

Short Communication

Presence of alkylresorcinols, potential whole grain biomarkers, in human adipose tissue

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Alkylresorcinols (AR) in plasma samples have been suggested to be short- to medium-term biomarkers of whole grain wheat and rye intake. In the present study, we investigated whether AR are present in human adipose tissues, and if content correlated with long-term whole grain bread intake. Furthermore, we investigated if the relative AR homologue composition reflected what has been found previously in the habitual diet of Swedes. Biopsy samples (10–25 mg) from free-living Swedish women (n 20) were analysed by GC–MS. The mean total AR concentration in the samples was 0.54 (SD 0.35) $\mu\text{g/g}$, ranging from below limit of quantification ($<0.08 \mu\text{g/g}$) to 1.50 $\mu\text{g/g}$. Whole grain bread intake was significantly correlated with plasma total AR content (r 0.48, $P < 0.05$), and the C17:0/C21:0 ratio was 0.35 (SD 0.24), which is similar to what is found in plasma among free-living subjects consuming a mixed whole grain wheat and rye diet. These results suggest that AR in the adipose tissue should be evaluated as a long-term biomarker of whole grain wheat and rye intake.

Alkylresorcinols: Biomarker: Long term: Whole grain: Adipose tissue

Whole grain intake has consistently been associated with reduced risk of CHD and type 2 diabetes in prospective cohort studies^(1,2). Results from these studies rely on self-reported intake, usually derived from FFQ, which may be subjected to considerable random and systematic measurement errors inherent to subjects' memory and motivation, use of different whole grain definitions and the limited number of questions available for the assessment of whole grain intake^(3,4).

Alkylresorcinols (AR), a group of phenolic lipids (1,3-dihydroxy-5-alkylbenzene derivatives with odd-numbered hydrocarbon chains, usually C17:0–C25:0), have been suggested as biomarkers of whole grain intake of wheat and rye, partly because they are only present in the outer parts of these cereals and essentially not in white flour or in other commonly consumed foods⁽⁴⁾. AR are absorbed from the intestine (40–60%) via the lymphatic system and transported in association with lipoproteins and erythrocyte membranes in the blood⁽⁵⁾. Plasma AR concentration has been shown to increase proportionally with whole grain intake of wheat and rye^(5–7). In cereals, the relative AR homologue composition

and the C17:0/C21:0 ratio are relatively consistent within species with a ratio of approximately 0.1 in wheat and approximately 1.0 in rye (Fig. 1(a))⁽⁸⁾. Plasma relative AR homologue composition and the C17:0/C21:0 ratio have been shown to roughly reflect those of wheat and rye under whole grain intervention conditions (Fig. 1(b)), although other factors seem to affect this ratio as well⁽⁹⁾.

Due to a rather short apparent elimination half-life (approximately 5 h), plasma AR may serve as short- to medium-term biomarkers of whole grain wheat and rye intake^(7,9). However, in epidemiological studies, intake during the aetiologically relevant time period is of interest, and for many diseases, this period is extended over a long period of time (years)⁽¹⁰⁾. Compounds that accumulate in adipose tissue, e.g. fatty acids and carotenoids, have been used as long-term biomarkers due to the slow turnover of this tissue^(11,12). Ross *et al.*⁽¹³⁾ showed that AR were present in adipose tissue of rats after feeding them diets rich in AR purified from rye for 4 weeks, but no such finding in human subjects has been reported before.

In the present study, we estimated AR content and relative homologue composition in adipose tissue biopsy samples from

Abbreviation: AR, alkylresorcinols.

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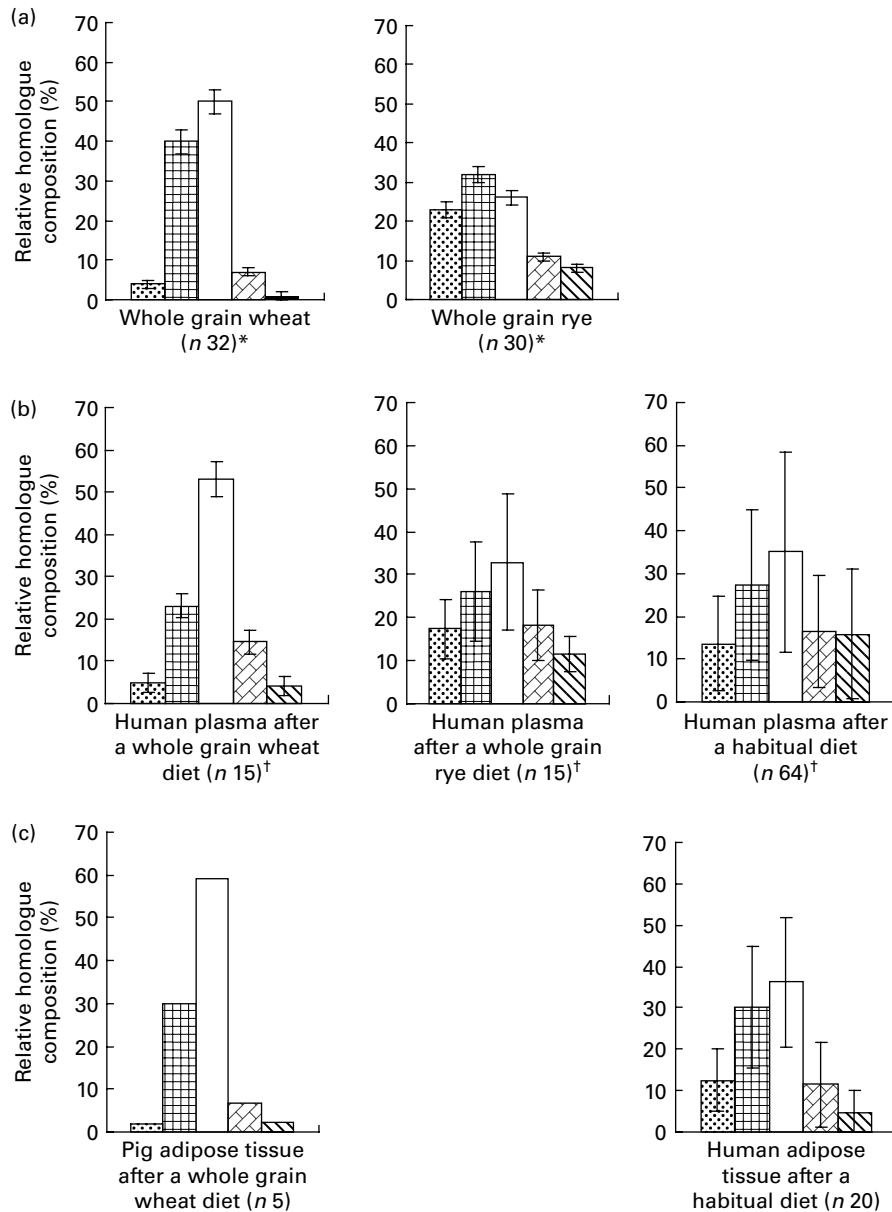


Fig. 1. Average relative alkylresorcinol homologue composition in (a) whole grain wheat and rye ^{*}(adopted from Chen *et al.*⁽⁶⁾), (b) human fasting plasma after 6 weeks on a whole grain wheat diet and 6 weeks on whole grain rye diet [†](adopted from Linko-Parvinen *et al.*⁽⁵⁾) and fasting plasma samples from free-living Swedish subjects consuming their habitual diet [†](adopted from Landberg *et al.*^(6,7,17)) and (c) pooled adipose tissue sample from pigs fed a whole grain wheat diet and human adipose tissue biopsy samples from free-living women. Bars denote standard deviation. □, C17:0; ▤, C19:0; □, C21:0; ▥, C23:0; ▦, C25:0.

free-living human subjects and in a pooled adipose tissue sample from pigs fed a constant whole grain wheat diet.

Methods

Adipose tissue biopsies from free-living women ($n = 20$) in the Swedish Mammography Cohort were analysed for AR concentration and homologue composition. The cohort has been described elsewhere⁽¹⁴⁾. Total daily whole grain bread intake (whole grain soft bread + dark crisp bread) was calculated from an FFQ with 123 food items using standard portion sizes⁽¹⁵⁾. Subcutaneous adipose tissue aspirate samples were taken from the outer upper quadrant of buttock using a needle, and a vacuum tube was used for blood samples. The samples

(approximately 10–50 mg) were left in the needle, and stored in a freezer at -70°C until analysis.

A sample (10–25 mg) was placed in a glass vial (15 ml) with a screw cap together with diethyl ether (2 ml) and internal standard (AR, C20:0, 15.75 ng in 75 μl methanol; ReseaChem LifeScience, Burgdorf, Switzerland). The sample was homogenised using a Heidolph DiAx 600 homogeniser (Heidolph, Kelheim, Germany) fitted to a dispersion head type 6 G. The homogenizer knife was rinsed with diethyl ether (1 ml) which was combined with the sample extract. The sample was ultrasonicated (20 min) and centrifuged (483 g for 10 min), and the clear supernatant was transferred to a 15 ml disposable test tube and evaporated to dryness under a gentle stream of N_2 . Sample extract was redissolved in methanol (1 ml) and

purified on Oasis-MAX[®] 3 cc/60 mg solid-phase extraction cartridges (Waters Corporation, Milford, MA, USA), silylated and analysed by GC-MS (Thermo Fisher Scientific, Waltham, MA, USA) as described elsewhere⁽¹⁶⁾. 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 Regional Ethical Review Board at Karolinska Institute (Stockholm, Sweden, Dnr KI 02-472). Written informed consent was obtained from all subjects.

For the evaluation of method precision and for the assessment of the reflection of relative AR homologue composition after whole grain wheat intake, adipose tissues taken from the shoulder in the split carcass from five individual pigs (Swedish Yorkshire dams × Swedish Yorkshire sires, 115 kg at slaughter) were pooled and used. Pigs of a live weight of 25 kg were fed *ad libitum* a diet rich in whole grain wheat until they gained a weight of 60 kg (corresponding to an age of 120 d). Thereafter, they were restrictively fed 3 kg/d in total twice a day until slaughter at an age of 170 d. The within-day variability assessed as the CV% (n 5) was 9.1% for total AR in the pooled pig sample. Relatively large variations were observed for C17:0, C23:0 and C25:0 due to concentrations near the limit of detection of these three homologues. The accuracy (analysed amount/added amount) × 100 and recovery (peak area of analyte after going through the entire method/peak area of analyte after direct injection for GC-MS) were determined for reference standards, without sample matrix, and were found to be 101 (SD 8) and 85 (SD 25)%, respectively. Total AR limit of quantification was estimated as the sum of concentrations from individual homologues showing signal-to-noise ratios of 5:1 compared with baseline. The animal study was carried out in accordance with the guidelines and approval of the Ethical Committee for Animal Experiments in the Uppsala region (Uppsala, Sweden). Pearson's correlation coefficient was used to investigate the association between whole grain bread intake and AR content in adipose tissue since no departure from normality was found (Shapiro-Wilk test, $P=0.11$).

Results and discussion

In adipose tissue biopsy samples from free-living Swedish women, total AR was on average 0.54 (SD 0.35) $\mu\text{g/g}$ tissue, and it showed a large variation between individuals (below limit of quantification ($<0.08 \mu\text{g/g}$) to 1.50 $\mu\text{g/g}$). A large variation was expected and probably reflected by differences in intake but also in bioavailability, pool size and/or other determinants. AR content was in the same range as that found for carotenoids in free-living subjects from nine countries (concentrations were typically in the range 0.04–1.4 $\mu\text{g/g}$ fatty acids, depending on specific carotenoid and country)⁽¹²⁾. Total daily whole grain bread intake (whole grain soft bread + dark crisp bread) estimated from an FFQ that reflected the intake during the past 6 months was significantly correlated with adipose tissue total AR content (r 0.48, $P<0.05$). This suggests that adipose tissue AR may be used as long-term biomarkers of whole grain intake of wheat and rye (Fig. 2). The relative AR homologue compositions in human adipose tissue biopsies were on average 12.4% (C17:0), 30.0% (C19:0), 36.0% (C21:0), 11.9% (C23:0) and 4.7%

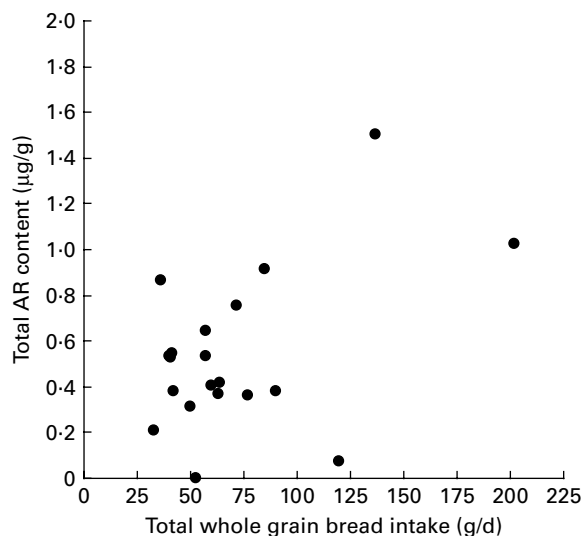


Fig. 2. Scatter plot of total whole grain bread intake (whole grain soft bread + dark crisp bread) estimated from an FFQ and total alkylresorcinol (AR) content in human adipose tissue biopsies. Pearson's r 0.48 ($P<0.05$), n 20. Limit of quantification $<0.08 \mu\text{g/g}$.

(C25:0), and the C17:0/C21:0 ratio was 0.35 (SD 0.24) (Fig. 1). The variation was high for homologues C23:0 and C25:0, probably due to measurement error caused by very low concentrations near the limit of quantification. However, the results are comparable to the average relative homologue composition in fasting baseline plasma samples from sixty-four Swedish subjects participating in whole grain intervention studies (Fig. 1)^(6,7,17).

The total AR content in the pooled adipose tissue from pigs fed a whole grain wheat diet was 1.50 (SD 0.22) $\mu\text{g/g}$. The levels were about half of what Ross *et al.*⁽¹³⁾ semi-quantitatively estimated in rats fed a diet rich in AR for 4 weeks (approximately 2–4 $\mu\text{g/g}$). The relative AR homologue composition in the pooled pig adipose tissue sample was 1.9% (C17:0), 29.9% (C19:0), 59.0% (C21:0), 6.8% (C23:0) and 2.4% (C25:0), and the C17:0/C21:0 ratio was 0.03 (Fig. 1(c)). This profile is similar to that of plasma samples from human subjects fed a diet rich in whole grain wheat⁽⁶⁾, and reflects that of whole grain wheat but with a somewhat lower relative abundance of shorter AR homologues present in adipose tissue (C17:0 and C19:0; Fig. 1). These differences might be due to more rapid metabolism for shorter AR homologues than for the longer ones as described by Landberg *et al.*⁽⁹⁾, less affinity of shorter homologues to be incorporated into adipose tissue and/or differences in analytical results derived from different laboratories and sample matrices.

In summary, we have shown that AR can be quantified in human adipose tissue biopsy samples, that intake of whole grain bread is correlated with the adipose tissue content and that relative AR homologue composition roughly reflects what has been found previously in plasma and diet of free-living Swedes. Future controlled feeding studies as well as population-based studies are needed to determine whether AR in adipose tissue of human subjects may be a useful long-term biomarker of whole grain wheat and rye intake.

In order to facilitate such investigations, the method used may need some modification to allow high sample throughput.

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