/or transformed by the intestinal microbiota. Consequently, the possibility that AR were partially broken down by bacteria in the ileostomy bags, although this is strongly warranted in further research to investigate their metabolism, biokinetics, biological activities and use as biomarkers of whole grain wheat and rye intake.

Because AR disappeared from the upper part of the gastrointestinal tract, it can be assumed that they were absorbed or perhaps transformed. AR have been detected unchanged in the intestinal tract, it can be assumed that they were absorbed or perhaps transformed. AR have been detected unchanged in the intestinal tract, it can be assumed that they were absorbed or perhaps transformed.

### Table 1

<table>
<thead>
<tr>
<th>AR homolog</th>
<th>Intake</th>
<th>Nibbling</th>
<th>Ordinary</th>
</tr>
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<tbody>
<tr>
<td>mg/d</td>
<td>% ileal digestibility</td>
<td></td>
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</tr>
<tr>
<td>17:0</td>
<td>38</td>
<td>59 ± 7.1 a,b</td>
<td>58 ± 9.3 a</td>
</tr>
<tr>
<td>19:0</td>
<td>51</td>
<td>60 ± 7.3 a,b</td>
<td>59 ± 9.5 a</td>
</tr>
<tr>
<td>21:0</td>
<td>34</td>
<td>60 ± 6.3 b</td>
<td>60 ± 8.1 a</td>
</tr>
<tr>
<td>23:0</td>
<td>12</td>
<td>50 ± 7.0 a,c</td>
<td>49 ± 8.7 b</td>
</tr>
<tr>
<td>25:0</td>
<td>10</td>
<td>44 ± 8.4 c</td>
<td>48 ± 11.0 b</td>
</tr>
<tr>
<td>Total</td>
<td>147</td>
<td>58 ± 6.7</td>
<td>57 ± 8.7</td>
</tr>
</tbody>
</table>

1 Values are means of three determinations of each sample ± SD, CV < 5%. Means in the same column without common letters differ, P < 0.05.
2 For details of diets and meal frequency see Materials and Methods.

**DISCUSSION**

Because AR disappeared from the upper part of the gastrointestinal tract, it can be assumed that they were absorbed or perhaps transformed. AR have been detected unchanged in the intestinal tract, it can be assumed that they were absorbed or perhaps transformed.

The amount of dietary AR has been suggested to affect the postileal stage and/or transformed by the intestinal microbiota. Consequently, the possibility that AR were partially broken down by bacteria in the ileostomy bags, although this is strongly warranted in further research to investigate their metabolism, biokinetics, biological activities and use as biomarkers of whole grain wheat and rye intake.

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