

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

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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 biomarker¹. 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. Other promising intake biomarkers were summarized as well.

1 Introduction

Whole grains (WGs) and their processed food contain a lot of non-nutrient compounds in their bran part. Besides providing carbohydrates which mostly locate in grains' endosperm, WGs may have other health beneficial effects. However, epidemiologic studies showed mixed results due to subjective self-report based food exposure measurement[**ISI:000447355100002**]. Using Biomarkers of Food Intake (BFIs) can potentially measure food exposure in population more objectively with accuracies and details[**Scalbert2014**].

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[**arreview2004**]. Therefore, ARs have the possibility to be used as biomarkers for whole grain wheat and rye intake.

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

¹In my project work, I actually proposed some biomarkers

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 (e.g. food diaries and food frequency questionnaires) used in observational studies could cause biases 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[Pratico2018]. The flowchart was included in Appendix (Fig-2)

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[Pratico2018] 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 (trial* 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[hmdb], FoodDB[foodb], PhenolExplorer[phenolexplorer], Dictionary of Food Compound[dictionary] 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 duplicates from merged 3 database 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[Baik2008].

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[ISI:000272990200002, Foster2003].

A 2-month intervention study[DeAngelis2015] incorporated 75% refined drum wheat and 25% WG barley. The fecal samples showed significant change in microbiota and metabolome after intervention[DeAngelis2015]. However, no specific metabolite can indicate WG barley intake.

ARs and their metabolites may not indicate WG barley intake. Several observation studies[ISI:000309032000011, ISI:000259554500019] investigated correlation between ARs metabolites and whole grain intake. Although these studies tried to cover more whole grain species, for example, one study[ISI:000259554500019] 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: Presence of ARs in Cereal Grains, adapted from [doi:10.1021/jf0340456, ANDERSSON2010794, BORDIGA201638](unit: $\mu\text{g/g dm}$), conc. varies due to different species and milling methods.

Most search results focused on barley's *effect biomarkers* as defined by Dragsted[Dragsted2017] and Gao[Gao2017], such as bowel health indicators[Bird2008], postprandial glucose and insulin response[Ames2015], lipid profiles and cardiovascular diseases (CVD) markers[Marungruang2018], 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

²Dark 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 | [Gurdeniz2016] |
| 4 | Hordatine A | C ₂₈ H ₃₈ N ₈ O ₅ | alkaloid | only reported in barley | FoodDB (002330) |
| 4 | Hordatine B | C ₂₉ H ₄₀ N ₈ O ₅ | alkaloid | only reported in barley | FoodDB (002328) |
| 2 | Distichonic acid A | C ₁₀ H ₁₈ N ₂ O ₈ | gamma amino acids and derivatives | only reported in barley | FoodDB (18164) |
| 3 | Distichonic acid B | C ₁₀ H ₁₈ N ₂ O 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

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.

3.2 WG wheat

3.2.1 Overview

The literature search got 312 results after removing duplicates from merged results. Some articles were found from the references of searched results. **One intervention study and one observation study** were included in the table.

Surprisingly few studies of whole grain wheat intake biomarkers were reported because whole grains seem to be the hotspot and mostly researched area of BFIs. 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 SUBTYPE whole grains’ intake.

FOR EXAMPLE, IN THIS STUDY[ISI:000348343300015], SUBJECTS WERE CLASSIFIED AS CONSUMER OF 'NONE-BREAD', 'WHITE BREAD' AND 'WHOLE GRAIN BREAD'. HOWEVER, WE DO NOT KNOW, WHICH TYPE OF CEREAL GRAINS ARE THE BREAD MADE OF. THESE BIOMARKERS ARE LESS USEFUL FOR OUR IDENTIFICATIONS.

In observational studies, estimations of food exposure are MOSTLY self-report BASED. IN SUCH KIND OF SELF-REPORTED SURVIES, RESULTS WERE NORMALLY NOT DETAILED TO EACH WHOLE-GRAIN SUBTYPE.

Therefore, it is difficult to assign the biomarker to the specific food.

Food frequency questionnaire causes high deviations distinguishing each subtype cereal by its nature. 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.

However, ARS can only indicate whole grain wheat and rye intake but not other whole grain cereals. assuming someone eats a lot of whole grain oat or

3.2.2 Alkylresorcinols (ARs) and AR metabolites

Alkylresorcinols (ARs) have the potential to indicate WG wheat and rye intake. Because within commonly consumed plant-based food, alkylresorcinols present high concentration exclusively in bran part of wheat and rye.

Total alkylresorcinols and their metabolites (3,5-DHPPTA, 3,5-DHPPA, 3,5-DHBA and 3,5-DHBA glycine) were not specific to WG wheat but got good correlations with WG wheat intake in rye rarely consumed countries, such as UK and USA. In these countries, WG wheat is the only alkylresorcinol source.

ARs are absorbed by humans and can be detected in blood plasma, erythrocytes, adipose tissue[ISI:000451002000016], and in the form of polar metabolites in urine

AR metabolites: urine/plasma: HPLC-COULOMETRIC ELECTRODE AR-RAY DETECTOR

longer apparent half-lives

3.2.3 AR homologous ratio C17:0/C21:0

In wheat and rye both consumed countries, total ARs and ARs metabolites indicate WG wheat and rye intake, while AR homologous ratio C17:0/C21:0 can indicate relative compositions of WG wheat and rye in the diet.

AR homologous ratio C17:0/C21:0 was first reported by cereal scientists in 2004 to distinguish WG rye and wheat cereal [Chen2004]. In 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 distinguish WG rye and wheat intake. In 2005, Linko[ISI:000376712600013] 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 [10.1093/jn/137.5.1137]. 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[ISI:000255012000007].

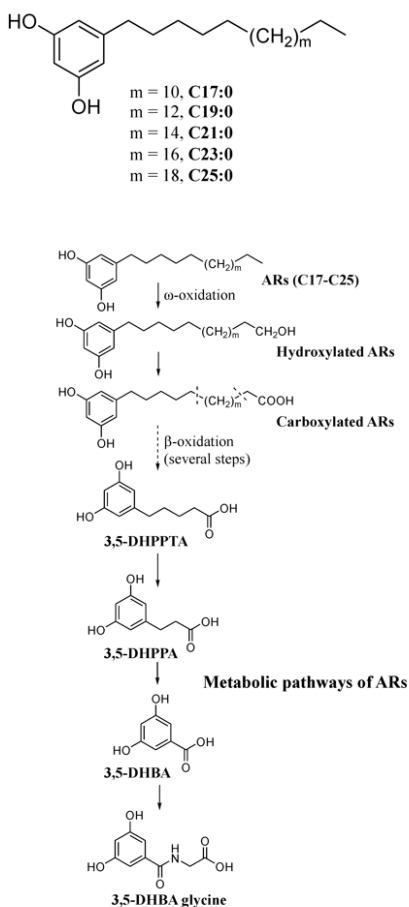


Figure 1: Structure of ARs and suggested metabolic pathway, adapted from [ISI:000447355100002]

But WG diet and refined diet can be distinguished by total ARs concentration in plasma.

An observational study further validated this marker. In 2014, EPIC³ 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 popula-

³European Prospective Investigation into Cancer and Nutrition

tion.

| 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 | [ISI:000376712600013] |

Table 3: Biomarkers of Wheat Intake Reported in Intervention study

| Type of WG | No. subjects | Sample type | Analytical method | Candidate biomarker(s) | Associated with | Reference |
|------------------|--------------|--------------------------------------|-------------------|------------------------|-----------------|-----------------------|
| WGs ⁴ | 2845 | fasting and non-fasting plasma | GC-MS | AR C17:0/C21:0 | FFQ | [ISI:000334172400017] |

Table 4: Biomarkers of Wheat Intake Reported in Observation study

3.2.4 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 relative compositions in the diet. This will provide more detailed information regarding intake amount of specific cereal species.

For example, an observation study showed, in Chinese population AR metabolite DHPPA was correlated with lower odds of type II diabetes and impaired glucose regulation[ISI:000430455900021]. However, DHPPA as AR metabolite can come from either whole grain wheat or rye.

Other two researches can better show that, actually WG rye could be more favorable for type II diabetes. An observational study showed in a population with metabolic syndrome, plasma AR C17:0/C21:0 was associated with increased insulin sensitivity[ISI:000333777700008]. Further, it was observed that in healthy Scandinavian populations, plasma total ARs concentration was not correlated with type II diabetes risk. However higher C17:0/C21:0 ratio (implicating more rye intake) was associated with increased insulin sensitivity[ISI:000378977200013]. These results 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. 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.

⁴This cohort studies investigated WGs conc. in different EU countries' population.

All these observation studies indicate that, application of biomarkers for sub-type cereal may be more powerful to rather than a overall WG intake.

3.2.5 Other potential markers

Searching results also showed some *Food compound intake biomarkers (FCIBs)* research as defined by Gao[Gao2017] such as phenolic compounds[ISI:000389134200003], benzoxazinoids (BXOs)[ISI:000394168100034, ISI:000348343300015], phytoestrogen[ISI:000384082300015], phytosterol and lignan[ISI:000387249200001], MICROBIOAL DERIVATIVES[ISI:000348343300015]. These compounds also present in other food, not specific for WG wheat. These results were summarized in Appendix.

In this article ISI:000387249200001, author performed an intervention study using refined wheat bread as control group, authors proposed a panel of metabolites consisting 7 AR metabolites, 5 BXO metabolites and 5 phenolic acid derivatives can be used to objectively monitor WG wheat intake. However, this conclusion needs to be further identified in other intervention and observation studies.

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 cereal. 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 [ISI:000447355100002], one of the challenges in BFIs discovery of WG is that the chemical compositions of most of WGs were not systematically studied. due to limited systematic research on phytochemicals

more studies should be conducted to investigate WG sub-type's intake biomarkers. because each whole grain sub-type may not benefit health equally in different population.

It worths pointing out that, in Chinese population WG study, to the author's knowledge, WG rye is rarely produced and consumed in Chinese population, therefore, DHPPA could primarily come from WG wheat ⁵ interesting conclusions may be drawn. WGs's health beneficial effects may be different in different populations.

In scandinavian population, WG rye and wheat. . therefore, in order to investigate intake biomarker for each sub-type cereals, single WG source country or WG low consumption country such as China could be a good choice.

not too many food compound and natural product, phytochemical database are available.

Using ARs to represent WG intake.

6 Appendix

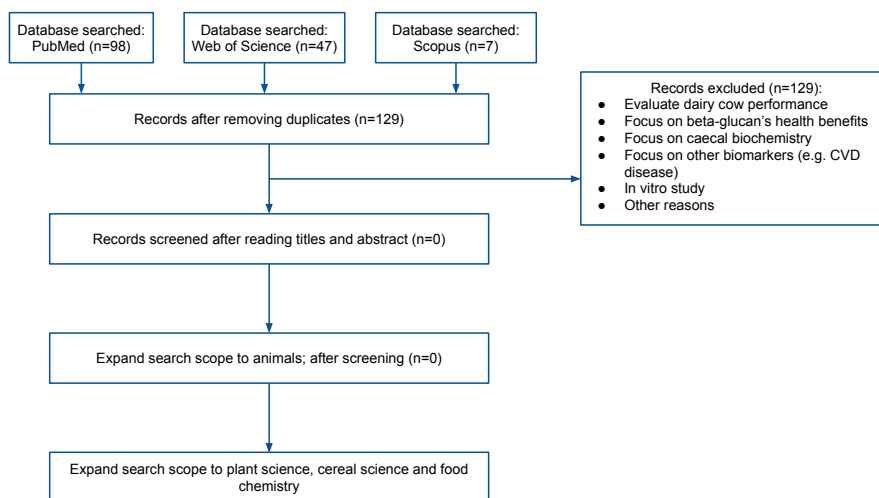


Figure 2: Flow chart of literature searching and screening for articles of barley intake biomarkers

⁵A letter has been sent to the author suggesting them to investigate the AR homologue ratio in plasma samples.

| Dietary factor | No. subjects | Study design | Sample type | Analytical method | Candidate biomarker(s) | Reference |
|-----------------------------------|--------------|--|-------------|--|---|-----------------------|
| Wheat bran, Wheat aleurone | 14+13 | randomized, cross-over, intervention | plasma | LC-MS/MS (Microbiology assay for folate) | betaine choline folate dimethylglycine (DMG) | [ISI:000350230300006] |
| None-bread, White bread, WG bread | 155 | observation ⁶ | urine | HPLC-qTOF-MS | Benzoxazinoid-related metabolites (HPPAA, HBOA glycoside) ARs-related metabolites (DHPPA glucuronide, DHPPTA sulphate microbial-derived metabolites (| [ISI:000348343300015] |
| WGs ⁷ | 104 | observation (FFQ) | spot urine | GC-MS | ARs metabolites (DHBA, DHPPA) | [ISI:000303089700010] |
| WGs ⁸ | 266 | randomized, parallel-group, intervention | plasma | GC-MS | Total ARs | [ISI:000298402100026] |

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

⁶dietary exposure measured from FFQ

⁷This 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.

⁸This 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