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Biomarkers of Whole Grain Intake

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ABSTRACT SUMMARY

Biomarkers of dietary intake have been long proposed as alternatives or complementary to questionnaire based forms of dietary assessment. In the case of a food category as diverse as whole grains the application of biomarkers may increase the robustness of estimations of whole grain intake.

Currently, alkylresorcinols—phenolic lipids present in the outer layers of wheat, rye, and barley—are promising biomarkers of whole grain cereal intake, meeting most of the suggested criteria for an intake biomarker. Initial studies demonstrate that plasma alkylresorcinols and their metabolites are highly responsive to alkylresorcinol intake and that they reflect whole grain intake. Further studies are required to test their application in observational cohorts and as biomarkers of compliance in intervention studies.

There is a need for more biomarkers to cover the other major food cereals such as oats, corn, and rice, and new technological developments will be integral in enabling this.

Introduction

While greater intake of whole grain foods is consistently associated with reduced risk of many diet-related diseases in epidemiological studies, uncertainty remains around how accurate questionnaire-based estimates of whole grain intake are. This uncertainty stems from the diversity of cereal grains, the rapidly expanding range of different products that may include whole grains, and the difficulty in determining what proportion of a product is whole grain. The use of biomarkers of whole grain intake may be an alternative or complement to traditional methods of assessing whole grain intake. They may also be an additional method for assessing compliance during intervention studies or estimating whole grain intake in subjects where no dietary records exist (e.g., biobank samples). Currently the most promising biomarkers of whole grain intake are the alkylresorcinols and their metabolites (9).

Alkylresorcinols

Alkylresorcinols are phenolic lipids present in the outer layers of wheat, rye, and barley, but not in the consumed parts of other food plants (with the exception of minor amounts in mango flesh). They are 1,3-dihydroxy-5-alkyl-benzene derivatives, with the alkyl-chain mainly ranging from C17 to C25 in wheat, rye, and barley. The ratio of these different homologues is specific to each cereal type and also differs between common and durum wheat. This is indicated using the ratio of homologues C17:0 and C21:0, with 0.01 for durum wheat, 0.1 for common wheat, and 1 for rye (the rye x wheat hybrid triticale has a ratio of 0.3–0.4) (2). This ratio is reflected to some extent when measuring plasma alkylresorcinols (7).

The amount of alkylresorcinols in white wheat flour is generally between 10–40 μg/g, with increasing amounts depending on the extraction rate. Whole grain common wheat flour generally ranges from 300–700 μg/g, while whole grain rye generally ranges from 500–1000 μg/g and whole grain barley between 40–120 μg/g (13, 14).

The estimated mean intake of alkylresorcinols is around 12 mg/d in a non-whole grain culture country (United Kingdom; where over 50% of the population had an intake <3 mg/d) and 23 mg/d in a whole grain culture country (Sweden) (11). While some *in vitro* studies ascribe potential bioactivities to alkylresorcinols, effective doses are high and it is unlikely that intakes are sufficiently high to make them important bioactive compounds in the general population (9).

Alkylresorcinols are readily absorbed from the small intestine, are transported in lipoproteins in plasma, incorporate into erythrocytes, and appear to be stored in adipose tissue as for other lipid soluble phytochemicals. They have a short-medium plasma $T_{1/2}$ of 5–8 h, with maximum concentrations at 6–7 hr after a meal. The percentage absorption appears to decrease with increasing doses of alkylresorcinols (4, 6). After absorption they can be metabolized by cytochrome P450 metabolism, mainly to 3,5-dihydroxybenzoic acid and 3,5-dihydroxyphenylpropianoic acid, with these metabolites having a longer plasma $T_{1/2}$ and representing an alternative to using intact alkylresorcinols as biomarkers (10).

To date, a relatively large number of intervention and observational trials have looked at the relationship between alkylresorcinol intake and plasma alkylresorcinols, and the correlation between mean intake and mean response is highly linear ($r^2 \approx 0.8$ over a wide range of intakes). It is notable, however, that interindividual variation is wide, with individuals having overlapping plasma alkylresorcinol concentrations possible across a relatively wide range of intakes. This indicates that alkylresorcinols are probably best suited for population-based studies while individual results in isolation need to be interpreted in the context of known intake. Similar trends are observed for alkylresorcinol metabolites, though not enough studies have been performed at ranges of intakes likely in a "normal" population (9).

An important criterion for the use of biomarkers of intake in

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observational studies is their long-term reproducibility. This can be determined using the intraclass correlation coefficient (ICC), and several recent studies indicate that this is highly dependent on study type (intervention vs. free-living), dose (high vs. low-moderate), dietary assessment method (weighbacks, diary, or questionnaire), and gender. Intervention studies with high doses of alkylresorcinols lead to excellent ICC values (0.8–0.9), while free-living studies lead to moderate mean ICC values (0.42–0.48), with females generally having higher ICC values than males (1, 5, 8, 12).

A few recent studies have used alkylresorcinols as biomarkers of compliance in large intervention studies (3, 12). While individual plasma alkylresorcinol concentrations may vary widely, there is now sufficient data to allow conservative thresholds to be used to identify subjects that have almost certainly not been compliant. However such an approach still needs further validation and should be interpreted with caution in conjunction with dietary intake data if available.

Other Potential Whole Grain Biomarkers

While alkylresorcinols are potentially good biomarkers of whole grain intake, they are only really applicable in populations where wheat and rye are commonly consumed. While wheat is common in large parts of the world, other cereals, notably rice, corn, and oats, are important common sources of whole grain in the diet. Presently there are no other candidate biomarkers of whole grain intake, and there is a need to cover these cereals in order to improve estimation of whole grain intake across the globe.

Ideally a biomarker should be unique to a particular cereal or group of cereals. Oats contain a group of unique polyphenols, the avenanthramides. These are implicated in anti-inflammatory processes and could be an excellent combination of a biomarker of intake and of biochemical benefit/exposure. However their concentrations in oats is low (50–200 μg/g) and clearance too rapid ($T_{1/2}$ 3 hr) for them to be effective biomarkers with current methods. Rice contains γ-oryzanols, a group of ferulic acid–phytosterol conjugates concentrated in the outer layers of rice at relatively high concentrations (100–700 μg/g). However recent research confirms that the γ-oryzanols are almost all cleaved to ferulic acid and phytosterol in *in vitro* models, both being found commonly in other plant foods.

Conclusion

Alkylresorcinols are currently the best candidate for biomarkers of whole grain intake and appear to perform well relative to biomarkers of intake proposed for other food groups. New research should focus on the application of alkylresorcinols and their metabolites in observational and intervention studies, as well as determining factors responsible for interindividual variation. New technologies and methods will be invaluable for the detection of biomarkers of other cereals.

References

1. Andersson A, Marklund M, Diana M, Landberg R. Plasma alkylresorcinol concentrations correlate with whole grain wheat and rye intake and show moderate reproducability over a 2- to 3-month period in free-living Swedish adults. Journal of Nutrition 2011;141:1712-8.
2. Chen Y, Ross AB, Åman P, Kamal-Eldin A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. J Agric Food Chem 2004;52:8242-6.
3. Kristensen M, Toubro S, Jensen MG, Ross AB, Riboldi G, Petronio M, Bügel S, Tetens I, Astrup A. Whole grain compared to refined wheat decreases the percentage body fat following a 12-week energy restricted dietary intervention in postmenopausal women. Journal of Nutrition 2012;142:710-6.
4. Landberg R, Åman P, Friberg LE, Vessby B, Adlercreutz H, Kamal-Eldin A. Dose response of whole-grain biomarkers: alkylresorcinols in human plasma and their metabolites in urine in relation to intake. Am J Clin Nutr 2009;89:290-6.
5. Landberg R, Kamal-Eldin A, Andersson SO, Johansson JE, Zhang JX, Hallmans G, Åman P. Reproducibility of plasma alkylresorcinols during a 6-week rye intervention study in men with prostate cancer. J Nutr 2009;139:975-80.
6. Landberg R, Linko AM, Kamal-Eldin A, Vessby B, Adlercreutz H, Åman P. Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. J Nutr 2006;136:2760-5.
7. Linko-Parvinen AM, Landberg R, Tikkanen MJ, Adlercreutz H, Penalvo JL. Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. J Nutr 2007;137:1137-42.
8. Montonen J, Landberg R, Kamal-Eldin A, Åman P, Knueppel S, Boeing H, Pischon T. Reliability of fasting plasma alkylresorcinol concentrations measured 4 months apart. European Journal of Clinical Nutrition 2010;64:698-703.
9. Ross AB. Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake. Journal of Nutrition and Metabolism 2012;2012:1-12.
10. Ross AB, Åman P, Kamal-Eldin A. Identification of cereal alkylresorcinol metabolites in human urine-potential biomarkers of wholegrain wheat and rye intake. J Chromatogr B Analyt Technol Biomed Life Sci 2004;809:125-30.
11. Ross AB, Becker W, Chen Y, Kamal-Eldin A, Åman P. Intake of alkylresorcinols from wheat and rye in the United Kingdom and Sweden. Br J Nutr 2005;94:496-9.
12. Ross AB, Bourgeois A, Macharia HN, Kochhar S, Jebb SA, Brownlee IA, Seal CJ. Plasma alkylresorcinols as a biomarker of whole grain food consumption in a large population - results from the WHOLEheart intervention study. Am J Clin Nutr 2012;95:204-11.
13. Ross AB, Kochhar S. Rapid and sensitive analysis of alkylresorcinols from cereal grains and products using HPLC-Coularray-based electrochemical detection. J Agric Food Chem 2009;57:5187-93.
14. Ross AB, Shepherd MJ, Schupphaus M, Sinclair V, Alfaro B, Kamal-Eldin A, Åman P. Alkylresorcinols in cereals and cereal products. Journal of Agricultural and Food Chemistry 2003;51:4111-8.