A Mini Review on Biomarkers of Whole Grain Barley and Whole Grain Wheat Intake*

Tu Hu

Mar, 2019

Abstract

Due to lacking objective food exposure measurement, epidemiologic studies showed mixed results on whole grains' health beneficial effects. Meanwhile, increasing evidence showed each whole grain type could benefit health differently. In order to accurately quantify whole grain exposure, there is a demand to discover intake biomarkers for each whole grain species. This mini review referred the systematic biomarkers for food intake literature review guidelines and examined biomarkers for whole grain barley and whole grain wheat intake. For whole grain barley, there is no reported intake biomarker[1]. For whole grain wheat, the homologous ratio of alkylresorcinols C17:0/C21:0 was proposed as biomarkers to distinguish wheat and rye relative composition in the diet.

1 Introduction

Whole grains (WGs) and their processed food contain a lot of non-nutrient compounds in their bran parts. Besides providing carbonhydrates which mostly locate in grains' endosperm, WGs may have other health beneficial effects such as disease prevention. However, epidemiologic studies showed mixed results due to subjective self-report based food exposure measurement[1]. Using Biomarkers of Food Intake (BFIs) can potentially measure food exposure in population more objectively with accuracies and details[2].

Alkylresorcinols (ARs) and their metabolites were widely reported and validated biomarkers for WGs intake. In plants commonly consumed for food, ARs only present high amounts in rye and wheat, especially concentrated in their bran parts[3]. Therefore, ARs have the possibility to be used as biomarkers for whole grain wheat and rye intake.

Increasing evidence showed that, different WG cereal types (such as wheat, rye, oat, barley etc.) could benefit health differently. However, classical self-reported measurement tools used in observational studies could cause biases

*This is part of Tu Hu's master thesis work, supervised by Lars Ove Dragsted and Gözde Gürdeniz

[1]In my project work, I actually proposed some biomarkers

and confoundings to distinguish each cereal type. Therefore, discovering BFIs of each whole grain type could potentially provide a tool to accurately quantify their exposures.

This mini-review aimed at systematically examining available literatures to obtain information of potential biomarkers for WG barley and wheat intake. This will prioritize further identification and validation of the thesis work.

2 Materials and Methods

This review referred the systematic BFIRev methodology[4]. The flowchart was included in Appendix (Fig-1)

The objective of this literature review was to identify and evaluate reported potential biomarkers for dietary assessment for whole grain wheat and whole grain barley.

Keywords as suggested in the guidelines[4] were used to search in 3 database (PubMed, Web of Science, Scopus). Keywords used for searing BFI barley in human: (barley) AND (biomarker* OR marker* OR metabolite* OR biokinetics OR biotransformation OR pharmacokinetics) AND (intake OR meal OR diet OR ingestion OR consumption OR eating OR food) AND (human* OR men OR women OR patient* OR volunteer* OR participant*) AND (trail* or experiment OR study) AND (urine OR plasma OR blood OR serum OR excretion OR hair OR toenail OR faeces OR faecal water). The first element was changed to wheat for wheat intake biomarker searching.

Due to limited amount of searching results, barley searching scope was expanded to animal studies. Therefore, the keyword (animal* OR goat OR sheep OR cow OR mice OR mouse* OR animal model* OR dog*) was used to replace the previous 'human*' subjects. In addition, 'feed' was added to 'food' entry.

Other database including HMDB[5], FoodDB[6], PhenolExplorer[7], Dictionary of Food Compound[8] were also used to explore compounds present exclusively in WG barley and wheat.

In order to verify the uniqueness of compound, the same keywords combinations were used but with compound name instead of 'wheat' and 'barley'.

3 Results

3.1 WG barley

The literature search got 129 records after removing duplicate records from merged 3 database search results. However, within them, none of the studies directly investigated WG barley intake biomarkers. This could be explained by limited consumption of barley in population. Although barley is the 4th most produced cereal grains worldwidely. Most of them is used for brewing or feed. Approximately only 4% is directly consumed[9].

When the scope expanded to animal studies, the search results still did not show any direct research about BFIs. Most of animal studies were interested

in how barley feed can benefit the growth of animals or quality improvement of animal-source products[10, 11].

A 2-month intervention study[12] incorporated 75% refined drum wheat and 25% WG barley. The fecal samples showed significant change in microbiota and metabolome after intervention[12]. However, no specific metabolite can indicate WG barley intake.

ARs and their metabolites may not indicate WG barley intake. Several observation studies[13, 14] investigated correlation between ARs metabolites and whole grain intake. Although these studies tried to cover more whole grain species, for example, one study[14] listed 7 types of commonly consumed WGs in American populations in the Food Frequency Questionnaire (FFQ)[2], barley was not solely listed. Therefore, although ARs and their metabolites got good correlation with these 'Whole-grain intake'. Readers should be cautious to apply these markers to WG barley intake. In addition, ARs concentration in cereal barley is much lower compared with WG wheat and rye, with similar concentration with refined wheat and rye flours (Table-1).

Cereal	Conc. range in cereal	Conc. average or range in WG flour	Conc. average in refined flour	Main homologues	C17:C21 homologues ratio
Rye	360-3200	972	90	C17, C19, C21	0.8-0.9
Wheat	761-8390	490-710	36	C19, C21	0.07-0.1
Barley	55.8-98.2	NA	NA	C19, C21, C25	NA

Table 1: Prensence of ARs in Cereal Grains, adapted from [15–17](unit: µg/g dm), conc. varies due to different species and milling methods.

Most search results focused on barley's *effect biomarkers* as defined by Dragsted[18] and Gao[19], such as bowel health indicators[20], postprandial glucose and insulin response[21], lipid profiles and cardiovascular diseases (CVD) markers[22], etc. However, in these intervention studies, compliance monitoring lacked objective markers.

Further search results in food chemistry, cereal science and plant science showed some compounds exclusively present in barley. These could give hints for further identification. The results were summarized in Table-2.

To conclude, barley, especially WG barley attracted a lot of interest due to its health beneficial effects for chronic disease. However, due to barley's limited consumption in the population, currently there is no reported biomarkers can indicate its intake. A lot of sparse information was reported from cereal and food chemistry could give hints for identification and validations of WG barley's intake biomarkers.

[2]Dark breads, High-fiber or bran cereals, Cooked cereals and grits, Regular granola, Granola bars and cereal bars, Plain popcorn (no butter) or low-fat microwave popcorn, Buttered or gular microwave popcorn

No	Candidate biomarker	Formula	Chemical group	Presence in Food	Reference
1	Hordenine	$C_{10}H_{15}NO$	alkaloid	germinating barley, beer and other plants	[23]
4	Hordatine A	$C_{28}H_{38}N_8O_5$	alkaloid	only reported in barley	FoodDB (002330)
4	Hordatine B	$C_{29}H_{40}N_8O_5$	alkaloid	only reported in barley	FoodDB (002328)
2	Distichonic acid A	$C_{10}H_{18}N_2O_8$	gamma amino acids and derivatives	only reported in barley	FoodDB (18164)
3	Distichonic acid B	$C_{10}H_{18}N_2O_8$	gamma amino acids and derivatives	only reported in barley	FoodDB (018165)
5	14,16-Nona cosanedione	$C_{29}H_{56}O_2$	ketone	only reported in barley	FoodDB (013891)
6	N-Norgramine	$C_{10}H_{12}N_2$	indole	only reported in barley	FoodDB (017815)

Table 2: Candidate Biomarkers for WG barley intake

3.2 WG wheat

3.2.1 Overview

The literature search got 312 results after removing duplicate records from merged results. Some articles were found from the references of searched results. Two intervention studies and one observation study were included in the table.

Surprisingly few studies of wheat were reported because whole grain seems to be the most mature and hotspot area of BFIs research. In fact, within searched results, most intervention studies used WG diet containing several types of cereals as a comparison with refined diet, most commonly using WG rye and wheat and reporting ARs as intake biomarkers for WG rye and wheat. Very few intervention studies investigated biomarkers for different whole grains' intake.

In observational studies, food frequency questionnaires naturally cause confounding distingushing each sub-type cereal. because participants had difficulty recalling and distinguishing the different cereal species. Those non-specific markers were listed in appendix. These ambiguous studies were excluded. However, still surprising to us, few researches were studied to distinguish wheat from other cereal grains.

3.3 Alkylresorcinols, homologous ratio C17:0/C21:0 and AR metabolites

Alkylresorcinols present exclusively in bran part of wheat and rye within commonly consumed plant based food. Therefore, alkylresorcinols have the potential to indicate WG wheat and rye intake.

In rye rarely consumed countries, i.e. WG wheat is sole alkylresorcinol source, total alkylresorcinols and their metabolites got good correlations with WG wheat intake.

The AR homologous ratio C17:0/C21:0 was the major reported, validated

and applied biomarker for WG wheat intake.

This marker was first reported by cereal scientists in 2004 to distinguish WG rye and wheat cereal [24]. In cereal grains, rye has homologous C17:0/C21:0 ratio close to 1.0, while wheat around 0.1, durum wheat around 0.01.

Further this marker was proposed by nutritionists to be capable of distinguishing WG rye and wheat intake. In 2005, Linko[25] first investigated this biomarker in human plasma to measure food exposure. The intervention study showed the potential of this marker AR C17:0/C21:0 to distinguish WG wheat and rye in diet in healthy postmenopausal women. For rye-dominated diet, the ratio was 0.84 and for WG wheat-dominated diet, the ratio was around 0.53. Further in 2007, Linko-Parvinen validated this marker in healthy adults by an intervention study [26]. In plasma, the value was 0.1 after WG wheat intake, 0.6 after WG rye intake. In erythrocytes, the value was 0.06, 0.33 respectively after WG wheat and rye intake. This study also implied ARs could be transported in human plasma lipoproteins.

However, the AR homologues ratio C17:0/C21:0 was not significantly different between WG diet and refined cereal diet as reported by Landsberg[27]. But WG diet and refined diet can be distinguished by total ARs concentration in plasma.

An observational study further validated this marker. In 2014, EPIC[3] cohort study investigated plasma ARs and the C17:0/C21:0 ratio of subjects from 10 European countries. The result showed that Greek, Italian, Dutch and UK participants of whom the diet was dominated by wheat, had low C17:0/C21:0 ratio in plasma. Whereas Danish, German and Swedish subjects had high C17:0/C21:0 ratio. French and Norwegian subjects had intermediate ratio. This marker seems reversely correlated with WG wheat consumption in the population.

Dietary factor	No. subjects	Study design	Sample type	Analytical method	Candidate biomarker(s)	Reference
WG wheat WG rye	39	intervention, cross-over, randomized	plasma	GC-MS	ratio of AR C17:0/C21:0	[25]
WGs[4]	266	randomized, parallel-group, intervention	plasma	GC-MS	Total ARs	[28]

Table 3: Biomarkers of Wheat Intake Reported in Intervention study

In whole grain (WG) source dominated by wheat, total ARs got good correlation with wheat intake. e.g. UK and America.

Meanwhile, ARs metabolites could also be a potential marker, but may not be a good marker in mixed-ARs source countries since ARs metabolites were

[3]European Prospective Investigation into Cancer and Nutrition

[4]This study was conducted in UK. WG wheat is the main WG source in British population. Considering this, although several types of WGs were used (WG wheat, corn, oats, barley and rice), WG wheat made up around 65% of the intervention

not specific to WG wheat. ARs from WG rye could also be metabolized to same products causing confounding.

In this study[28], total ARs, rather than the ratio (C17:0/C21:0), were reported as WG wheat intake biomarkers. This intervention was conducted in UK. In British population, the major whole grain source is wheat. Rye was rarely consumed. Therefore, plasma ARs got a good correlation with whole grain (WG) wheat intake.

3.3.1 Applications in Type II diabetes research

Combining plasma total ARs concentration and the ratio (C17:0/C21:0) can subjectively estimate WG wheat and rye intake and approximate the composition.

Two studies already showed the power of BFIs for each sub-type cereal.

It was observed that,

The total ARs concentration was not correlated with type II diabetes risk.

higher C17:0/C21:0 ratio (increase of rye intake) is associated with increased insulin sensitivity in a population with metabolic syndrome.

This may imply that, a whole grain diet dominated by rye could be favourable for type II diabetes prevention.

In EPIC cohort study, an interesting phenomena could also imply XXX. Rye has higher constitutions in Danish populations's WG source (70%) than Swedish(55%) on average. However, regarding C17:0/C21:0 ratio, Danish participants in EPIC cohort showed lower value (0.37) than Swedish participants (0.43). However, Swedish participants were healthy adults, while participants from Denmark in EPIC study were obese or over-weights subjects. Those participants may have different dietary habits and consume less rye than average Danes. This may imply rye could also be favorable in weight control.

Type of WG	No. subjects	Sample type	Analytical method	Candidate biomarker(s)	Associated with	Reference
WGs[5]	104	spot urine	GC-MS	ARs metabolites (DHBA, DHPPA)	FFQ	[29]
WGs[6]	2845	fasting and non-fasting plasma	GC-MS	AR C17:0/C21:0	FFQ	[30]

Table 4: Biomarkers of Wheat Intake Reported in Observation study

Searching results also showed some *Food compound intake biomarkers (FCIBs)* research as defined by Gao[19] such as phenolic compounds[31], benzoxazinoids[32] and phytoestrogen[33]. These compounds also present in other food, not specific for WG wheat. These results were summarized in Appendix.

[5]This study was conducted in US. WG wheat is the major WG source in US populations. However, these two metabolites were not specific to WG wheat. Because other cereals containing ARs could also be metabolized to these metabolites.

[6]This cohort studies investigated WGs conc. in different EU countries' population.

Their concentrations varied in different cereal grains. Therefore, a combination of their metabolites could potentially indicate intake of different cereals.

to conclude, AR 17/21 seems promising

4 Conclusions

Currently, there's no biomarkers reported for WG barley intake both in human and animal studies.

Total ARs and their metabolites were reported to potentially indicate WG wheat and rye intake. The homologues ratio of ARs C17:0/C21:0 was proposed to distinguish which whole grain type dominates in the diet.

Several phytochemicals could potential become candidate markers of WG wheat and barley intake. However, they need to be further validated.

5 Discussions

In order to clarify each sub-type of cereal's health beneficial effects, it is important to accurately quantify exposure amount of each sub-type. BFIs showed their strengths and potentials in studying WGs.

it is essential to discover intake biomarker for each sub-type cereal grain. Currently, most studies showed interest in WG effect biomarkers.

As discussed in [1], one of the challenges in BFIs discovery of WG is that the chemical compositions of most of WGs were not systematically

due to limited systematic research on phytochemicals

6 Appendix

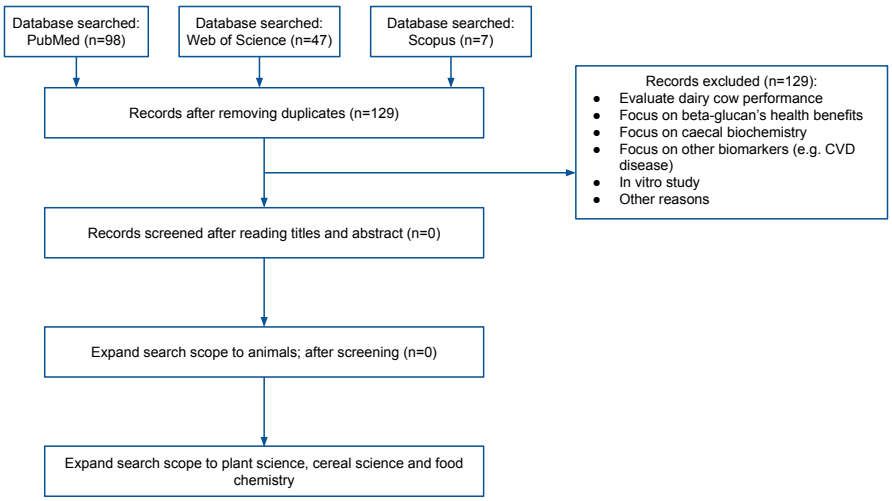

Figure 1: Flow chart of literature searching and screening for articles of barley intake biomarkers

Dietary factor	No. subjects	Study design	Sample type	Analytical method	Candidate biomarker(s)
Wheat bran, Wheat aleurone	14+13	randomized, cross-over, intervention	plasma	LC-MS/MS (Microbiology assay for folate)	betaine choline folate dimethylglycine (DMG)
None-bread, White bread, WG bread	155	observation[7]	urine	HPLC-qTOF-MS	Benzoxazinoid-related metabolites (HHPAA, HBOA glycoside) ARs-related metabolites(DHPPA glucuronide, DHPPTA sulphate), microbial

Table 5: Reported markers distinguishing WG wheat intake, but NOT specific

[7]dietary exposure measured from FFQ

References

1. Sang, S. Biomarkers of Whole Grain Intake. *JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY* **66,** 10347–10352. ISSN: 0021-8561 (Oct. 2018).
2. Scalbert, A. *et al.* The food metabolome: a window over dietary exposure. *The American Journal of Clinical Nutrition* **99,** 1286–1308. ISSN: 0002-9165. `https://academic.oup.com/ajcn/article/99/6/1286/4577352` (June 2014).
3. Ross, A. B., Kamal-Eldin, A. & Åman, P. *Dietary Alkylresorcinols: Absorption, Bioactivities, and Possible Use as Biomarkers of Whole-grain Wheat- and Rye-rich Foods* 2004.
4. Praticò, G. *et al.* Guidelines for Biomarker of Food Intake Reviews (BFIRev): How to conduct an extensive literature search for biomarker of food intake discovery. *Genes and Nutrition* **13.** ISSN: 18653499 (2018).
5. Wishart, D. S. *et al.* HMDB 4.0: the human metabolome database for 2018. eng. *Nucleic acids research* **46,** D608–D617. ISSN: 1362-4962 (Electronic) (Jan. 2018).
6. *FooDB* `http://foodb.ca/`.
7. Medina-Remón, A. *et al.* Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. *Database* **2013.** ISSN: 1758-0463. `https://dx.doi.org/10.1093/database/bat070` (2013).
8. Dictionary of food compounds with CD-ROM. *Choice Reviews Online.* ISSN: 0009-4978 (2013).
9. Baik, B.-k. & Ullrich, S. E. Barley for food : Characteristics , improvement , and renewed interest. *Journal of Cereal Science* **48,** 233–242 (2008).
10. Abidi, S., Ben Salem, H., Vasta, V. & Priolo, A. Supplementation with barley or spineless cactus (Opuntia ficus indica f. inermis) cladodes on digestion, growth and intramuscular fatty acid composition in sheep and goats receiving oaten hay. *SMALL RUMINANT RESEARCH* **87,** 9–16. ISSN: 0921-4488 (Nov. 2009).
11. Foster, A. P. *et al.* Serum IgE and IgG responses to food antigens in normal and atopic dogs, and dogs with gastrointestinal disease. eng. *Veterinary immunology and immunopathology* **92,** 113–124. ISSN: 0165-2427 (Print) (May 2003).
12. De Angelis, M. *et al.* Effect of Whole-Grain Barley on the Human Fecal Microbiota and Metabolome. eng. *Applied and environmental microbiology* **81,** 7945–7956. ISSN: 1098-5336 (Electronic) (Nov. 2015).
13. Ma, J. *et al.* Plasma Alkylresorcinols, Biomarkers of Whole-Grain Intake, Are Related to Lower BMI in Older Adults. *JOURNAL OF NUTRITION* **142,** 1859–1864. ISSN: 0022-3166 (Oct. 2012).

14. Guyman, L. A. *et al.* Urinary 3-(3,5-dihydroxyphenyl)-1-propanoic acid, an alkylresorcinol metabolite, is a potential biomarker of whole-grain intake in a US population. *JOURNAL OF NUTRITION* **138,** 1957–1962. ISSN: 0022-3166 (Oct. 2008).

15. Ross, A. B. *et al.* Alkylresorcinols in Cereals and Cereal Products. *Journal of Agricultural and Food Chemistry* **51,** 4111–4118. https://doi.org/10.1021/jf0340456 (2003).

16. Andersson, A. A. M., Åman, P., Wandel, M. & Frølich, W. Alkylresorcinols in wheat and rye flour and bread. *Journal of Food Composition and Analysis* **23,** 794–801. ISSN: 0889-1575. http://www.sciencedirect.com/science/article/pii/S0889157510001341 (2010).

17. Bordiga, M. *et al.* Alkylresorcinol content in whole grains and pearled fractions of wheat and barley. *Journal of Cereal Science* **70,** 38–46. ISSN: 0733-5210. http://www.sciencedirect.com/science/article/pii/S0733521016300790 (2016).

18. Dragsted, L. O. *et al.* Dietary and health biomarkers—time for an update. *Genes and Nutrition* **12,** 1–7. ISSN: 18653499 (2017).

19. Gao, Q. *et al.* A scheme for a flexible classification of dietary and health biomarkers. *Genes & Nutrition* **12,** 34. ISSN: 1865-3499. https://doi.org/10.1186/s12263-017-0587-x (Dec. 2017).

20. Bird, A. R. *et al.* Wholegrain foods made from a novel high-amylose barley variety (Himalaya 292) improve indices of bowel health in human subjects. eng. *The British journal of nutrition* **99,** 1032–1040. ISSN: 0007-1145 (Print) (May 2008).

21. Ames, N. *et al.* A double-blind randomised controlled trial testing the effect of a barley product containing varying amounts and types of fibre on the postprandial glucose response of healthy volunteers. eng. *The British journal of nutrition* **113,** 1373–1383. ISSN: 1475-2662 (Electronic) (May 2015).

22. Marungruang, N., Tovar, J., Bjorck, I. & Hallenius, F. F. Improvement in cardiometabolic risk markers following a multifunctional diet is associated with gut microbial taxa in healthy overweight and obese subjects. eng. *European journal of nutrition* **57,** 2927–2936. ISSN: 1436-6215 (Electronic) (Dec. 2018).

23. Gurdeniz, G. *et al.* Detecting Beer Intake by Unique Metabolite Patterns. eng. *Journal of proteome research* **15,** 4544–4556. ISSN: 1535-3907 (Electronic) (Dec. 2016).

24. Chen, Y., Ross, A. B., Åman, P. & Kamal-Eldin, A. Alkylresorcinols as Markers of Whole Grain Wheat and Rye in Cereal Products. *Journal of Agricultural and Food Chemistry* **52,** 8242–8246. ISSN: 0021-8561. https://doi.org/10.1021/jf049726v (Dec. 2004).

25. Raninen, K. J. *et al.* Fiber content of diet affects exhaled breath volatiles in fasting and postprandial state in a pilot crossover study. *NUTRITION RESEARCH* **36,** 612–619. ISSN: 0271-5317 (June 2016).

26. Adlercreutz, H., Peñalvo, J. L., Linko-Parvinen, A.-M., Tikkanen, M. J. & Landberg, R. Alkylresorcinols from Whole-Grain Wheat and Rye Are Transported in Human Plasma Lipoproteins. *The Journal of Nutrition* **137,** 1137–1142. ISSN: 0022-3166. `https://dx.doi.org/10.1093/jn/137.5.1137` (2007).

27. Landberg, R., Kamal-Eldin, A., Andersson, A., Vessby, B. & Aman, P. Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. *AMERICAN JOURNAL OF CLINICAL NUTRITION* **87,** 832–838. ISSN: 0002-9165 (Apr. 2008).

28. Ross, A. B. *et al.* Plasma alkylresorcinols as a biomarker of whole-grain food consumption in a large population: results from the WHOLEheart Intervention Study. *AMERICAN JOURNAL OF CLINICAL NUTRITION* **95,** 204–211. ISSN: 0002-9165 (Jan. 2012).

29. Landberg, R. *et al.* Alkylresorcinol Metabolite Concentrations in Spot Urine Samples Correlated with Whole Grain and Cereal Fiber Intake but Showed Low to Modest Reproducibility over One to Three Years in U.S. Women. *JOURNAL OF NUTRITION* **142,** 872–877. ISSN: 0022-3166 (May 2012).

30. Kyro, C. *et al.* Plasma alkylresorcinol concentrations, biomarkers of whole-grain wheat and rye intake, in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *BRITISH JOURNAL OF NUTRITION* **111,** 1881–1890. ISSN: 0007-1145 (May 2014).

31. Bresciani, L. *et al.* Bioavailability and metabolism of phenolic compounds from wholegrain wheat and aleurone-rich wheat bread. *MOLECULAR NUTRITION & FOOD RESEARCH* **60,** 2343–2354. ISSN: 1613-4125 (Nov. 2016).

32. Jensen, B. M. *et al.* Quantitative analysis of absorption, metabolism, and excretion of benzoxazinoids in humans after the consumption of high- and low-benzoxazinoid diets with similar contents of cereal dietary fibres: a crossover study. *EUROPEAN JOURNAL OF NUTRITION* **56,** 387–397. ISSN: 1436-6207 (Feb. 2017).

33. Nybacka, S., Forslund, H. B. & Hedelin, M. Validity of a web-based dietary questionnaire designed especially to measure the intake of phytooestrogens. *JOURNAL OF NUTRITIONAL SCIENCE* **5.** ISSN: 2048-6790 (Sept. 2016).

34. Keaveney, E. M. *et al.* Postprandial plasma betaine and other methyl donor-related responses after consumption of minimally processed wheat bran or wheat aleurone, or wheat aleurone incorporated into bread. *BRITISH JOURNAL OF NUTRITION* **113,** 445–453. ISSN: 0007-1145 (Feb. 2015).

35. Garcia-Aloy, M. *et al.* Nutrimetabolomics fingerprinting to identify biomarkers of bread exposure in a free-living population from the PREDIMED study cohort. *METABOLOMICS* **11,** 155–165. ISSN: 1573-3882 (Feb. 2015).