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Alkylresorcinols as Biomarkers of Whole-Grain Intake

Michael Robert MacArthur
University of Rhode Island, mrj55@wildcats.unh.edu

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ALKYLRESORCINOLS AS BIOMARKERS OF WHOLE-GRAIN INTAKE

BY

MICHAEL ROBERT MACARTHUR

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE**

**IN
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OF
MICHAEL ROBERT MACARTHUR

APPROVED:

Thesis Committee:

Major Professor: Ingrid Lofgren

Cathy English

Angela Slitt

Nasser H. Zawia

DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

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ABSTRACT

Background: In order to attenuate the measurement error inherent in dietary recall methods, alkylresorcinols (ARs) have been proposed as a biomarker of whole-grain (WG) intake (1, 2). Although ARs have shown promise in experimental (3) and observational studies (4), most of this research has been conducted in Nordic populations that consume relatively high amounts of WG (5).

Objectives: The objectives of this study were to assess the utility of plasma AR concentration as a WG intake biomarker and identify the primary dietary and non-dietary determinants of plasma AR concentration in a population that consumes relatively few WG.

Methods: This ancillary study used data from first-year college students (n=122) who completed three interviewer-administered 24-hour recalls and had two fasting venous blood draws performed on non-consecutive days. Dietary data was compiled using the Nutrition Data System for Research (6). Blood lipids and glucose were determined via enzymatic assay and plasma AR concentrations were determined by gas chromatography-mass spectrometry (7). Correlation coefficients (rs) were calculated between plasma AR concentrations and dietary intake variables and cardiometabolic risk factors. One-way ANOVA and Tukey's post-hoc test were used to assess group differences across plasma AR quartiles. Determinants of plasma AR concentration were assessed using multiple regression models. Statistical analyses were performed using R version 3.2.3.

Results: Plasma AR concentration was significantly positively correlated with WG intake (rs = 0.24, p <0.01). The correlation was stronger in males (n=36, rs = 0.33, p <

0.05) than females (n=86, $r_s = 0.18$, $p = 0.08$). One-way ANOVA across plasma AR quartiles was significant for fiber intake [$F(3,118) = 8.58$, $p < 0.001$] and Tukey's HSD test showed that the mean daily fiber intake for the highest AR quartile was significantly greater than for the lowest ($M = 20.2 \pm 1.9$ g, 14.7 ± 1.4 g respectively). The only cardiometabolic risk factor that was significantly associated with plasma AR concentration was triglycerides ($r_s = 0.27$, $p < 0.01$). Multiple regression models including log-transformed dietary variables showed that WG bread was the only significant food-item predictor of plasma AR concentration ($\beta = 0.54$, $p < 0.01$).

Conclusions: Although plasma AR concentration was significantly associated with WG and fiber intake, triglycerides were the primary determinant and the relationships were not strong enough to produce accurate WG intake prediction models. More research is needed on the use of AR as a quantitative biomarker in low WG-consuming populations.

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MANUSCRIPT

INTRODUCTION

Although the inverse association between whole-grain (WG) intake and chronic disease risk is well-established (1-3), a number of challenges still exist in assessing WG intake. These include systematic and random measurement errors inherent in dietary reporting tools (4, 5), and consumer confusion in identifying WG products and evaluating serving sizes (6, 7). To deal with these challenges and improve the detection of relationships between WG intake and disease, alkylresorcinols (ARs) have been proposed as an objective biomarker of WG intake (8-10).

Alkylresorcinols are phenolic lipids consumed almost exclusively from wheat and rye bran (11), and are absorbed and metabolized after consumption with a plasma half-life of ~5h (8, 12, 13). Intervention (14-18) and free-living (9, 19-22) studies have shown that plasma AR concentration is a valid, dose-responsive biomarker of WG intake and has moderate reproducibility up to 4 months. Correlation coefficients range from 0.24 – 0.56 for the relationship between plasma AR and total WG, WG wheat, WG rye and fiber intakes. The correlation between plasma AR and WG intake may be stronger in groups that consume more WG. This was shown with both acute consumption in a controlled feeding trial (14) and habitual consumption in observational and long-term intervention studies (16, 20). Many of the studies testing AR as a WG intake biomarker have been done in Nordic populations (9, 17, 19-21, 23, 24) which have relatively high habitual WG intake and consume more rye-based WG which has a higher AR content by weight than wheat (25, 26).

Few studies have tested plasma AR as a WG intake biomarker in American populations which consume relatively few WG, and those WG are primarily from wheat (27). To be a universally valid WG intake biomarker, plasma AR concentration should also reflect WG intake in populations that consume few WG from primarily non-rye sources. In this study, we examined the relationship between plasma AR concentrations and self-reported WG intake in a group of free-living first year American college students who consumed relatively few WG. Our objectives were to assess the utility of plasma AR concentration as a WG intake biomarker and to identify the primary dietary and non-dietary determinants of plasma AR concentration in this population.

MATERIALS AND METHODS

This ancillary study was completed using blood samples and data from a cross-sectional study done at the University of Rhode Island with first-year college students from spring 2008 through fall 2009. A subset of 130 students was randomly selected from the original study sample. Students between 18 and 24 years of age were eligible for the original study. Exclusion criteria included being pregnant or lactating, or self-report of one of the following conditions: eating disorder, liver disease, bleeding disorder, diabetes, cancer, or CHD. Both the original and ancillary studies were approved by the University of Rhode Island Institutional Review Board.

Anthropometric measures:

All measurements were performed by trained study staff using standardized protocols. Measurements were performed in duplicate. Additional measures were performed if the variance in duplicate measures was outside pre-established standards.

Averages of repeated measures with acceptable variances were reported. Height was measured to the nearest 0.1 cm using a Seca 220 stadiometer. Weight was measured to the nearest 0.1 kg using a calibrated digital Seca 760 Scale. Body mass index was calculated as weight in kg/height in meters². Waist circumference was measured to the nearest 0.1 cm at the top of the iliac crest using a Gulick fiberglass, non-stretchable tape measure (Patterson Medical Products).

Biochemical measures:

A trained phlebotomist completed two 12-hour fasting venous blood draws on two non-consecutive mornings in the same week. Plasma was obtained via centrifugation of whole blood for 20 minutes at 2200 RPM at 4°C. A preservation cocktail was added to the plasma containing: 0.01g/100g phenylmethylsulfonyl fluoride (Roche), 0.01g/100g sodium azide (Fisher) and 0.05g/100g aprotinin (Fisher). Samples were stored in a -80°C freezer until analysis.

Enzymatic assays were performed to determine TC, HDL-C, TAG and glucose concentrations. A Roche diagnostic Chol kit was used to measure TC concentrations (28). A Roche/Hitachi Chol kit was used to measure HDL-C after apo-B containing lipoproteins were precipitated using dextran sulfate and magnesium chloride (Acros Organics) (29). A Roche/Hitachi Trig/GB (Roche) kit was used to measure TAG concentrations (30). The Friedewald equation was used to calculate LDL-C concentrations (31). An Autokit Glucose kit (Wako Diagnostics) was used to measure glucose concentrations. Plates were read in a Biotek ELX 808 plate reader.

Blood pressure was measured by a trained exercise physiologist after a five-minute seated resting period using a Littman Select stethoscope and a Welch-Allyn blood pressure cuff. Measures were taken in duplicate with one minute between measurements. If systolic or diastolic values varied by greater than 2 mmHg then the measurement was repeated.

Plasma samples were shipped to the Swedish University of Agricultural Science in Uppsala, Sweden for AR quantification as previously described (32). Briefly, samples were incubated at 37°C with an internal standard. Plasma proteins were precipitated to release bound AR and the organic phase was extracted using diethyl ether. Samples were then cleaned using Oasis MAX cartridges (Waters Corporation). Gas chromatography-mass spectrometry analysis was then performed on a TR-5 column using a Finnigan Trace GC Ultra Gas chromatograph coupled to a Finnigan Trace DSQ II mass detector. Homologues C17:0 to C25:0 were quantified using molecular ions. Minimum and maximum quantification concentrations were 7 and 870 nmol/L.

Dietary measures:

Each participant completed three 24-hour recalls administered by trained staff using the multiple pass method in conjunction with the Nutrition Data System for Research (NDS-R) software versions 2007 and 2008 (University of Minnesota) (33). The first recall was completed in person and two over the phone on non-consecutive days including two weekdays and one weekend day (34). NASCO food models (NASCO) were available during the first 24-hour recall to help visualize and estimate portion sizes. Food booklets designed by the Nutrition Coordinating Center at the

University of Minnesota were also provided to participants to help with portion size estimation.

Statistical analyses:

Data are represented as means $\pm$ SD unless stated otherwise. Non-normal variables were log-transformed before analysis to reduce skewness. Independent samples t-tests were performed to test for differences between genders in relevant dietary and biochemical measures using a Bonferroni correction for multiple comparisons. Spearman's r_s were calculated to assess the correlation between plasma AR concentration and dietary and biochemical measures. Spearman's r_s were also used to assess correlation between individual AR homologue concentrations and WG intake measures (total WG, WG cereal, WG bread, fiber). Univariate and multivariate regression models were used to determine the dietary and non-dietary determinants of plasma AR concentrations. In all analyses, dietary intake variables were means of three 24-hour recalls. Analyses were performed using R version 3.2.3.

RESULTS

Participant characteristics:

The mean participant age was 18.3 ± 0.5 y and 70% (n=86) were females (Table 2). The average BMI was 23.4 ± 3.8 ; 23% of participants were overweight and 6.5% were obese. The majority of participants (62.3%) did not show any metabolic syndrome criteria (35) and only 3 participants showed 3 or more criteria (Table 1). The most common metabolic syndrome criteria was low HDL-C (19.6%) followed by elevated TAG (13.1%) and elevated blood glucose (11.5%).

WG intake and plasma AR concentration:

The mean daily intake of WG was 1.59 ± 1.86 oz servings and the mean daily fiber intake was 16.9 ± 8.6 g. Twenty-one participants reported consuming no WG. The primary dietary sources of WG were breads and cereals, which accounted for 70.0% of WG intake (Figure 1). Males consumed 23% more fiber than females (19.6 ± 8.6 g, 15.9 ± 7.2 g, $p=0.01$) and 33% more WG (1.95 ± 2.71 oz, 1.45 ± 1.35 oz, $p=0.09$). The mean plasma AR concentration was 61.6 ± 71.0 nmol/L and there was no significant difference between males and females (59.9 ± 76.1 nmol/L, 62.3 ± 69.3 nmol/L). C17:0 was the predominant AR homologue and the mean C17:0:C21:0 ratio was 0.12 ± 0.09 indicating that participants consumed wheat as their primary WG (18) (Figure 2).

WG intake and plasma AR concentration:

The correlation between WG intake and total plasma AR concentration was significant and positive ($r_s = 0.24$, $p < 0.01$). A significant positive correlation between fiber intake and total plasma AR concentration was also observed ($r_s = 0.27$, $p < 0.01$) (Table 3). All plasma AR homologue concentrations were significantly correlated with WG intake ($r_s = 0.20 - 0.25$) and with fiber intake ($r_s = 0.20 - 0.32$). Total plasma AR concentration was significantly correlated with WG bread intake ($r_s = 0.27$, $p < 0.01$) and WG cereal intake ($r_s = 0.20$, $p < 0.05$). The only WG intake category that did not include wheat or rye was popcorn, which was not significantly correlated with total plasma AR concentration ($r_s = -0.11$, $p = 0.24$). One-way ANOVA results showed that the highest quartile of AR concentration had significantly higher WG and fiber intake than the lowest quartile.

When examining associations by gender, males showed stronger correlations between WG intake measures and plasma AR concentrations. The correlation between total WG intake and total plasma AR was significant for males ($r_s = 0.33$, $p > 0.05$), but not for females ($r_s = 0.18$, $p = 0.11$) (Table 3). All plasma AR homologue concentrations were significantly correlated with total WG intake in males ($r_s = 0.30 - 0.38$) while none were significantly correlated in females ($r_s = 0.10 - 0.19$) (Figure 3). All plasma AR homologue concentrations were also significantly correlated with WG bread intake in males ($r_s = 0.35 - 0.44$) while only C19:0 and C21:0 were significantly correlated in females ($r_s = 0.26, 0.21$ respectively).

Classification of subjects into the same or adjacent WG intake quartile based on total plasma AR quartile ranged from 58% to 78% across quartiles (Table 4). The gross misclassification rate, defined as subjects in the first or fourth WG quartile being classified in the opposite AR quartile, was 9.7% to 13.8%. Cohen's weighted κ for agreement was 0.15 indicating slight agreement between quartile classification.

Associations with cardiometabolic risk factors

The only metabolic syndrome criteria that was significantly associated with total plasma AR concentration was TAG ($r_s = 0.27$, $p < 0.01$). One-way ANOVA results showed that the highest quartile of plasma AR concentration had significantly higher TAG concentrations than the lowest quartile (Table 4). WG intake was only significantly correlated with HDL ($r_s = 0.20$, $p > 0.05$). Neither WG intake nor total plasma AR concentration was significantly associated with BMI.

DISCUSSION

This study was one of the first to assess plasma AR concentration as a WG intake biomarker in a low-WG consuming Western population. The mean WG intake in this study was 1.51 ± 1.81 oz servings per day (Figure 1). Although this is higher than the American average of 0.97 oz servings per day (36), it is lower than reported intakes in Nordic countries where most AR studies have been conducted. Nordic population-based studies have reported average intakes of at least 2 oz servings per day and observational studies investigating ARs in Nordic populations have reported average intakes of 4-8 oz servings per day (19, 23, 25, 37).

The mean total plasma AR concentration in this sample was 61.6 ± 71.0 nmol/L. This is lower than averages from many observational studies in Nordic populations which ranged from 66-115 nmol/L (19, 20, 37). Additionally, the mean plasma C17:0 concentration in this sample was 2.35 ± 3.51 nmol/L, while averages from Nordic samples ranged from 5-10.5 nmol/L (9, 20). The C17:0 homologue is more prevalent in rye bran compared to wheat (17, 18), indicating lower rye consumption in this sample. This observation is consistent with previous work showing higher rye intake in Nordic compared to Western populations (25).

Despite relatively low intake, WG and fiber intake were significantly positively correlated with total plasma AR concentration ($r_s = 0.24, 0.28$ respectively) (Table 3, 4), and with all individual homologues ($r_s = 0.20-0.32$) (Table 4). These correlation coefficients are consistent with some studies done in Nordic populations ($r_s = 0.24-0.25$) (19, 23) and slightly lower than others ($r_s = 0.41-0.57$) (20, 21). When examined by gender these correlations were all significant in males (total AR $r_s =$

0.33, homologues $r_s = 0.30-0.38$) and all non-significant in females (total AR $r_s = 0.18$, homologues $r_s = 0.10-0.19$) (Table 4).

There are potential physiologic and non-physiologic explanations for the observed gender-specific differences in WG-AR correlations. First, previous research has shown that females tend to under-report dietary intake to a greater extent than men (38, 39). Gender differences in lipid metabolism may also explain the differences in correlations (40). Plasma ARs are transported in the blood in lipoproteins, primarily very low density lipoprotein (VLDL) (18). Plasma TAG concentration can act as a direct estimator of VLDL concentration (41) and in this sample women had significantly higher plasma TAG concentrations. This indicates that women may have had higher VLDL concentrations leading to more efficient transport and metabolism of ARs. Women also metabolize γ -tocopherol, a proposed model molecule for AR metabolism (37, 42, 43), more efficiently (44) which may further explain these differences. Tocopherols are also transported in VLDL particles (45), but whether gender differences in metabolism are due to general differences in lipid metabolism or differences in activity and expression of tocopherol-specific enzymes remains to be determined (44).

The primary sources of WG intake in this sample were bread and cereal, which accounted for 70% of total WG intake (Figure 1). Multivariate regression models predicting total AR and homologue concentrations that included whole- and refined-grain bread and cereal explained 6-13% of the variation in AR concentrations (Table 6). These models identified WG bread as the major food-item determinant of plasma AR concentrations. In models including dietary and non-dietary variables, TAG was

shown to be the primary determinant of plasma AR concentrations. The positive correlation between total AR and TAG was significant ($r_s = 0.27$) and predictive models including TAG achieved R^2 values approaching 0.20 while models including only dietary variables did not surpass $R^2 = 0.14$.

CONCLUSION

In conclusion, the results of this study demonstrate that plasma AR concentrations are positively correlated with WG intake in a low WG-consuming population. The relationship between WG intake and AR concentrations was stronger in males than females which could be due to a number of physiologic or non-physiologic reasons. The primary dietary determinant of AR concentrations was WG bread and models including only dietary variables explained about 14% of the variation in AR concentrations. Plasma TAG was the strongest non-dietary determinant of AR concentrations and had the highest predictive power in regression models. Models including TAG and dietary variables explained about 20% of the variation in AR concentrations. Although there was a notable linear relationship between WG intake and AR concentrations, the relationship was not strong enough to produce good predictive WG intake models. This study's findings of correlations coefficients $r_s = 0.20-0.40$ and R^2 values of 0.15-0.20 are consistent with findings from Nordic populations that consume more WG. This suggests that plasma ARs function equally well across a range of WG intakes.

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TABLES AND FIGURES

Table 1: Demographics, biochemical and dietary descriptives

	Total (n=122)	Males (n=36)	Females (n=86)
Age (y)	18.3±0.5	18.3±0.5	18.3±0.5
BMI (kg/m ²)	23.4±3.8	24.0±3.4	23.2±4.0
Total cholesterol (mg/dL)	147.8±28.7	134.6±19.8	153.4±30.0
Triglycerides (mg/dL)	89.0±50.5	74.8±43.0	95.0±52.5
HDL-C (mg/dL)	58.4±14.6	51.8±9.8	61.1±15.4
LDL-C (mg/dL)	71.7±26.2	67.9±21.0	73.2±28.0
Blood glucose (mg/dL)	89.2±9.0	90.2±8.4	88.8±9.3
Total plasma AR (nmol/L)	61.6±71.0	59.9±76.1	62.3±69.3
WG intake (oz servings/day)	1.59±1.86	1.95±2.71	1.45±1.35
Total dietary fiber (g/day)	16.9±8.6	19.6±10.9	15.9±7.2

Values are means ± standard deviation, BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; AR, alkylresorcinol; WG, whole-grain

Table 2: Metabolic syndrome characteristics

Number of MetS criteria	n (%)	MetS criteria	n (%)
0	76 (62.3)	Waist Circumference	9 (7.4)
1	32 (26.2)	TAG	16 (13.1)
2	11 (9.0)	HDL-C	24 (19.6)
3	3 (2.5)	Glucose	14 (11.5)
		Blood Pressure	0 (0)

Values are counts (percent), MetS, metabolic syndrome; TAG, triglycerides; HDL-C, high-density lipoprotein cholesterol

Figure 1: Average WG intake by source

Bars represent means $\pm$ standard error, values were compiled from food group level subject averages from NDSR

Figure 2: Average concentration of total AR and AR homologues

Bars represent means $\pm$ standard errors

Figure 3: Log total WG intake vs log total plasma AR concentration

Spearman's $r = 0.24$, $p < 0.01$

Table 3: Correlation coefficients (r_s) between plasma AR concentration and various measures of WG intake

	Total (n=122)	Males (n=36)	Females (n=86)
Total WG intake	0.24**	0.33*	0.18
Total fiber intake	0.28**	0.34*	0.24*
WG breads	0.27**	0.39*	0.21*
WG cereals	0.20*	0.25	0.14

* $p < 0.05$, ** $p < 0.01$

Table 4: Correlation coefficients (r_s) between WG and AR by gender

	Total (n=122)	Males (n=36)	Females (n=86)
Total AR	0.24**	0.33*	0.18
C17:0	0.20*	0.35*	0.10
C19:0	0.24**	0.38*	0.18
C21:0	0.25**	0.39*	0.19
C23:0	0.22*	0.36*	0.11
C25:0	0.20*	0.30*	0.13

* $p < 0.05$, ** $p < 0.01$

Figure 4: Correlation coefficients (r_s) between plasma AR concentrations and WG intake measures in males and females

Bars represent correlation coefficients (r_s) between individual AR homologues and WG intake source, * $p < 0.05$

Table 5: A) Participant characteristics by plasma AR concentration quartile and B) WG intake quartile agreement with plasma AR concentration quartile

A)	Q1 (n=31)	Q2 (n=30)	Q3 (n=30)	Q4 (n=31)
AR (nmol/L)	13.4 ± 0.84	27.7 ± 0.74	52.3 ± 2.19	151.7 ± 16.2
WG (Oz servings/day)	1.12 ^a ± 0.35	1.30 ^{ab} ± 0.19	1.7 ^{ab} ± 0.34	1.9 ^b ± 0.39
TAG (mg/dL)	77.9 ^a ± 8.1	78.41 ^{ab} ± 8.2	86.9 ^{ab} ± 7.6	112.5 ^b ± 11.1
TC (mg/dL)	144.2 ± 4.2	147.5 ± 5.4	142.8 ± 3.9	156.6 ± 6.6
BMI (mg/dL)	23.6 ± 0.72	22.7 ± 0.48	24.2 ± 0.85	23.3 ± 0.67
Fiber (g/day)	14.7 ^a ± 1.4	14.9 ^{ab} ± 0.9	17.9 ^{ab} ± 1.6	20.2 ^b ± 1.9
B)				
WG Intake (servings/d)	1.12 ± 0.35	1.30 ± 0.19	1.72 ± 0.34	1.91 ± 0.39
Same or adjacent Q (%)	67.7	73.3	78.1	58.6
Gross misclassification (%)	9.7			13.8

Different a,b indicates significant difference by Tukey's post hoc test, $p < 0.05$, Cohen's weighted kappa for agreement between AR and WG quartiles = 0.15

Table 6: Food determinants of plasma AR concentration

Intake Variable	Univariate Analysis	Multivariate Analysis	Intake Variable	Univariate Analysis	Multivariate Analysis
<i>WG Bread</i>			<i>WG Cereal</i>		
C17:0	0.12 (0.09)	0.11 (0.10)	C17:0	0.17 (0.09)	0.15 (0.09)
C19:0	0.25 (0.10)*	0.26 (0.09)*	C19:0	0.18 (0.09)	0.13 (0.09)
C21:0	0.25 (0.10)*	0.27 (0.09)*	C21:0	0.23 (0.09)*	0.18 (0.09)*
C23:0	0.19 (0.09)*	0.19 (0.09)*	C23:0	0.21 (0.09)*	0.18 (0.09)*
C25:0	0.17 (0.09)	0.17 (0.10)	C25:0	0.15 (0.09)	0.13 (0.09)
Total AR	0.23 (0.09)*	0.25 (0.09)*	Total AR	0.19 (0.09)*	0.15 (0.09)
<i>RG Bread</i>			<i>RG Cereal</i>		
C17:0	0.04 (0.08)	0.08 (0.10)	C17:0	0.00 (0.09)	0.00 (0.09)
C19:0	0.06 (0.09)	0.14 (0.09)	C19:0	0.04 (0.08)	0.04 (0.08)
C21:0	0.11 (0.09)	0.19 (0.09)*	C21:0	0.07 (0.10)	0.07 (0.08)
C23:0	0.08 (0.09)	0.14 (0.09)	C23:0	0.02 (0.07)	0.03 (0.09)
C25:0	0.05 (0.08)	0.11 (0.09)	C25:0	0.03 (0.09)	0.04 (0.08)
Total AR	0.10 (0.09)	0.18 (0.09)*	Total AR	0.04 (0.09)	0.04 (0.09)
Multivariate R ²					
C17:0	0.056				
C19:0	0.099				
C21:0	0.134				
C23:0	0.091				
C25:0	0.064				
Total AR	0.113				

Values are standardized beta coefficients (standard errors) for univariate and multivariate models, multivariate models included whole-grain and refined-grain bread and cereal as predictors

REFERENCES

1. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu SM. Greater Whole-Grain Intake Is Associated with Lower Risk of Type 2 Diabetes, Cardiovascular Disease, and Weight Gain. *J Nutr* 2012;142(7):1304-13. doi: 10.3945/jn.111.155325.
2. Liu SM, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am J Clin Nutr* 2003;78(5):920-7.
3. Sahyoun NR, Jacques PF, Zhang XLL, Juan WY, McKeown NM. Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. *Am J Clin Nutr* 2006;83(1):124-31.
4. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing With Dietary Measurement Error in Nutritional Cohort Studies. *J Natl Cancer Inst* 2011;103(14):1086-92. doi: 10.1093/jnci/djr189.
5. Livingstone MBE, Black AE. Markers of the validity of reported energy intake. *J Nutr* 2003;133(3):895S-920S.
6. De Moura FF, Lewis KD, Falk MC. Applying the FDA Definition of Whole Grains to the Evidence for Cardiovascular Disease Health Claims. *J Nutr* 2009;139(11):2220S-6S. doi: 10.3945/jn.109.112383.
7. Jones JM. Regulatory Aspects of Whole Grain and Whole Grain Foods: Definitions and Labeling. *Cereal Chem* 2010;87(2):150-4. doi: 10.1094/cchem-87-2-0150.
8. Landberg R, Linko AM, Kamal-Eldin A, Vessby B, Adlercreutz H, Aman P. Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. *J Nutr* 2006;136(11):2760-5.
9. 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. *Am J Clin Nutr* 2008;87(4):832-8.
10. van Dam RM, Hu FB. Are alkylresorcinols accurate biomarkers for whole grain intake? *Am J Clin Nutr* 2008;87(4):797-8.
11. Ross AB, Kamal-Eldin A, Aman P. Dietary alkylresorcinols: Absorption, bioactivities, and possible use as biomarkers of whole-grain wheat- and rye-rich foods. *Nutr Rev* 2004;62(3):81-95. doi: 10.1301/nr.2004.mar.81-95.

12. Ross AB, Shepherd MJ, Knudsen KEB, Glitz LV, Bowey E, Phillips J, Rowland I, Guo ZX, Massy DJR, Aman P, et al. Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats. *Br J Nutr* 2003;90(4):787-94. doi: 10.1079/bjn2003965.
13. Ross AB, Kamal-Eldin A, Lundin EA, Zhang JX, Hallmans G, Aman P. Cereal alkylresorcinols are absorbed by humans. *J Nutr* 2003;133(7):2222-4.
14. Landberg R, Aman 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 (vol 89, pg 290, 2009). *Am J Clin Nutr* 2009;89(5):1478. doi: 10.3945/ajcn.2009.27667.
15. Landberg R, Kamal-Eldin A, Andersson SO, Johansson JE, Zhang JX, Hallmans G, Aman P. Reproducibility of Plasma Alkylresorcinols during a 6-Week Rye Intervention Study in Men with Prostate Cancer. *J Nutr* 2009;139(5):975-80. doi: 10.3945/jn.108.099952.
16. 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(1):204-11. doi: 10.3945/ajcn.110.008508.
17. Linko AM, Juntunen KS, Mykkanen HM, Adlercreutz H. Whole-grain rye bread consumption by women correlates with plasma alkylresorcinols and increases their concentration compared with low-fiber wheat bread. *J Nutr* 2005;135(3):580-3.
18. Linko-Parvinen AM, Landberg R, Tikkarinen MJ, Adlercreutz H, Penalvo JL. Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. *J Nutr* 2007;137(5):1137-42.
19. Andersson A, Marklund M, Diana M, Landberg R. Plasma Alkylresorcinol Concentrations Correlate with Whole Grain Wheat and Rye Intake and Show Moderate Reproducibility over a 2-to 3-Month Period in Free-Living Swedish Adults. *J Nutr* 2011;141(9):1712-8. doi: 10.3945/jn.111.139238.
20. Aubertin-Leheudre M, Koskela A, Marjamaa A, Adlercreutz H. Plasma alkylresorcinols and urinary alkylresorcinol metabolites as biomarkers of cereal fiber intake in Finnish women. *Cancer Epidemiol Biomarkers Prev* 2008;17(9):2244-8. doi: 10.1158/1055-9965.epi-08-0215.
21. Ross AB, Pineau N, Kochhar S, Bourgeois A, Beaumont M, Decarli B. Validation of a FFQ for estimating whole-grain cereal food intake. *Br J Nutr* 2009;102(11):1547-51. doi: 10.1017/s0007114509990845.

22. Montonen J, Landberg R, Kamal-Eldin A, Aman P, Knueppel S, Boeing H, Pischon T. Reliability of fasting plasma alkylresorcinol concentrations measured 4 months apart. *Eur J Clin Nutr* 2010;64(7):698-703. doi: 10.1038/ejcn.2010.71.
23. Landberg R, Kamal-Eldin A, Aman P, Christensen J, Overvad K, Tjønneland A, Olsen A. Determinants of plasma alkylresorcinol concentration in Danish post-menopausal women. *Eur J Clin Nutr* 2011;65(1):94-101. doi: 10.1038/ejcn.2010.193.
24. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Akesson B, Onning G, Jonsdottir SE, Rosqvist F, Schwab U, Herzig KH, et al. Plasma Alkylresorcinols Reflect Important Whole-Grain Components of a Healthy Nordic Diet. *J Nutr* 2013;143(9):1383-90. doi: 10.3945/jn.113.175588.
25. Ross AB, Becker W, Chen Y, Kamal-Eldin A, Aman P. Intake of alkylresorcinols from wheat and rye in the United Kingdom and Sweden. *Br J Nutr* 2005;94(4):496-9. doi: 10.1079/bjn20051511.
26. Ross AB, Shepherd MJ, Schupphaus M, Sinclair V, Alfaro B, Kamal-Eldin A, Aman P. Alkylresorcinols in cereals and cereal products. *J Agric Food Chem* 2003;51(14):4111-8. doi: 10.1021/jf0340456.
27. Reicks M, Jonnalagadda S, Albertson AM, Joshi N. Total dietary fiber intakes in the US population are related to whole grain consumption: results from the National Health and Nutrition Examination Survey 2009 to 2010. *Nutr Res* 2014;34(3):226-34. doi: 10.1016/j.nutres.2014.01.002.
28. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. ENZYMATIC DETERMINATION OF TOTAL SERUM-CHOLESTEROL. *Clin Chem* 1974;20(4):470-5.
29. Warnick GR, Nauck M, Rifai N. Evolution of methods for measurement of HDL-cholesterol: From ultracentrifugation to homogeneous assays. *Clin Chem* 2001;47(9):1579-96.
30. Kohlmeier M. DIRECT ENZYMATIC MEASUREMENT OF GLYCERIDES IN SERUM AND IN LIPOPROTEIN FRACTIONS. *Clin Chem* 1986;32(1):63-6.
31. Friedewald WT, Fredrickson DS, Levy RI. ESTIMATION OF CONCENTRATION OF LOW-DENSITY LIPOPROTEIN CHOLESTEROL IN PLASMA, WITHOUT USE OF PREPARATIVE ULTRACENTRIFUGE. *Clin Chem* 1972;18(6):499.

32. Landberg R, Aman P, Kamal-Eldin A. A rapid gas chromatography-mass spectrometry method for quantification of alkylresorcinols in human plasma. *Anal Biochem* 2009;385(1):7-12. doi: 10.1016/j.ab.2008.10.031.
33. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, Paul DR, Sebastian RS, Kuczynski KJ, Ingwersen LA, et al. The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. *Am J Clin Nutr* 2008;88(2):324-32.
34. Nelson M, Black AE, Morris JA, Cole TJ. BETWEEN-SUBJECT AND WITHIN-SUBJECT VARIATION IN NUTRIENT INTAKE FROM INFANCY TO OLD-AGE - ESTIMATING THE NUMBER OF DAYS REQUIRED TO RANK DIETARY INTAKES WITH DESIRED PRECISION. *Am J Clin Nutr* 1989;50(1):155-67.
35. Grundy SM, Cleeman JJ, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, et al. Diagnosis and management of the metabolic syndrome - An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112(17):2735-52. doi: 10.1161/circulationaha.105.169404.
36. Albertson AM, Reicks M, Joshi N, Gugger CK. Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional National Health and Nutrition Examination Survey 2001-2012. *Nutr J* 2016;15:14. doi: 10.1186/s12937-016-0126-4.
37. Marklund M, Landberg R, Andersson R, Aman P, Kamal-Eldin A. Alkylresorcinol Metabolism in Swedish Adults Is Affected by Factors Other Than Intake of Whole-Grain Wheat and Rye. *J Nutr* 2012;142(8):1479-86. doi: 10.3945/jn.112.159244.
38. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, Sharbaugh CO, Trabulsi J, Runswick S, Ballard-Barbash R, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: The OPEN Study. *Am J Epidemiol* 2003;158(1):1-13. doi: 10.1093/aje/kwg092.
39. Beaton GH, Milner J, Corey P, McGuire V, Cousins M, Stewart E, Ramos MD, Hewitt D, Grambsch PV, Kassim N, et al. SOURCES OF VARIANCE IN 24-HOUR DIETARY RECALL DATA - IMPLICATIONS FOR NUTRITION STUDY DESIGN AND INTERPRETATION. *Am J Clin Nutr* 1979;32(12):2546-59.
40. Blaak E. Gender differences in fat metabolism. *Curr Opin Clin Nutr* 2001;4(6):499-502. doi: 10.1097/00075197-200111000-00006.

41. Wilson PW, Abbott RD, Garrison RJ, Castelli WP. ESTIMATION OF VERY-LOW-DENSITY LIPOPROTEIN CHOLESTEROL FROM DATA ON TRIGLYCERIDE CONCENTRATION IN PLASMA. *Clin Chem* 1981;27(12):2008-10.
42. Ross AB, Chen Y, Frank J, Swanson JE, Parker RS, Kozubek A, Lundh T, Vessby B, Aman P, Kamal-Eldin A. Cereal alkylresorcinols elevate gamma-tocopherol levels in rats and inhibit gamma-tocopherol metabolism in vitro. *J Nutr* 2004;134(3):506-10.
43. Marklund M, McKeown NM, Blumberg JB, Chen CYO. Hepatic biotransformation of alkylresorcinols is mediated via cytochrome P450 and beta-oxidation: A proof of concept study. *Food Chem* 2013;139(1-4):925-30. doi: 10.1016/j.foodchem.2013.01.122.
44. Frank J, Lee S, Leonard SW, Atkinson JK, Kamal-Eldin A, Traber MG. Sex differences in the inhibition of gamma-tocopherol metabolism by a single dose of dietary sesame oil in healthy subjects. *Am J Clin Nutr* 2008;87(6):1723-9.
45. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. *J Physiol Biochem* 2001;57(1):43-56.

APPENDIX A: LITERATURE REVIEW

Introduction

Although the inverse association between whole-grain intake and chronic disease is well established (1-3), many challenges still exist in measuring whole-grain intake, particularly in large studies (4, 5). Whole-grain intake is associated with lower risk of cardiovascular disease (CVD), type 2 diabetes and obesity (3, 6, 7). Increased consumption of whole-grains has also been associated with lower risk of metabolic syndrome, which is a significant predictor of heart disease, diabetes and stroke (1, 8). However, commonly used dietary data collection methods such as food frequency questionnaires and 24 hour recalls are prone to estimation error and are usually time intensive for both data collectors and respondents (9). This is further complicated by the numerous definitions of “whole-grain” that are used by researchers when formulating questionnaires and study criteria (4). The Food and Drug Administration has attempted to implement a universal definition, but many organizations still maintain their own (5). In light of the challenges associated with accurately collecting and assessing dietary data, many researchers have turned to biomarkers as objective indicators of nutrient intake. This is an active area of research and examples of successfully implemented biomarkers include urinary nitrogen to assess protein intake and doubly labeled water to assess total energy intake (10, 11).

Researchers have begun to explore the use of plasma alkylresorcinol (AR) concentrations as a biomarker of whole-grain wheat and rye intake (12-14). Alkylresorcinols are phenolic lipids that are obtained in the diet almost exclusively from the bran of wheat and rye. Numerous controlled feeding trials have found that

plasma AR levels reflect whole-grain wheat and rye consumption with r values ranging from 0.25 to 0.97 (10). Additionally, in a review of the validity of over 20 novel biomarkers, Hedrick et al. (10) found that plasma AR level was only one of two biomarkers to meet acceptable standards in three critical criteria: validity, reproducibility and sensitivity. Due to these positive initial results researchers have tried to correlate plasma AR concentrations with the same positive health outcomes associated with whole-grain intake. So far, the fewer than 10 studies conducted with this objective have found that higher plasma AR concentration is associated with lower incidence of colorectal cancer, higher insulin sensitivity, slightly lower body fat percent and lower BMI (15-19).

This literature review will explore the utility of ARs as a biomarker of whole-grain intake and will discuss the necessity of a whole-grain biomarker, the characteristics of ARs, their use in research to date and limitations in their use.

Whole-Grain Intake and Chronic Disease Risk

Negative associations between whole-grain intake and CVD (20-24), type 2 diabetes (25-28), metabolic syndrome (1, 29, 30) and all-cause mortality (31, 32) have been reported for decades. These results have been collected through primary research and confirmed through meta-analyses, which represent hundreds of thousands of subjects and efficiently convey cardiometabolic-protective effects.

Whole-Grain Intake and Cardiovascular Disease

In 2014 Wu et al. (33) published a meta-analysis examining the association between dietary fiber intake and coronary heart disease (CHD) in cohort studies. The systematic literature search was done on PubMed and EMBASE covering all articles

published through May 2013. In total 532 relevant articles were identified, of which 18 met all inclusion criteria, including availability of relative risks or hazard ratios for the primary outcome and a number of relevant confounders. The primary outcomes were pooled relative risk for CHD incidence and mortality in the highest versus lowest intake groups. This analysis was also performed for each dietary fiber subtype (cereal, fruit and vegetable). The secondary outcome was to quantify a dose-response relationship between fiber intake and CHD incidence and mortality in the 13 studies for which this data was available.

The multivariate adjusted risk ratios showed a significant inverse association between cereal fiber intake and CHD incidence (pooled RR = 0.92; 95% CI [0.85-0.99], P = 0.032) and between cereal fiber intake and CHD mortality (pooled RR = 0.81; [0.72-0.92], P = 0.001). The only other significant finding in the fiber subtype analyses was an inverse association between fruit fiber and CHD incidence (pooled RR = 0.92; [0.86-0.98], P = 0.01). Significant dose-response relationships were observed for total fiber intake. Each 10 gram per day increase in total fiber was associated with an 8% decrease in the risk of a coronary event (pooled RR = 0.92; [0.87-0.97]) and a 24% decrease in risk of death (pooled RR = 0.76; [0.65-0.88]). Dose-response analyses were not performed on fiber subtypes due to limited availability of data.

In 2006 Mellen et al. (2) performed a meta-analysis on prospective cohort studies that looked at associations between whole-grain intake and cardiac events. Their systematic literature review was done on MEDLINE covering from 1966 through April 2006. Eight studies from seven cohorts met all inclusion criteria, which

included having a prospective design, quantitative measures of whole-grain intake and clinical confirmation of cardiovascular events. For analyses, participants were split into whole-grain intake quintiles within study and odds ratios for cardiac events were computed using models adjusted for demographic characteristics and cardiovascular risk factors. The variance weighted odds ratios were then combined to determine an overall effect.

The demographic-adjusted model showed a 37% decrease in risk of incident cardiovascular disease between the highest and lowest quintiles of whole-grain intake (OR 0.63; 95% CI [0.58-0.68]). The

Figure 1 Odds ratios of incident CVD in high vs low whole-grain intake quartiles from studies in meta-analysis by Mellen et al. ¹Demographic-adjusted model. ²Demographic and cardiovascular risk factor adjusted model.

demographic and cardiovascular risk factor-adjusted model showed a 21% decrease in risk (OR 0.79; [0.73-0.85]) (Figure). They also investigated a number of secondary outcomes and found similar results when analyses were limited to men (OR 0.82; [0.73-0.92]), women (OR 0.79; [0.68-0.91]), fatal endpoints (OR 0.78; [0.70-0.88]) and incident coronary heart disease (OR 0.76; [0.69-0.83]). They also analyzed risk of cardiovascular event based on refined grain intake and found no significance (OR 1.07; [0.94-1.22]).

Whole-Grain Intake and Metabolic Syndrome/Obesity

In 2006 Sahyoun et al. (1) published a cross-sectional study looking at the association between whole-grain intake and metabolic syndrome prevalence and mortality in older adults. Initial data collection took place between 1981-1984 on 747 community-living adults aged > 59 years. Participants kept 3-day food records which were reviewed by a registered dietitian for completeness and accuracy. Biochemical values were assessed from a fasting venous blood draw, and demographics and anthropometric measures were collected by trained nurse practitioners. Metabolic syndrome was defined as having at least three of the follow risk factors: triglycerides ≥ 150 mg/dL, HDL-C <40 mg/dL in men or <50mg/dL in women, blood pressure $\geq 130/85$ mm Hg or on blood pressure lowering medications, fasting blood glucose >100 mg/dL and BMI ≥ 31 in men or ≥ 27 in women (34). In 1995, the vital status of the participants was assessed by examining the annual national index of death and obtaining death certificates to confirm death index entries. Cause of death was attained from death certificates. If vital status was unascertainable then the participant was assumed to be alive.

Fasting blood glucose and BMI significantly decreased across whole-grain intake quartiles ($p = 0.01$ and 0.03 respectively) in ANCOVA adjusting for demographic and lifestyle factors. Odds ratios for having metabolic syndrome were 0.58-0.41 for whole-grain intake quartiles 2-4 compared to quartile 1 (p trend = 0.005) in logistic regression adjusted for relevant risk factors. There was a significant inverse trend between whole-grain intake and CVD-related mortality. Compared to whole-grain intake quartile 1, quartile 4 had a significantly lower risk of CVD-related

mortality (relative risk (RR); 95% CI = 0.48; 0.25-0.96). No associations between whole-grain intake and all-cause mortality were observed.

A number of studies have also looked at associations between whole-grain intake and weight status. In 2003 Liu et al. (7) performed an analysis on data from the Nurses' Health Study focusing on associations between whole-grain and fiber intake and weight changes and BMI. The analyzed sample consisted of 74,091 female registered nurses between the ages of 30-55 years at time of recruitment. Dietary intake was assessed by a 60-item semiquantitative FFQ that was administered in 1984, 1986, 1990 and 1994. Body weight was self-reported at the same time points and weight change was assessed as the differences between two year intervals and overall difference from 1984 to 1994. Analyses were performed across quintiles of dietary intake variables and were adjusted for age, exercise, smoking, hormone replacement therapy, alcohol and caffeine intake.

At baseline, women in the highest quintile of whole-grain intake weighed significantly less and had significantly lower BMIs compared to women in the lowest intake quintile (p trend < 0.0001). Over the 10 years of observation, women in the highest quintile of whole-grain intake gained significantly less weight than women in the lowest quintile (1.07 vs 1.58 kg, p trend < 0.0001). Similar results were observed for dietary fiber intake; over the 10 years of follow up the women who increased their dietary fiber intake the most, gained an average of 1.52 kg less than those who increased their fiber intake the least (p trend < 0.0001). The risk for developing obesity (BMI $\geq$ 30) was also significantly lower in the highest whole-grain intake quintile compared to the lowest (odds ratio; 95% CI = 0.81; 0.73-0.91).

Whole-Grain Intake and Type 2 Diabetes

In 2014 Yao et al. (35) conducted high versus low and dose-response meta-analyses of prospective cohort studies that looked at dietary fiber intake and incidence of type 2 diabetes. Systematic literature searches were conducted on EMBASE covering 1974 through April 2013 and PubMed covering 1966 through April 2013. Seventeen studies met the inclusion criteria, which included having a prospective design, using dietary fiber or a dietary fiber subtype as the main exposure, using type 2 diabetes as the primary outcome and including adjusted risk ratios with 95% confidence intervals. To assess risk with high versus low whole-grain intake a combined measure was calculated using the inverse variance-weighted mean of the natural log-transformed covariate-adjusted risk ratio of the highest compared to lowest intake group in each study. Both linear and non-linear methods were used to assess dose response.

Figure 2 Dose-response between total fiber intake and risk of type 2 diabetes from meta-analysis by Yao et al. Solid line represents the linear model, dashed line is nonlinear model with 95% CI.

The final analysis included 17 prospective studies including 488,293 participants published between 1997-2013. The high versus low

analysis showed that the low cereal fiber intake group had a significantly lower risk of developing type 2 diabetes (RR; 95% CI = 0.77; 0.69-0.85). The risk reduction for

cereal fiber was greater than for fruit fiber (RR = 0.94) and vegetable fiber (RR = 0.95). Linear dose response modeling showed a 6% decrease in risk of type 2 diabetes for every 2g/day increase in cereal fiber (RR; 95% CI = 0.94; 0.93-0.96).

Randomized Controlled Feeding Trials

A limited number of randomized controlled trials have shown potential mechanisms underlying the inverse association between whole-grain

Figure 3 Fasting insulin concentrations from overweight or obese adults (N=11) during refined grain or whole-grain intervention. Values are means $\pm$ SE. Table from Pereira et al.

intake and cardiometabolic risk seen in epidemiologic studies (26, 36-38). In 2002 Pereira et al. (26) tested the effects of a six week whole-grain vs. refined grain diet intervention on insulin sensitivity measured by a euglycemic hyperinsulinemic clamp test and fasting insulin. Participants (N=11) were hyperinsulinemic, nondiabetic, overweight or obese and 25-56 years old. The researchers provided all foods for the entire 6 weeks and participants were asked to consume no other food. All participants received both diets in a random order with a 6-9 week washout period between. Both diets used identical food items with the only difference being the use of whole or refined grain. The whole-grain diet was higher in fiber (28.0 vs 17.8 g/day) and slightly higher in some minerals, but the diets had otherwise identical nutrient

contents. During the whole-grain diet, fasting insulin levels were significantly lower and insulin sensitivity was significantly higher compared to baseline and the refined-grain diet at weeks 2, 4 and 6. Additionally, the area under a 2-hour insulin concentration curve after a liquid non-grain test meal tended to be lower after six weeks of the whole-grain diet compared to the refined-grain diet. Fasting blood glucose also tended to be lower after the whole-grain diet but the difference was not significant.

In addition to euinsulinemic effects, others have hypothesized that the benefits of whole-grains are related to bioactive compounds such as lignins and phenols that have shown antioxidant effects in animal and cell models (39, 40). These results have been difficult to translate into humans and only a limited number of studies have been attempted with mixed results (36-38). Part of the difficulty in conducting these studies are the numerous operational definitions of “antioxidant status”. It is also difficult, logistically and financially, to model the long-term moderate doses seen in epidemiologic studies in an experimental design.

These data, comprised of observations on over 600,000 individuals, provide strong evidence that increasing intake of whole-grains may decrease risk of a number of chronic diseases. However, whole-grain intake was quantified in all of these studies using self-reported dietary intake data which presents some unique methodological challenges.

Challenges in Quantifying Dietary Intake

Quantifying whole-grain intake in free-living populations is a challenging process that is complicated by a number of factors. First, the most widely used

methods of collecting self-reported dietary data are subject to systemic biases that can attenuate or altogether hide nutrient-disease relationships (11, 41, 42). The second issue is that there is not a universal definition of “whole-grain” used in research or industry (43). This lack of a universal standard leads to inconsistency across research and reporting, particularly regarding enrichment with components of whole-grains. Industrial labeling practices can also cause consumer confusion leading to inaccurate reporting of whole-grain intake (5). Many consumers do not have a firm grasp on what constitutes a whole-grain product and when that is combined with nebulous labeling and multi-grain formulations the chance for errant reporting increases.

Challenges in Quantifying Dietary Intake

Food frequency questionnaires (FFQ) and 24-hour dietary recalls (24HR) are two of the most widely used methods of collecting dietary intake data, and both are subject to substantial systematic and random error (41, 42, 44). Systematic error is inherent to the specific tool being used, has a non-zero mean and is very difficult to identify without using another validation tool (45). This type of error will affect all observations, or large subsets of observations, in a similar way, creating distinct patterns of error. An example of this type of error in dietary reporting is the systemic bias of under-reporting. In 2003 Livingstone et al. (11) conducted a meta-analysis of 43 studies that compared self-reported energy intake to observed energy intake using doubly labelled water (DLW), an objective biomarker of energy intake (46). They found that the average percent of energy reported across all studies was $83 \pm 14\%$ of that observed with DLW, indicating average under-reporting of 10-20%. They also noted that women and individuals with higher BMI under-reported to a greater extent.

Additionally, every method of dietary self-reporting (weighed records, estimated records, diet history, 24HR and FFQ) showed similar under-reporting, ranging from 13-16%.

Random error can affect any tool being used and has a mean zero, affecting all observations in a non-uniform manner (47, 48). In dietary self-reporting random error usually comes from unpredictable variation in intake across days or seasons. For example, a participant may perform a 24HR on an unusual, non-representative day which will introduce some error that cannot be accounted for. Although it is difficult to mode random error out of data, in a study with a large sample and repeated measures it does not have as strong of an influence on the results as systematic error (49).

Food frequency questionnaires (FFQ) are widely used in epidemiologic studies because of their low researcher burden, however, they are subject to both systematic and random error (41, 42, 44). The FFQ is a tool that measures usual dietary intake over a defined time period and can be used on a large scale due to its relatively low burden on researchers. This has made it the primary tool of choice for most large epidemiologic studies examining diet-disease relationships. However, the FFQ is subject to bias because it asks participants to recall intakes over long periods of time leading to error-prone estimation. This estimation leads to a “flattened slope” phenomenon wherein subjects with high intake of an item tend to underestimate their intake and subjects with low intake tend to overestimate, reducing the true range of intakes (50). This phenomenon effectively reduces the power to detect diet-disease

relationships in a sample and, when coupled with classical random error, has the potential to attenuate any underlying relationships (9).

The other major method, 24HR, is considered a more accurate measure of short-term intake and is often used to “calibrate” FFQs to remove some of the systematic error (49). Although this method is considered more accurate, it is often interviewer administered which puts greater burden on researchers and is still subject to considerable systemic error (11). The sources of systematic error are different with the 24HR method, and are suspected to include consistent underestimation of portion sizes and potential misreporting to meet perceived expectations of interviewers (51).

These data show that both FFQs and 24HRs are subject to systematic and random error and generally tend to show underreporting of total energy and individual food items and nutrients. These biases have the potential to attenuate true diet-disease relationships that may exist in epidemiologic studies and are part of the reason for inconsistent, conflicting results.

Challenges in Quantifying Whole-Grain Intake

The quantification of whole-grain intake by self-report is also strongly affected by the lack of a universal definition of whole-grain and by consumer inability to interpret nutrition labels and identify whole-grain products. Within the United States, a number of governing bodies maintain whole-grain definitions. However, the most widely used definition is the Food and Drug Administration’s which was adopted from the American Association of Cereal Chemists and states that whole-grains consist of “intact, ground, cracked or flaked fruit of the grains whose principal components – the starchy endosperm, germ and bran – are present in the same relative proportions as

they exist in the intact grain.” (4). This definition was adopted in 2006 and subsequently the FDA approved a number of label statement health claims regarding the relation of whole-grain intake and cancer and CVD. However, most of the research that the FDA based the approval of these claims on was done using broader definitions of whole-grain. In 2009, De Moura et al. (4) revisited the evidence the FDA used to approve these claims and found that only 2 out of 29 studies adhered to the 2006 FDA definition. This led the authors to conclude that there is insufficient evidence to substantiate most whole-grain related health claims when the FDA definition is applied to research. A number of leading cereal grain researchers recently collaborated on a paper addressing the issue of whole-grain definitions in research and came up with five recommendations for rigorous quantification of whole-grain intake (43). These include quantifying whole-grain content in food on a dry weight basis, identifying the whole-grain definition used, reporting different types of grains with enriched germ and bran reported separately and noting the milling and processing of the grains. These standard would reduce or eliminate most of the inconsistency in whole-grain research, however, exceedingly few studies meet all of these reporting recommendations.

These reporting recommendations are also not met on most commercially available food products, which leads to consumer confusion and inability to identify whole-grain items (43). Many different terms are used to describe grain products on labels including whole-grain, whole-meal, multi-grain, cereal, 100% whole-grain and partial whole-grain. The rise of alternative grains and pseudograins like couscous, buckwheat and quinoa have also led to consumer confusion due to difficulties in

identifying items, first as a grain and second as a whole-grain (5). All of these factors combine to make it very difficult to accurately quantify whole-grain intake in free-living populations in order to elucidate diet-disease relationships. This has led researchers to seek an objective biomarker of whole-grain intake to attenuate or circumvent these challenges completely.

Alkylresorcinols

Biomarker Characteristics

Biomarkers can help circumvent many of the issues with self-reported diet intake assessment and are an important research area in nutritional science (52). Dietary intake biomarkers are objectively measureable molecules that can be used to quantify recent intake of a specific food item or nutrient and are most commonly measured in blood and urine (11, 53). Intake biomarkers help to assess actual intake versus self-reported intake, validate compliance during controlled feeding trials, evaluate intake of specific nutrients when nutrition research databases are inadequate and reduce error-induced bias when evaluating diet-disease relationships (10). In 2007 during a meeting on establishing dietary reference intakes, the Institute of Medicine noted that the lack of dietary intake biomarkers is an important research gap that must be addressed in order to better evaluate diet-disease relationships and improve overall nutritional assessment (52).

Dietary biomarkers can be grouped into two classes; concentration and recovery (54). Concentration biomarkers are based on a concentration measurement at a single timepoint in a body fluid or tissue sample. These biomarkers provide a rough estimate of intake, but are subject to higher inter-individual variability and cannot be

translated into an absolute quantitative measure of intake (55). Factors such as endogenous production, enzymatic metabolism or absorption may affect the amount of these biomarkers that are found in samples between individuals (56). Another class, recovery biomarkers, exhibit a defined and well-characterized balance between intake and excretion for all individuals in a population (57). Because recovery is always a fixed proportion of intake for all individuals, intakes can be accurately computed from measures of these biomarkers(58). Relatively few recovery biomarkers have been discovered and widely used. The three most commonly used are urinary nitrogen to measure protein intake, urinary potassium to measure potassium intake and doubly labelled water to measure total energy intake (10).

Biomarkers are further classified into time classes by the period of time over which they reflect intake. Short term biomarkers reflect intake over hours to days, medium term reflect weeks to months and long term reflect months to years (10). The time class is primarily determined by the type of sample required to detect biomarker. Short to medium term biomarkers are usually detected free in plasma or urine. Medium to long term biomarkers are usually detected in red blood cells, hair or adipose tissue biopsies. The rate of metabolism and extent of storage of biomarkers are the primary determinants of their time class (10).

Alkylresorcinols

Recently a group of phenolic lipids called alkylresorcinols (ARs) has been proposed as a short to medium term concentration biomarker of whole-grain intake (59-61). These molecules are dihydroxylated phenols with an alkyl side chain of variable length. The length of the alkyl chain determines the AR homologue identity.

Dietary ARs are obtained almost exclusively in the bran of whole-grain wheat and rye. Refined wheat and rye have very low AR contents due to the removal of the bran during processing, making AR a potentially good biomarker for intake of these whole-grains. The ratio of AR homologues varies between grain species with rye having a C:17/C:21 ratio near 1 and wheat having a C:17/C:21 ratio near 0.1, allowing for further specificity in quantifying intake (62).

Absorption

Alkylresorcinols are readily absorbed and systemically distributed in the body with consistent pharmacokinetics. Confirmation of AR absorption was provided in studies by Ross et al. (63, 64) which used an ileostomy model in pigs and humans. In the pig study, four experimental diets comprised of bread made from differing milled rye fractions were fed to ileal cannulated pigs three times per day for two weeks. Ileostomy effluent was collected on days 12 and 14 and analyzed for AR recovery. No AR was detected in effluent from pigs consuming bread made with endosperm only. Recovery values from bran-containing treatment groups ranged from 20-40% indicating that 60-80% of ingested AR was absorbed in the proximal small intestine. A dose effect was observed, with the highest AR treatments showing lower absorption than low AR treatments. No major differences in homologue absorption were observed.

These results were corroborated in crossover study in 10 human ileostomy patients ages 34-51 years (63). This study consisted of a high and low AR diet with two meal frequencies within each diet. Each participant completed two weeks of the high and low AR diets with one week of each meal frequency within the diets. There

was a two week AR-free diet run-in and a one week washout between diet treatments. When consuming about 215 mg/day AR via rye bread on the high AR diet, AR recovery in ileostomy effluent was about 40%, indicating about 60% absorption in the proximal small intestine. There was no effect of meal frequency on absorption, but longer chain homologues (C:23 and C:25) showed significantly lower absorption than shorter chain homologues (C:17-C:21).

Despite strong evidence that AR are absorbed in the small intestine, the molecular mechanism for absorption is not known. It has been hypothesized that, due to their structural similarities, AR may be absorbed in a similar manner to tocopherols which are taken up by scavenger receptor class B and incorporated into chylomicrons for lymphatic transport or directly transferred to HDL particles (65). Although the mode of absorption is not definitively known, ARs show consistent plasma kinetics after ingestion.

Metabolism and Excretion

The catabolic mechanisms for AR are not fully understood, but limited experimental evidence and inferences from structurally similar molecules have given some clues. *In vitro* studies have provided strong evidence for the first step in the AR metabolic pathway (66). In a series of experiments published in 2013, Marklund et al. (66) used three *in vitro* models to investigate the initial steps of AR metabolism. The three model systems were insect microsomes expressing human CYP4F2, human liver subcellular fraction S9 and HepG2 human hepatocytes. Each system was incubated with the AR homologue C19:0 at multiple combinations over multiple time courses. Alkylresorcinol metabolites were analyzed in media samples using gas

chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography with electrochemical detection. Due to structural similarities, it was hypothesized that ARs are metabolized similar to tocopherols, which are biotransformed to allow for β -oxidation, resulting in the formation of two metabolites that are excreted in the urine.

It was hypothesized that the first step in AR catabolism is CYP-mediated ω -oxidation. To test this, CYP4F2-expressing microsomes were screened for hydroxylated AR after an incubation with C19:0. Hydroxylated AR was found in incubated samples and was absent in control samples, supporting ω -oxidation as the first step of AR catabolism. This result was confirmed in human liver S9, supporting ω -oxidation of ARs by human hepatic microsomal enzymes. Alkyl chains must ultimately be carboxylated to allow for β -oxidation, and carboxylated AR was also observed at lower concentrations in S9 fractions after AR incubation. Finally, researchers checked for 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-propanoic acid (DHPPA), which are the end products of β -oxidation of AR, in HepG2 cells incubated with C19:0. They found DHPPA but were not able to detect DHBA, possibly because of its higher limit of detection. These results support hepatic ω - and β -oxidation as primary AR catabolic pathways resulting in DHPPA and DHBA formation which have been proposed as the primary excretory metabolites (67-69).

Pharmacokinetics

Despite incomplete information on their biotransformation, AR pharmacokinetics have been characterized (66, 70-74). In 2006 Linko et al. (70)

performed two experiments investigating the appearance and clearance of ARs in plasma using a pig model under controlled feeding conditions. In the first experiment, four pigs were fed a refined-grain wheat enriched diet or a whole-grain rye enriched diet for one week in a crossover design. The diets were approximately equivalent in most macro- and micronutrients except ARs (1158 vs 0 $\mu\text{g/g}$ in rye vs wheat). Multiple blood samples were taken on days 5 and 7 around feeding at -30, 0, 30 and 60 minutes then every 60 minutes up 480 minutes post-feeding. In the second experiment, four pigs were fed an AR-free diet for 5 days, then on the morning of day 6 were fed the whole-grain rye enriched diet. Blood samples were taken around feeding at -30, 0, 30 and 60 minutes, then every 60 minutes up to 960 minutes post-feeding. This experiment was performed twice in the same animals.

Figure 4 Appearance of AR in plasma from the portal vein and mesenteric arteries of pigs (N=4) after a single rye meal intake. Values are means $\pm$ SD. From Linko et al.

In the first experiment pigs fed the refined wheat enriched diet showed basal AR concentrations of 35 ± 4 nmol/L before feeding and a peak concentration

of 74 ± 7.5 nmol/L at 180 minutes post feeding despite having no detectable ARs in the feed. Pigs fed the rye enriched diet consumed approximately 1244 μmol of AR per feeding. They showed a basal concentration of 260 ± 77 nmol/L before feeding and a peak concentration of 662 ± 92 nmol/L at 240 minutes post feeding. At 480 minutes, concentrations were still significantly elevated compared to baseline (370 ± 52 nmol/L).

In the second study, pigs consumed an approximately 1471 μmol of ARs in a single feeding. Baseline AR concentrations were 30 ± 3.7 nmol/L before feeding. Levels began to rise at 60 minutes post feeding and peaked at 180-240 minutes post feeding (666 ± 35 nmol/L). At 960 minutes post-feeding, concentrations were still significantly elevated compared to baseline (136 ± 34 nmol/L) (figure 4). Blood was drawn from mesenteric arteries and portal veins with no observed differences in AR concentrations between the two sources. This suggests that ARs are not directly absorbed into capillaries at the basolateral aspect of intestinal absorptive enterocytes and must enter the blood via lymphatic circulation. The relatively long times needed reach peak plasma concentrations (3-4 hours) also support lymphatic uptake.

In 2006 Landberg et al. (71) confirmed these finding in humans. Participants (N=6) were 50% female with an average age of 26 ± 1.1 years and an average weight of 74 ± 3.9 kg. One week before the study participants were instructed to avoid any foods containing whole-grain wheat or rye or bran from wheat or rye. On the test day participants were given a 120 g portion of rye bran containing approximately 495 μg of ARs. Participants consumed at least 200 mL of 3% fat yogurt with the rye bran because fat is hypothesized to aid in AR absorption (75). Blood samples were taken at baseline and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hours post intake. Participants were fed a standardized, AR-free lunch four hours after intake, then all dietary restrictions were lifted.

Baseline samples showed less than 2 nmol/L ARs after the one week run-in. After intake, two major concentration peaks were identified. The first peak occurred at 2.4-3.4 hours post intake with a concentration of 1253 ± 123 nmol/L. The second peak occurred at 6.5 hours post intake with a concentration of 3365 ± 309 nmol/L

Figure 5 Total plasma AR concentrations from human plasma (N=6) after a single rye bran flake meal. From Landberg et al, 2006.

(figure). The observed half-life was 4.8 hours. Differences were noted in bioavailability with longer chain lengths showing higher bioavailability. This trend fit an exponential curve and was significant ($R^2=0.97$, $P<0.05$). The relatively short plasma half-lives observed in these experiments show that ARs may function best as a short to medium term whole-grain intake biomarker.

Once in circulation ARs have been shown to travel in lipoproteins. In 2007 Linko-Parvinen et al. (14) conducted a study looking at alkylresorcinol distribution in components of blood after controlled feeding of wheat and rye breads. Fifteen healthy, normal weight ($BMI\ 24.4 \pm 0.99\ kg/m^2$) Nordic men and women (age 21-36 years) were included in the randomized crossover design. Participants consumed only refined breads for a one week baseline period then were randomized to either 100 g/day of whole-grain wheat or rye crisp bread for one week. The first treatment was followed by a one week washout then the second treatment. Blood samples were taken before the refined grain baseline, after each one week period and one week after completion

of the trial. Alkylresorcinol concentrations were determined plasma, erythrocytes and lipoproteins via gas chromatography–mass spectrometry.

Results showed that whole-grain wheat and rye treatments both increased overall plasma AR concentrations compared to habitual diet and baseline/washout periods. Over 70% of ARs measured in plasma were accounted for in the lipoprotein fraction. When accounting for loss of sample in lipoprotein isolation the authors speculated that over 80% of ARs could be contained in the lipoprotein fraction. Lipoprotein-deficient plasma showed no detectable ARs, strengthening the hypothesis that ARs are transported almost exclusively in lipoproteins. Within the lipoprotein fraction in fasting blood samples, very low-density lipoprotein (VLDL) particles contained the highest proportion of ARs ($46\pm2.1\%$) followed by HDL particles ($33\pm1.5\%$) and LDL particles ($20\pm1.3\%$). The authors also speculated that ARs are likely absorbed, incorporated into chylomicrons, transported into circulation via the lymphatic system and incorporated into VLDL in the liver, similar to tocopherols. These data illustrate the lipophilic nature of ARs and suggest that changes in blood lipid profile may affect AR metabolism.

In addition to incorporation into lipid fractions, acute and habitual consumption of ARs has been shown to result in their accumulation in adipose in rats, pigs and humans. In 2004 Ross et al. (76) conducted an experiment to examine the effects of ARs on growth and tocopherol metabolism in rats. Rats (N=32) were fed ARs isolated from rye bran at 0, 1, 2 or 4 g/kg diet for four weeks. After four weeks, rats were fasted for 12 hours, euthanized, and blood, liver, lung and perirenal adipose samples were collected. Samples were analyzed for lipid, AR and tocopherol content.

Analyses showed that ARs accumulated in the perirenal fat pads of the rats in concentrations as high as 4 µg/g fresh tissue. Diets higher in ARs also increased serum, lung and liver tocopherol concentrations by inhibiting tocopherol-degrading enzymes. This effect has not been well-characterized, but fits with other *in vitro* findings such as suppression of lipolysis via inhibition of hormone sensitive lipase in 3T3-L1 adipocytes (77). Increased ARs also strongly decreased liver cholesterol concentrations, but did not affect serum cholesterol levels. These data demonstrate that ARs are sequestered in lipid depots at relatively high concentrations and may be able to elicit systemic effects even after serum levels return to baseline. The liver-specific reductions in cholesterol and systemic increases in tocopherols may also be part of the mechanism underlying the cardioprotective effect of whole-grain wheat and rye.

Jansson et al. (78) also showed AR accumulation in adipose tissue of free-living humans. In this study women (N=20) from the Swedish Mammography Cohort had adipose tissue samples taken from an outer upper buttock quadrant, blood samples taken and whole-grain intake quantified using a 123-item FFQ that reflected intake over the prior six months. Alkylresorcinol content was quantified in the adipose and blood samples using GC-MS.

Alkylresorcinol quantification in human adipose samples showed an average of 0.54 ± 0.35 µg/g dry tissue with a range of below the limit of detection to 1.50 µg/g. The authors speculated that the large variation could be due to a number of factors including intake, bioavailability and adipose depot size. Alkylresorcinol content in human adipose samples showed a significant positive correlation with total daily whole-grain bread intake as quantified by FFQ ($r = 0.48$). These results confirm that

ARs are stored in adipose tissue depots in humans, and suggest that AR concentrations in adipose tissue may reflect longer term intake of whole-grains.

Once AR are absorbed they are rapidly metabolized and cleared from circulation with a half-life of 5-7 hours (71). The full catabolic mechanism is not known, but it is speculated to be similar to that of tocopherols which includes conversion of the carbon chain to an acyl-CoA ester and subsequent β -oxidation (64, 79). This supposition has been bolstered by the discovery of AR metabolites (DHBA and DHPPA) that are analogous to tocopherol catabolic end products (80). These metabolites have been identified in plasma and urine and are the major form of AR excreted in the urine (81).

Figure 6 After ingestion ARs are absorbed in the intestine and packaged into chylomicrons for lymphatic circulation (A). Once in the blood ARs circulate in VLDL particles and are distributed to other compartments like adipose tissue (B, C). ARs are metabolized in the liver to two major metabolites which are excreted in the urine (E, D). ARs can also be excreted via biliary secretion (E,F).

Collectively, these data show that dietary ARs are absorbed and metabolized in the body and that they show adequate specificity to act as a biomarker for whole-wheat and rye intake. In addition to specificity, good biomarkers must show dose-dependency and reproducibility. These characteristics have been demonstrated in a number of human trials.

Validation of Alkylresorcinols as a Biomarker

Controlled Feeding Trials

In 2009 Landberg et al. (59) conducted a controlled feeding trial to assess the dose response of plasma ARs to increasing intakes of whole-grain rye. Fifteen participants (N=9 women) with an average age of 30.6 ± 10.3 years and an average BMI of 23 ± 3.3 kg/m² were recruited from the Uppsala region of Sweden. The study used a randomized, non-blinded, 3-way crossover design in which each participant consumed three levels of rye bran for one week each with a one week washout period between treatments. Fasting blood samples were taken before and after the one week run in period and after each treatment and washout period. Participants were instructed to maintain their habitual diet with the exception to avoid all whole-grain or bran of wheat and rye during the week before and throughout the study. The treatments were 7.5, 15 or 30 g doses of rye bran flakes, containing 11, 22 and 44 mg ARs respectively, which were taken three times per day.

Blood samples prior to the run in period showed an average plasma AR concentration of 117 ± 69 nmol/L which dropped to 68 ± 33 after the one week run in period. Plasma concentrations increased significantly between each increasing treatment (148 ± 60 , 210 ± 81 , 455 ± 189 , $P > 0.05$) as assessed by mixed linear model using period and dose as fixed factors

Figure 7 Total plasma AR concentration by quartile of daily AR intake estimated from weighed food records. $N = 112$.

and participants as a random factor. Within subject variability in AR concentration was moderate at approximately 30%, as assessed using washout period values.

However, there was considerable between-subjects variability which was greatest at the highest AR doses. Although a clear dose-response relationship was shown,

variability in the data complicated predictive modeling. The variability between subjects forced the researchers to group participants into AR intake quartiles rather than using AR as a continuous variable in the regression model, which made it

impossible to explicitly define a linear AR dose-plasma concentration relationship.

Additionally, predicted plasma AR concentrations derived from regression models

showed high variability despite attempting to keep intake at a steady state through the study design. These complications led the researchers to conclude that plasma AR

concentration may be a good concentration biomarker, but is not a potential recovery biomarker for whole-grain intake.

In 2009 Landberg et al. (82) also investigated the reproducibility of plasma ARs during a randomized non-blinded crossover whole-grain rye intervention in male prostate cancer patients. Participants (N=17) were male and had clinically diagnosed prostate cancer. Average age was 73.5 ± 4.6 years and average BMI was 27.5 ± 4.6 kg/m². Participants received a whole-grain rye and a refined wheat diet separated by a two week washout period. There was no run in period. Each intervention lasted six weeks and participants were given 300g (3 pieces) of bread, 100 g (10 pieces) of crisp bread, 50 g of breakfast cereal, 33 g porridge and 58 g of table spread each day. Participants were instructed to consume the intervention products during breakfast, lunch, dinner and two or three snacks each day. Participants were also instructed to eliminate all other cereal products from their diets during the intervention periods. Total AR content was approximately 6,800 µg/day for the whole-grain rye diet and 70 µg/day for the refined wheat diet. Fasting blood samples were taken before and after the second, fourth and sixth weeks of each treatment period to measure plasma AR concentration.

Actual daily AR intakes were 552 ± 65 and 8.2 ± 2.0 during rye and wheat treatments respectively as assessed by multiple 4 day food logs. Average plasma AR concentrations were 991 ± 794 and 75 ± 92 nmol/L during rye and wheat treatments respectively. The intraclass correlation coefficient (ICC) was 0.90 (95% CI; [0.82, 0.98]) during the first intervention period and 0.99 (95% CI; [0.78, 0.98]) during the second intervention period. Although the ICCs were high, variability in the data still made modeling exact intake difficult, even under controlled conditions. Confidence interval modeling showed that during the first intervention period nearly 20 plasma

samples would be required to ascertain true mean plasma AR concentrations with a 5% precision (D) level and 95% confidence.

In 2012 Ross et al. (12) conducted an extended feeding trial comprised of 252 overweight or obese participants over a 16 week treatment period. Participants were recruited at two centers in Newcastle and Cambridge, England. Participants were overweight but otherwise healthy with low habitual whole-grain intake (< 30 g/day). Participants were randomized into one of three treatment groups. The control group was instructed to maintain their habitual diet and was not told that the study was investigating whole-grains. The first treatment group was instructed to consume three 20 g servings of whole-grain foods per day along with their normal diet. The second treatment group was instructed to consume three 20 g servings of whole-grain foods during weeks 1-8 and to increase their intake to six 20 g servings during weeks 9-16. All whole-grain foods were provided to the participants. Fasting blood samples were taken at baseline and during weeks 8 and 16. Self-reported whole-grain intake was also assessed at baseline and during weeks 8 and 16 via a 149 item FFQ.

Total whole-grain intake was significantly correlated with plasma AR levels in all participants at week 16 ($r = 0.35$). Approximately 65% of the whole-grains consumed throughout the study were from wheat, therefore the correlation between whole-grain wheat intake and plasma AR levels was stronger than for overall whole-grain intake at week 16 ($r = 0.43$). Correlations were strongest in the overall study population when whole-grain intakes were highest at week 16. Linear modeling identified five significant predictors of AR concentration; whole-grain intake, gender, non-esterified free fatty acids, triglycerides and study center location. Although

triglycerides were a significant predictor in the overall model, they were only correlated with plasma AR levels in males. This led to speculation that differences between males and females in the overall model may be due to gender-specific differences in lipid metabolism and distribution. As with other studies, interindividual variability and confounding variables made it impossible to define a quantitative model to predict whole-grain intake based on plasma AR levels. However, quartiles of plasma AR levels were able to predict whole-grain intake quartile; 70-79% of participants were classified in the same or adjacent quartiles and only 9-12% were grossly misclassified into a non-adjacent quartile.

Although this was an intervention study it contained a number of naturalistic elements that made it more applicable to free-living and epidemiologic studies. First, compliance was measured through self-report using an FFQ rather than through collection of unused food products. Second, other whole-grain products were not excluded from participants' diets and the control group was not restricted from consuming whole-grains. Third, the study specifically targeted overweight and obese individuals which is a group known to consistently misreport their intake to a greater extent than other groups (11, 41, 83, 84). Despite the inclusion of these naturalistic and confounding elements significant associations were still observed, strengthening the case for plasma ARs as a whole-grain concentration biomarker.

Studies in Free-Living Populations

The ultimate goal of identifying dietary intake biomarkers is to better assess habitual dietary intake in free-living populations, therefore, findings under controlled feeding conditions must be replicable in non-experimental designs. Numerous studies

have investigated the validity of ARs as a whole-grain intake biomarker in free-living populations.

In 2008 Aubertin-Leheudre et al. (85) investigated the relationship between cereal fiber intake and plasma AR levels in a group of Finnish women. Participants (N=56) were an average age of 46 ± 13 years, with an average BMI of 22.5 ± 2.7 kg/m² and 43% were post-menopausal. Participants filled out 5-day food records including at least one weekend day on two occasions approximately 6 months apart. Fasting blood samples were taken on the third day of the 5-day food record.

Results showed a significant correlation between total plasma AR and cereal fiber intake ($r = 0.379$) (figure) as well as between each individual AR homologue (C:17-C:25) and cereal fiber intake. Interestingly, vegetarians (N=20) had a higher

Figure 8 Pearson's correlation between cereal fiber intake and total plasma AR. Cereal fiber intake assessed by 3d diet record. From Aubertin-Leheudre et al. 2008.

correlation between total plasma AR and cereal fiber intake than omnivores ($r = 0.548$ vs 0.372) and also had higher cereal fiber intake (11.0 ± 3.6 vs 9.5 ± 2.6 g/day). These results suggest that plasma AR may work better as a biomarker in individuals who consume more whole-grains. This is consistent with findings from Ross et al. (12) and invalidates plasma ARs as a recovery biomarker because it is not equally robust across all populations. Regression analysis showed that plasma AR levels were not highly influenced by age or BMI and they also did not significantly correlate with other types

of fiber such as berry, legume and vegetable fiber. This shows that plasma ARs are robust to confounding by age and BMI, and show adequate specificity in reflecting only whole-grain intake. Although this study did show significant moderate correlations between total plasma AR and individual homologues and cereal fiber intake, the study design did not exactly reflect most dietary data collection methods used in epidemiologic studies. Plasma AR levels were measured during the time that diet was being monitored using food recalls, hence the measure was not reflecting habitual diet in the same way that an FFQ usually does.

In 2011 Andersson et al. (60) conducted a similar study investigating the effectiveness and reproducibility of plasma AR levels in a free-living population. Participants (N=72) were recruited in and around Uppsala, Sweden and had an average age of 42 ± 17 years, an average BMI of 24.3 ± 3.9 kg/m² and were 76% female. Participants completed two 3-day weighted food records 2-3 months apart after receiving oral and written instructions on keeping food records and being provided with a portable scale for measuring their intakes. At the end of each food record period, the majority of participants (N=51) provided one fasting blood sample. A subset of the participants (N=18) provided fasting blood samples on each day of the first food record period and non-fasting blood samples on each day of the second food record period.

Neither whole-grain intake or total fiber intake were significantly correlated to total plasma AR levels at either time point ($r = 0.24, 0.16$ respectively). Total cereal fiber intake and combined intake of whole-grain wheat and rye were significantly correlated to total plasma AR levels ($r = 0.32, 0.54$ respectively). Fasting total plasma

AR measures were significantly correlated over 2-3 months ($r_s = 0.38$). Over 2-3 days fasting plasma AR levels also showed high reproducibility (ICC = 0.60, 95% CI [0.36 – 0.80]) but non-fasting levels showed low reproducibility (ICC = 0.18, 95% CI [0.03 – 0.62]). All correlations between intake and plasma AR levels were only significant when comparing intakes and AR measures from the same testing period. Reported whole-grain intakes and AR measures from different periods did not show any significant associations. However, AR measures from both periods were significantly correlated with whole-grain intake when it was averaged across both periods.

These results show that fasting plasma AR levels have high reproducibility over a period of days and moderate reproducibility over a period of months. They also confirm that non-fasting AR levels should not be used as an indicator of habitual intake because they are greatly influenced by recent intake. This study was conducted in Sweden where the general population consumes relatively high amounts of whole-grains and rye. The average total fiber intake in the study population was 30 ± 14 g/day, the average cereal fiber intake was 11 ± 12 g/day and the average whole-grain rye intake was 29 ± 27 g/day. This may affect the generalizability of these results due to the fact that other research has suggested that plasma AR levels are a more effective biomarker at higher whole-grain intake levels (12, 85).

In 2009 Ross et al. (86) used a novel whole-grain focused FFQ to test the associations between self-reported whole-grain intake and plasma AR levels in a free-living population. Participants (N=33) were recruited from the area surrounding Lausanne, Switzerland and were 58% female with an average age of 38 ± 12 years and an average BMI of 23 ± 3 kg/m². Participants completed a 43 item whole-grain focused

FFQ on days 1 (FFQ1) and 14 (FFQ2), and a 3 day weighed food record sometime in between. Upon completion of the 3 day weighed food record participants provided a fasting blood sample for AR analysis.

Measures of total whole-grain intake as measured by the weighed 3 day food record and both rounds of the FFQ were significantly correlated ($r = 0.72, 0.81$ respectively). All three measures of total whole-grain intake were also significantly correlated with plasma AR levels ($r = 0.57$ 3 day record (figure), 0.54 FFQ1, 0.55 FFQ2). In attempting to create a quantifiable model of whole-grain intake using plasma AR levels, researchers found that AR levels could only distinguish between low ($<16\text{g/day}$) and moderate/high ($>29\text{ g/day}$) whole-grain consumers. The study was not powered to conduct gender-specific analyses.

These results in free-living populations highlight two major points about the use of ARs. First, both controlled and free-living studies have shown that plasma ARs are a more effective biomarker in individuals with higher whole-grain intakes (12, 59, 85).

Figure 9 Correlation between whole-grain intake and log total plasma AR. Whole-grain intake assessed by 3d weighed food record. Shading indicates tertiles.

Many of these studies (60, 61, 85-89) were conducted in Nordic populations that consume more whole-grains than US populations; Americans consume an average of 0.97 oz servings of whole-grains per day (90), while observational studies investigating ARs in Nordic populations have reported average intakes of 4-8 oz servings per day (60, 87, 91, 92). This may be positively biasing the overall body of research. Second, plasma AR levels are influenced by factors other than whole-grain intake. The most prominent factors seem to be plasma lipid levels (specifically triglycerides) and gender, although it has been proposed that gender-specific differences in plasma lipids are responsible for the gender effect (12).

Diet-Disease Relationships

One of the major goals in developing dietary intake biomarkers is to make more accurate and statistically powerful assessments of diet-disease relationships by eliminating some of the error that is inherent in self-reported dietary data. It is expected that a good whole-grain intake biomarker would strengthen the diet-disease relationships seen in epidemiologic studies. However, only a limited number of studies have looked at the associations between plasma AR concentrations and cardiometabolic risk factors (18, 19, 93)

In 2012 Ma et al. (19) examined the relationships between self-reported whole-grain intake, plasma AR levels and cardiometabolic risk factors in older adults. The study was an ancillary analysis on data from a vitamin K supplementation intervention trial. Participants (N=407) were 66% female with an average age of 68±6 years and a median BMI of 27 kg/m². Dietary intake was assessed using the Harvard semiquantitative 126 item FFQ which was completed at baseline and at 1 year. Fasting

blood samples used for AR analyses were taken at 6 months. Plasma AR concentration was significantly associated with both whole-grain rich food and bran intake ($r_s = 0.31, 0.27$ respectively). There were no significant associations between plasma AR concentrations and BMI, fasting blood glucose or insulin. After adjusting for age, sex, energy intake, physical activity, smoking status, alcohol intake, vegetable intake and fruit intake, one-way ANOVA showed a significant decrease in BMI across plasma AR quartiles (p trend = 0.04). Although the highest plasma AR quartile had a significantly lower average BMI than the lowest plasma AR quartile, the two groups differed by less than 1 kg/m² (26.7 vs 27.6 kg/m²).

In 2014 Magnustdottir et al. (18) looked at associations between plasma AR concentration and glucose and insulin homeostasis by performing a subanalysis on data from the SYSDIET Study (94). The SYSDIET Study was a randomized controlled dietary intervention in nondiabetic participants with metabolic syndrome conducted in six centers across Denmark, Sweden, Finland and Iceland. It examined the effects of an 18 week Nordic Diet intervention which included $\geq 25\%$ of energy from whole-grains of which at least 50% were rye, barley or oats. Participants in the Nordic diet group (N=96) received whole-grain products including oats, barley, rye crisp bread and whole-grain wheat and rye bread. Participants in the control group (N=70) received refined grain versions of similar products. Diet intake was assessed from multiple four day food records kept every 1-2 weeks throughout the study and checked by dietitians for completeness and accuracy. Biochemical measures were taken at baseline, week 12 and week 24 and included blood lipids, fasting glucose and

insulin. Participants also underwent an oral glucose tolerance test (OGTT) at the same timepoints.

There were no significant associations observed between total plasma AR or any individual homologues and fasting blood glucose, insulin or indices of insulin sensitivity. The C17:0/C21:0 was significantly inversely associated with fasting insulin and also significantly positively associated with the Matsuda ISI and DI indices of insulin sensitivity in covariate-adjusted models.

In another 2014 study Magnusdottir et al. (93) looked at associations between plasma AR concentrations and blood lipid outcomes in the same SYSDIET study population. Total plasma AR concentration was not significantly associated with any blood lipid measures (LDL-C, HDL-C, LDL-C/HDL-C ratio, TAG, apolipoprotein A1 or apolipoprotein B) after adjusting for confounders. The C17:0/C21:0 ratio was significantly inversely associated with LDL-C, LDL-C/HDL-C ratio, non-HDL-C, TAG and apolipoprotein B, and significantly positively associated with HDL-C in covariate adjusted models. Most of these associations were lost when a Bonferroni correction for multiple testing was applied and only HDL-C and TAG were robust to the correction.

These results show weak associations between plasma AR concentrations and the C17:0/C21:0 ratio and cardiometabolic risk factors. Most of the significant models relied heavily on confounding adjustments and, although the associations were significant, most of the achieved effect sizes were small. However, these three studies were all done in older adults with notable cardiometabolic risk. Over 90% of the participants in the SYSDIET studies (18, 93) had metabolic syndrome and in the study

by Ma et al. (19) over 70% of participants were overweight or obese. These results warrant further study in younger, healthier populations.

Conclusion

The research presented provides strong evidence supporting the use of plasma AR concentration as a concentration biomarker of whole-grain intake. Experimental and observational studies have shown that ARs are valid (71, 89), reproducible (60, 95) and dose-responsive (59). Although ARs meet these broad criteria, research has also shown that interindividual variability makes using ARs for precise predictive modeling nearly impossible (12). This implies that ARs may be best used as broader indicators of overall whole-grain intake, or predictors of membership in a whole-grain intake group.

A number of unanswered questions remain in AR research. The first is whether ARs perform equally well across a range of whole-grain intakes. The majority of research done (60, 61, 85-89) was conducted in Nordic populations that consume relatively high amounts of whole-grains. To confirm that ARs are a universally valid biomarker, more validation needs to be done in populations that consume few whole-grains, primarily from wheat. The second unanswered question is whether ARs can strengthen the diet-disease relationships seen with whole-grains in epidemiologic studies (1, 2, 35). If ARs are a valid biomarker it is expected that they will inversely correlate with a number of cardiometabolic risk factors. Limited research has focused on this question, and the studies that have been done have used samples of older, unhealthy adults (18, 19, 93). More research is needed in these two areas to further validate and strengthen ARs as whole-grain intake biomarkers.

Table 1 Experimental and observational studies validating plasma AR concentration as a whole-grain intake biomarker

Reference	No. of Subjects	Gender	Country	Study Type	Intake Measured	Plasma AR Correlation
Landberg et al. (42)	15	M/F	Sweden	Intervention	Rye bran	NR
Landberg et al. (61)	17	M	Sweden	Intervention	Total WG	NR
Ross et al. (11)	252	M/F	United Kingdom	Intervention	Total WG	0.32
Aubertin-Leheudre et al. (64)	56	F	Finland	Free-living	Cereal fiber	0.38
Andersson et al. (43)	51	M/F	Sweden	Free-living	Total WG	0.53
Ross et al. (65)	29	M/F	Switzerland	Free-living	Total WG	0.55
Landberg et al. (44)	30	M/F	Sweden	Intervention	Total WG	0.58
Landberg et al. (66)	360	F	Denmark	Free-living	WG Rye bread	0.25
Magnusdottir et al. (67)	126	M/F	Finland, Sweden, Denmark, Iceland	Intervention	Total fiber	0.26-0.57
Linko et al. (68)	39	F	Finland	Intervention	WG Rye bread	0.34
Ma et al. (18)	407	M/F	United States	Free-living	WG Wheat foods	0.31

REFERENCES

1. Sahyoun NR, Jacques PF, Zhang XLL, Juan WY, McKeown NM. Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. *Am J Clin Nutr* 2006;83(1):124-31.
2. Mellen PB, Walsh TF, Herrington DM. Whole grain intake and cardiovascular disease: A meta-analysis. *Nutr Metab Cardiovas* 2008;18(4):283-90. doi: 10.1016/j.numecd.2006.12.008.
3. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu SM. Greater Whole-Grain Intake Is Associated with Lower Risk of Type 2 Diabetes, Cardiovascular Disease, and Weight Gain. *J Nutr* 2012;142(7):1304-13. doi: 10.3945/jn.111.155325.
4. De Moura FF, Lewis KD, Falk MC. Applying the FDA Definition of Whole Grains to the Evidence for Cardiovascular Disease Health Claims. *J Nutr* 2009;139(11):2220S-6S. doi: 10.3945/jn.109.112383.
5. Jones JM. Regulatory Aspects of Whole Grain and Whole Grain Foods: Definitions and Labeling. *Cereal Chem* 2010;87(2):150-4. doi: 10.1094/cchem-87-2-0150.
6. Ross AB, Bruce SJ, Blondel-Lubrano A, Oguey-Araymon S, Beaumont M, Bourgeois A, Nielsen-Moennoz C, Vigo M, Fay LB, Kochhar S, et al. A whole-grain cereal-rich diet increases plasma betaine, and tends to decrease total and LDL-cholesterol compared with a refined-grain diet in healthy subjects. *Br J Nutr* 2011;105(10):1492-502. doi: 10.1017/s0007114510005209.
7. Liu SM, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am J Clin Nutr* 2003;78(5):920-7.
8. Jackson KH, West SG, Vanden Heuvel JP, Jonnalagadda SS, Ross AB, Hill AM, Grieger JA, Lemieux SK, Kris-Etherton PM. Effects of whole and refined grains in a weight-loss diet on markers of metabolic syndrome in individuals with increased waist circumference: a randomized controlled-feeding trial. *Am J Clin Nutr* 2014;100(2):577-86. doi: 10.3945/ajcn.113.078048.
9. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing With Dietary Measurement Error in Nutritional Cohort Studies. *J Natl Cancer Inst* 2011;103(14):1086-92. doi: 10.1093/jnci/djr189.

10. Hedrick VE, Dietrich AM, Estabrooks PA, Savla J, Serrano E, Davy BM. Dietary biomarkers: advances, limitations and future directions. *Nutr J* 2012;11:14. doi: 10.1186/1475-2891-11-109.
11. Livingstone MBE, Black AE. Markers of the validity of reported energy intake. *J Nutr* 2003;133(3):895S-920S.
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(1):204-11. doi: 10.3945/ajcn.110.008508.
13. Andersson AAM, Dimberg L, Aman P, Landberg R. Recent findings on certain bioactive components in whole grain wheat and rye. *J Cereal Sci* 2014;59(3):294-311. doi: 10.1016/j.jcs.2014.01.003.
14. Linko-Parvinen AM, Landberg R, Tikkarren MJ, Adlercreutz H, Penalvo JL. Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. *J Nutr* 2007;137(5):1137-42.
15. Knudsen MD, Kyro C, Olsen A, Dragsted LO, Skeie G, Lund E, Aringman P, Nilsson LM, Bueno-de-Mesquita HB, Tjonneland A, et al. Self-Reported Whole-Grain Intake and Plasma Alkylresorcinol Concentrations in Combination in Relation to the Incidence of Colorectal Cancer. *Am J Epidemiol* 2014;179(10):1188-96. doi: 10.1093/aje/kwu031.
16. Kyro C, Olsen A, Landberg R, Skeie G, Loft S, Aman P, Leenders M, Dik VK, Siersema PD, Pischon T, et al. Plasma Alkylresorcinols, Biomarkers of Whole-Grain Wheat and Rye Intake, and Incidence of Colorectal Cancer. *JNCI- Natl Cancer Inst* 2014;106(1):9. doi: 10.1093/jnci/djt352.
17. Kristensen M, Toubro S, Jensen MG, Ross AB, Riboldi G, Petronio M, Bugel S, Tetens I, Astrup A. Whole Grain Compared with Refined Wheat Decreases the Percentage of Body Fat Following a 12-Week, Energy-Restricted Dietary Intervention in Postmenopausal Women. *J Nutr* 2012;142(4):710-6. doi: 10.3945/jn.111.142315.
18. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Akesson B, Landin-Olsson M, Rosqvist F, Iggman D, Schwab U, Herzig KH, et al. Plasma alkylresorcinols C17:0/C21:0 ratio, a biomarker of relative whole-grain rye intake, is associated to insulin sensitivity: a randomized study. *Eur J Clin Nutr* 2014;68(4):453-8. doi: 10.1038/ejcn.2014.12.
19. Ma JT, Ross A, Bruce SJ, Jacques P, Lichtenstein AH, Saltzman E, Booth S, McKeown N. Biomarker of whole grain wheat intake associated lower BMI in older adults. *Faseb J* 2012;26:1.

20. Liu SM, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, Hennekens CH, Willett WC. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* 1999;70(3):412-9.
21. Liu SM, Manson JE, Stampfer MJ, Rexrode KM, Hu FB, Rimm EB, Willett WC. Whole grain consumption and risk of ischemic stroke in women - A prospective study. *JAMA-J Am Med Assoc* 2000;284(12):1534-40. doi: 10.1001/jama.284.12.1534.
22. Mozaffarian D, Kumanyika SK, Lemaitre RN, Olson JL, Burke GL, Siscovick DS. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. *JAMA-J Am Med Assoc* 2003;289(13):1659-66. doi: 10.1001/jama.289.13.1659.
23. Jensen MK, Koh-Banerjee P, Hu FB, Franz M, Sampson L, Gronbaek M, Rimm EB. Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. *Am J Clin Nutr* 2004;80(6):1492-9.
24. Liu SM, Buring JE, Sesso HD, Rimm EB, Willett WC, Manson JE. A prospective study of dietary fiber intake and risk of cardiovascular disease among women. *J Am Coll Cardiol* 2002;39(1):49-56. doi: 10.1016/s0735-1097(01)01695-3.
25. Liu SM, Manson JE, Stampfer MJ, Hu FB, Giovannucci E, Colditz GA, Hennekens CH, Willett WC. A prospective study of whole-grain intake and risk of type 2 diabetes mellitus in US women. *Am J Public Health* 2000;90(9):1409-15. doi: 10.2105/ajph.90.9.1409.
26. Pereira MA, Jacobs DR, Pins JJ, Raatz SK, Gross MD, Slavin JL, Seaquist ER. Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. *Am J Clin Nutr* 2002;75(5):848-55.
27. de Munter JSL, Hu FB, Spiegelman D, Franz M, van Dam RM. Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *PLoS Med* 2007;4(8):1385-95. doi: 10.1371/journal.pmed.0040261.
28. Fung TT, Hu FB, Pereira MA, Liu SM, Stampfer MJ, Colditz GA, Willett WC. Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. *Am J Clin Nutr* 2002;76(3):535-40.
29. McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PWF, Jacques PF. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic

syndrome in the Framingham Offspring Cohort. *Diabetes Care* 2004;27(2):538-46. doi: 10.2337/diacare.27.2.538.

30. Esmailzadeh A, Mirmiran P, Azizi F. Whole-grain consumption and the metabolic syndrome: a favorable association in Tehranian adults. *Eur J Clin Nutr* 2005;59(3):353-62. doi: 10.1038/sj.ejcn.1602080.
31. Steffen LM, Jacobs DR, Stevens J, Shahar E, Carithers T, Folsom AR. Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) study. *Am J Clin Nutr* 2003;78(3):383-90.
32. Kant AK, Schatzkin A, Graubard BI, Schairer C. A prospective study of diet quality and mortality in women. *JAMA-J Am Med Assoc* 2000;283(16):2109-15. doi: 10.1001/jama.283.16.2109.
33. Wu YH, Qian YF, Pan YW, Li PW, Yang J, Ye XH, Xu G. Association between dietary fiber intake and risk of coronary heart disease: A meta-analysis. *Clin Nutr* 2015;34(4):603-11. doi: 10.1016/j.clnu.2014.05.009.
34. Zhu SK, Wang ZM, Heshka S, Heo MS, Faith MS, Heymsfield SB. Waist circumference and obesity-associated risk factors among whites in the third National Health and Nutrition Examination Survey: clinical action thresholds. *Am J Clin Nutr* 2002;76(4):743-9.
35. Yao BD, Fang H, Xu WH, Yan YJ, Xu HL, Liu YN, Mo M, Zhang H, Zhao YP. Dietary fiber intake and risk of type 2 diabetes: a dose-response analysis of prospective studies. *Eur J Epidemiol* 2014;29(2):79-88. doi: 10.1007/s10654-013-9876-x.
36. Andersson A, Tengblad S, Karlstrom B, Kamal-Eldin A, Landberg R, Basu S, Aman P, Vessby B. Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. *J Nutr* 2007;137(6):1401-7.
37. Chen CYO, Milbury PE, Collins FW, Blumberg JB. Avenanthramides are bioavailable and have antioxidant activity in humans after acute consumption of an enriched mixture from oats. *J Nutr* 2007;137(6):1375-82.
38. Wang Q, Han PH, Zhang MW, Xia M, Zhu HL, Ma J, Hou MJ, Tang ZH, Ling WH. Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. *Asia Pac J Clin Nutr* 2007;16:295-301.

39. Parikka K, Rowland IR, Welch RW, Wahala K. In vitro antioxidant activity and antigenotoxicity of 5-n-alkylresorcinols. *J Agric Food Chem* 2006;54(5):1646-50. doi: 10.1021/jf052486e.
40. Labaj J, Slamenova D, Lazarova M, Kosikova M. Lignin-stimulated reduction of oxidative DNA lesions in testicular cells and lymphocytes of Sprague-Dawley rats in vitro and ex vivo. *Nutr Cancer* 2004;50(2):198-205. doi: 10.1207/s15327914nc5002_10.
41. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, Sharbaugh CO, Trabulsi J, Runswick S, Ballard-Barbash R, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: The OPEN Study. *Am J Epidemiol* 2003;158(1):1-13. doi: 10.1093/aje/kwg092.
42. Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, Bingham S, Schoeller DA, Schatzkin A, Carroll RJ. Structure of dietary measurement error: Results of the OPEN biomarker study. *Am J Epidemiol* 2003;158(1):14-21. doi: 10.1093/aje/kwg091.
43. Ross AB, Kristensen M, Seal CJ, Jacques P, McKeown NM. Recommendations for reporting whole-grain intake in observational and intervention studies. *Am J Clin Nutr* 2015;101(5):903-7. doi: 10.3945/ajcn.114.098046.
44. Black AE, Cole TJ. Biased over- or under-reporting is characteristic of individuals whether over time or by different assessment methods. *J Am Diet Assoc* 2001;101(1):70-80. doi: 10.1016/s0002-8223(01)00018-9.
45. Freedman LS, Carroll RJ, Wax Y. ESTIMATING THE RELATION BETWEEN DIETARY-INTAKE OBTAINED FROM A FOOD FREQUENCY QUESTIONNAIRE AND TRUE AVERAGE INTAKE. *Am J Epidemiol* 1991;134(3):310-20.
46. Schoeller DA, Ravussin E, Schutz Y, Acheson KJ, Baertschi P, Jequier E. ENERGY-EXPENDITURE BY DOUBLY LABELED WATER - VALIDATION IN HUMANS AND PROPOSED CALCULATION. *Am J Physiol* 1986;250(5):R823-R30.
47. Rossato SL, Fuchs SC. Handling random errors and biases in methods used for short-term dietary assessment. *Revista De Saude Publica* 2014;48(5):845-50. doi: 10.1590/s0034-8910.2014048005154.
48. Rosner B, Willett WC, Spiegelman D. CORRECTION OF LOGISTIC-REGRESSION RELATIVE RISK ESTIMATES AND CONFIDENCE-

INTERVALS FOR SYSTEMATIC WITHIN-PERSON MEASUREMENT ERROR. *Stat Med* 1989;8(9):1051-69.

49. Kipnis V, Carroll RJ, Freedman LS, Li L. Implications of a new dietary measurement error model for estimation of relative risk: Application to four calibration studies. *Am J Epidemiol* 1999;150(6):642-51.
50. Wacholder S. WHEN MEASUREMENT ERRORS CORRELATE WITH TRUTH - SURPRISING EFFECTS OF NONDIFFERENTIAL MISCLASSIFICATION. *Epidemiology* 1995;6(2):157-61. doi: 10.1097/00001648-199503000-00012.
51. Lichtman SW, Pisarska K, Berman ER, Pestone M, Dowling H, Offenbacher E, Weisel H, Heshka S, Matthews DE, Heymsfield SB. DISCREPANCY BETWEEN SELF-REPORTED AND ACTUAL CALORIC-INTAKE AND EXERCISE IN OBESE SUBJECTS. *New Engl J Med* 1992;327(27):1893-8. doi: 10.1056/nejm199212313272701.
52. Dietary Reference Intakes Research Synthesis: Workshop Summary. Washington, DC: National Academies Press, 2007.
53. Hardin DS. Validating Dietary Intake with Biochemical Markers. *J Am Diet Assoc* 2009;109(10):1698-9. doi: 10.1016/j.jada.2009.07.014.
54. Kaaks RJ. Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: Conceptual issues. *Am J Clin Nutr* 1997;65(4):1232-9.
55. Potischman N. Biologic and methodologic issues for nutritional biomarkers. *J Nutr* 2003;133(3):875S-80S.
56. Kaaks R, Ferrari P, Ciampi A, Plummer M, Riboli E. Uses and limitations of statistical accounting for random error correlations, in the validation of dietary questionnaire assessments. *Public Health Nutr* 2002;5(6A):969-76. doi: 10.1079/phn2002380.
57. Tasevska N, Runswick SA, Bingham SA. Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. *J Nutr* 2006;136(5):1334-40.
58. Bingham SA. Urine nitrogen as a biomarker for the validation of dietary protein intake. *J Nutr* 2003;133(3):921S-4S.
59. Landberg R, Aman 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 (vol 89, pg 290, 2009). *Am J Clin Nutr* 2009;89(5):1478-. doi: 10.3945/ajcn.2009.27667.

60. Andersson A, Marklund M, Diana M, Landberg R. Plasma Alkylresorcinol Concentrations Correlate with Whole Grain Wheat and Rye Intake and Show Moderate Reproducibility over a 2-to 3-Month Period in Free-Living Swedish Adults. *J Nutr* 2011;141(9):1712-8. doi: 10.3945/jn.111.139238.
61. 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. *Am J Clin Nutr* 2008;87(4):832-8.
62. Chen Y, Ross AB, Aman P, Kamal-Eldin A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. *J Agric Food Chem* 2004;52(26):8242-6. doi: 10.1021/jf049726v.
63. Ross AB, Kamal-Eldin A, Lundin EA, Zhang JX, Hallmans G, Aman P. Cereal alkylresorcinols are absorbed by humans. *J Nutr* 2003;133(7):2222-4.
64. Ross AB, Shepherd MJ, Knudsen KEB, Glitz LV, Bowey E, Phillips J, Rowland I, Guo ZX, Massy DJR, Aman P, et al. Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats. *Br J Nutr* 2003;90(4):787-94. doi: 10.1079/bjn2003965.
65. Rigotti A. Absorption, transport, and tissue delivery of vitamin E. *Mol Asp Med* 2007;28(5-6):423-36. doi: 10.1016/j.mam.2007.01.002.
66. Marklund M, McKeown NM, Blumberg JB, Chen CYO. Hepatic biotransformation of alkylresorcinols is mediated via cytochrome P450 and beta-oxidation: A proof of concept study. *Food Chem* 2013;139(1-4):925-30. doi: 10.1016/j.foodchem.2013.01.122.
67. Aubertin-Leheudre M, Koskela A, Samaletdin A, Adlercreutz H. Responsiveness of urinary and plasma alkylresorcinol metabolites to rye intake in finnish women. *Cancers* 2010;2(2):513-22. doi: 10.3390/cancers2020513.
68. Guyman LA, Adlercreutz H, Koskela A, Li L, Beresford SAA, Lampe JW. Urinary 3-(3,5-dihydroxyphenyl)-1-propanoic acid, an alkylresorcinol metabolite, is a potential biomarker of whole-grain intake in a US population. *J Nutr* 2008;138(10):1957-62.
69. Marklund M, Landberg R, Andersson A, Aman P, Kamal-Eldin A. Alkylresorcinol metabolites in urine correlate with the intake of whole grains and cereal fibre in free-living Swedish adults. *Br J Nutr* 2013;109(1):129-36. doi: 10.1017/s0007114512000621.

70. Linko AM, Ross AB, Kamal-Eldin A, Serena A, Kjaer AKB, Jorgensen H, Penalvo JL, Adlercreutz H, Aman P, Knudsen KEB. Kinetics of the appearance of cereal alkylresorcinols in pig plasma. *Br J Nutr* 2006;95(2):282-7. doi: 10.1079/bjn20051642.
71. Landberg R, Linko AM, Kamal-Eldin A, Vessby B, Adlercreutz H, Aman P. Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. *J Nutr* 2006;136(11):2760-5.
72. Soderholm PP, Koskela AH, Lundin JE, Tikkanen MJ, Adlercreutz HC. Plasma pharmacokinetics of alkylresorcinol metabolites: new candidate biomarkers for whole-grain rye and wheat intake. *Am J Clin Nutr* 2009;90(5):1167-71. doi: 10.3945/ajcn.2009.28290.
73. Marklund M, Stromberg EA, Laerke HN, Knudsen KEB, Kamal-Eldin A, Hooker AC, Landberg R. Simultaneous Pharmacokinetic Modeling of Alkylresorcinols and Their Main Metabolites Indicates Dual Absorption Mechanisms and Enterohepatic Elimination in Humans. *J Nutr* 2014;144(11):1674-80. doi: 10.3945/jn.114.196220.
74. Zhu YD, Shurlknight KL, Chen XX, Sang SM. Identification and Pharmacokinetics of Novel Alkylresorcinol Metabolites in Human Urine, New Candidate Biomarkers for Whole-Grain Wheat and Rye Intake. *J Nutr* 2014;144(2):114-22. doi: 10.3945/jn.113.184663.
75. Borel P. Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clin Chem Lab Med* 2003;41(8):979-94. doi: 10.1515/cclm.2003.151.
76. Ross AB, Chen Y, Frank J, Swanson JE, Parker RS, Kozubek A, Lundh T, Vessby B, Aman P, Kamal-Eldin A. Cereal alkylresorcinols elevate gamma-tocopherol levels in rats and inhibit gamma-tocopherol metabolism in vitro. *J Nutr* 2004;134(3):506-10.
77. Andersson U, Dey ES, Holm C, Degerman E. Rye bran alkylresorcinols suppress adipocyte lipolysis and hormone-sensitive lipase activity. *Mol Nutr Food Res* 2011;55:S290-S3. doi: 10.1002/mnfr.201100231.
78. Jansson E, Landberg R, Kamal-Eldin A, Wolk A, Vessby B, Aman P. Presence of alkylresorcinols, potential whole grain biomarkers, in human adipose tissue. *Br J Nutr* 2010;104(5):633-6. doi: 10.1017/s0007114510001169.
79. Birringer M, Drozan D, Brigelius-Flohe R. Tocopherols are metabolized in HepG2 cells by side chain omega-oxidation and consecutive beta-oxidation.

- Free Radic Biol Med 2001;31(2):226-32. doi: 10.1016/s0891-5849(01)00574-3.
80. Ross AB, Aman P, Kamal-Eldin A. Identification of cereal alkylresorcinol metabolites in human urine - potential biomarkers of wholegrain wheat and rye intake. *J Chromatogr B* 2004;809(1):125-30. doi: 10.1016/j.jchromb.2004.06.015.
 81. Marklund M, Landberg R, Aman P, Kamal-Eldin A. Determination of alkylresorcinol metabolites in human urine by gas chromatography-mass spectrometry. *J Chromatogr B* 2010;878(11-12):888-94. doi: 10.1016/j.jchromb.2010.02.007.
 82. Landberg R, Kamal-Eldin A, Andersson SO, Johansson JE, Zhang JX, Hallmans G, Aman P. Reproducibility of Plasma Alkylresorcinols during a 6-Week Rye Intervention Study in Men with Prostate Cancer. *J Nutr* 2009;139(5):975-80. doi: 10.3945/jn.108.099952.
 83. Paalanen L, Mannisto S, Virtanen MJ, Knekt P, Rasanen L, Montonen J, Pietinen P. Validity of a food frequency questionnaire varied by age and body mass index. *J Clin Epidemiol* 2006;59(9):994-1001. doi: 10.1016/j.jclinepi.2006.01.002.
 84. Braam L, Ocke MC, Bueno-de-Mesquita HB, Seidell JC. Determinants of obesity-related underreporting of energy intake. *Am J Epidemiol* 1998;147(11):1081-6.
 85. Aubertin-Leheudre M, Koskela A, Marjamaa A, Adlercreutz H. Plasma alkylresorcinols and urinary alkylresorcinol metabolites as biomarkers of cereal fiber intake in Finnish women. *Cancer Epidemiol Biomarkers Prev* 2008;17(9):2244-8. doi: 10.1158/1055-9965.epi-08-0215.
 86. Ross AB, Pineau N, Kochhar S, Bourgeois A, Beaumont M, Decarli B. Validation of a FFQ for estimating whole-grain cereal food intake. *Br J Nutr* 2009;102(11):1547-51. doi: 10.1017/s0007114509990845.
 87. Landberg R, Kamal-Eldin A, Aman P, Christensen J, Overvad K, Tjønneland A, Olsen A. Determinants of plasma alkylresorcinol concentration in Danish post-menopausal women. *Eur J Clin Nutr* 2011;65(1):94-101. doi: 10.1038/ejcn.2010.193.
 88. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Akesson B, Onning G, Jonsdottir SE, Rosqvist F, Schwab U, Herzig KH, et al. Plasma Alkylresorcinols Reflect Important Whole-Grain Components of a Healthy Nordic Diet. *J Nutr* 2013;143(9):1383-90. doi: 10.3945/jn.113.175588.

89. Linko AM, Juntunen KS, Mykkanen HM, Adlercreutz H. Whole-grain rye bread consumption by women correlates with plasma alkylresorcinols and increases their concentration compared with low-fiber wheat bread. *J Nutr* 2005;135(3):580-3.
90. Albertson AM, Reicks M, Joshi N, Gugger CK. Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional National Health and Nutrition Examination Survey 2001-2012. *Nutr J* 2016;15:14. doi: 10.1186/s12937-016-0126-4.
91. Ross AB, Becker W, Chen Y, Kamal-Eldin A, Aman P. Intake of alkylresorcinols from wheat and rye in the United Kingdom and Sweden. *Br J Nutr* 2005;94(4):496-9. doi: 10.1079/bjn20051511.
92. Marklund M, Landberg R, Andersson R, Aman P, Kamal-Eldin A. Alkylresorcinol Metabolism in Swedish Adults Is Affected by Factors Other Than Intake of Whole-Grain Wheat and Rye. *J Nutr* 2012;142(8):1479-86. doi: 10.3945/jn.112.159244.
93. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Akesson B, Rosqvist F, Schwab U, Herzig KH, Hukkanen J, Savolainen MJ, et al. Whole Grain Rye Intake, Reflected by a Biomarker, Is Associated with Favorable Blood Lipid Outcomes in Subjects with the Metabolic Syndrome - A Randomized Study. *PLoS One* 2014;9(10):8. doi: 10.1371/journal.pone.0110827.
94. Uusitupa M, Hermansen K, Savolainen MJ, Schwab U, Kolehmainen M, Brader L, Mortensen LS, Cloetens L, Johansson-Persson A, Oumlnning G, et al. Effects of an isocaloric healthy Nordic diet on insulin sensitivity, lipid profile and inflammation markers in metabolic syndrome - a randomized study (SYSDIET). *J Int Med* 2013;274(1):52-66. doi: 10.1111/joim.12044.
95. Montonen J, Landberg R, Kamal-Eldin A, Aman P, Knueppel S, Boeing H, Pischon T. Reliability of fasting plasma alkylresorcinol concentrations measured 4 months apart. *Eur J Clin Nutr* 2010;64(7):698-703. doi: 10.1038/ejcn.2010.71.

BIBLIOGRAPHY

1. Sahyoun NR, Jacques PF, Zhang XLL, Juan WY, McKeown NM. Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. *Am J Clin Nutr* 2006;83(1):124-31.
2. Mellen PB, Walsh TF, Herrington DM. Whole grain intake and cardiovascular disease: A meta-analysis. *Nutr Metab Cardiovas* 2008;18(4):283-90. doi: 10.1016/j.numecd.2006.12.008.
3. Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu SM. Greater Whole-Grain Intake Is Associated with Lower Risk of Type 2 Diabetes, Cardiovascular Disease, and Weight Gain. *J Nutr* 2012;142(7):1304-13. doi: 10.3945/jn.111.155325.
4. De Moura FF, Lewis KD, Falk MC. Applying the FDA Definition of Whole Grains to the Evidence for Cardiovascular Disease Health Claims. *J Nutr* 2009;139(11):2220S-6S. doi: 10.3945/jn.109.112383.
5. Jones JM. Regulatory Aspects of Whole Grain and Whole Grain Foods: Definitions and Labeling. *Cereal Chem* 2010;87(2):150-4. doi: 10.1094/cchem-87-2-0150.
6. Ross AB, Bruce SJ, Blondel-Lubrano A, Oguey-Araymon S, Beaumont M, Bourgeois A, Nielsen-Moennoz C, Vigo M, Fay LB, Kochhar S, et al. A whole-grain cereal-rich diet increases plasma betaine, and tends to decrease total and LDL-cholesterol compared with a refined-grain diet in healthy subjects. *Br J Nutr* 2011;105(10):1492-502. doi: 10.1017/s0007114510005209.
7. Liu SM, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am J Clin Nutr* 2003;78(5):920-7.
8. Jackson KH, West SG, Vanden Heuvel JP, Jonnalagadda SS, Ross AB, Hill AM, Grieger JA, Lemieux SK, Kris-Etherton PM. Effects of whole and refined grains in a weight-loss diet on markers of metabolic syndrome in individuals with increased waist circumference: a randomized controlled-feeding trial. *Am J Clin Nutr* 2014;100(2):577-86. doi: 10.3945/ajcn.113.078048.
9. Freedman LS, Schatzkin A, Midthune D, Kipnis V. Dealing With Dietary Measurement Error in Nutritional Cohort Studies. *J Natl Cancer Inst* 2011;103(14):1086-92. doi: 10.1093/jnci/djr189.

10. Hedrick VE, Dietrich AM, Estabrooks PA, Savla J, Serrano E, Davy BM. Dietary biomarkers: advances, limitations and future directions. *Nutr J* 2012;11:14. doi: 10.1186/1475-2891-11-109.
11. Livingstone MBE, Black AE. Markers of the validity of reported energy intake. *J Nutr* 2003;133(3):895S-920S.
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(1):204-11. doi: 10.3945/ajcn.110.008508.
13. Andersson AAM, Dimberg L, Aman P, Landberg R. Recent findings on certain bioactive components in whole grain wheat and rye. *J Cereal Sci* 2014;59(3):294-311. doi: 10.1016/j.jcs.2014.01.003.
14. Linko-Parvinen AM, Landberg R, Tikkarren MJ, Adlercreutz H, Penalvo JL. Alkylresorcinols from whole-grain wheat and rye are transported in human plasma lipoproteins. *J Nutr* 2007;137(5):1137-42.
15. Knudsen MD, Kyro C, Olsen A, Dragsted LO, Skeie G, Lund E, Aringman P, Nilsson LM, Bueno-de-Mesquita HB, Tjonneland A, et al. Self-Reported Whole-Grain Intake and Plasma Alkylresorcinol Concentrations in Combination in Relation to the Incidence of Colorectal Cancer. *Am J Epidemiol* 2014;179(10):1188-96. doi: 10.1093/aje/kwu031.
16. Kyro C, Olsen A, Landberg R, Skeie G, Loft S, Aman P, Leenders M, Dik VK, Siersema PD, Pischon T, et al. Plasma Alkylresorcinols, Biomarkers of Whole-Grain Wheat and Rye Intake, and Incidence of Colorectal Cancer. *JNCI- Natl Cancer Inst* 2014;106(1):9. doi: 10.1093/jnci/djt352.
17. Kristensen M, Toubro S, Jensen MG, Ross AB, Riboldi G, Petronio M, Bugel S, Tetens I, Astrup A. Whole Grain Compared with Refined Wheat Decreases the Percentage of Body Fat Following a 12-Week, Energy-Restricted Dietary Intervention in Postmenopausal Women. *J Nutr* 2012;142(4):710-6. doi: 10.3945/jn.111.142315.
18. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Akesson B, Landin-Olsson M, Rosqvist F, Iggman D, Schwab U, Herzig KH, et al. Plasma alkylresorcinols C17:0/C21:0 ratio, a biomarker of relative whole-grain rye intake, is associated to insulin sensitivity: a randomized study. *Eur J Clin Nutr* 2014;68(4):453-8. doi: 10.1038/ejcn.2014.12.
19. Ma JT, Ross A, Bruce SJ, Jacques P, Lichtenstein AH, Saltzman E, Booth S, McKeown N. Biomarker of whole grain wheat intake associated lower BMI in older adults. *Faseb J* 2012;26:1.

20. Liu SM, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, Hennekens CH, Willett WC. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am J Clin Nutr* 1999;70(3):412-9.
21. Liu SM, Manson JE, Stampfer MJ, Rexrode KM, Hu FB, Rimm EB, Willett WC. Whole grain consumption and risk of ischemic stroke in women - A prospective study. *JAMA-J Am Med Assoc* 2000;284(12):1534-40. doi: 10.1001/jama.284.12.1534.
22. Mozaffarian D, Kumanyika SK, Lemaitre RN, Olson JL, Burke GL, Siscovick DS. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. *JAMA-J Am Med Assoc* 2003;289(13):1659-66. doi: 10.1001/jama.289.13.1659.
23. Jensen MK, Koh-Banerjee P, Hu FB, Franz M, Sampson L, Gronbaek M, Rimm EB. Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. *Am J Clin Nutr* 2004;80(6):1492-9.
24. Liu SM, Buring JE, Sesso HD, Rimm EB, Willett WC, Manson JE. A prospective study of dietary fiber intake and risk of cardiovascular disease among women. *J Am Coll Cardiol* 2002;39(1):49-56. doi: 10.1016/s0735-1097(01)01695-3.
25. Liu SM, Manson JE, Stampfer MJ, Hu FB, Giovannucci E, Colditz GA, Hennekens CH, Willett WC. A prospective study of whole-grain intake and risk of type 2 diabetes mellitus in US women. *Am J Public Health* 2000;90(9):1409-15. doi: 10.2105/ajph.90.9.1409.
26. Pereira MA, Jacobs DR, Pins JJ, Raatz SK, Gross MD, Slavin JL, Seaquist ER. Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. *Am J Clin Nutr* 2002;75(5):848-55.
27. de Munter JSL, Hu FB, Spiegelman D, Franz M, van Dam RM. Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *PLoS Med* 2007;4(8):1385-95. doi: 10.1371/journal.pmed.0040261.
28. Fung TT, Hu FB, Pereira MA, Liu SM, Stampfer MJ, Colditz GA, Willett WC. Whole-grain intake and the risk of type 2 diabetes: a prospective study in men. *Am J Clin Nutr* 2002;76(3):535-40.
29. McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PWF, Jacques PF. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic

syndrome in the Framingham Offspring Cohort. *Diabetes Care* 2004;27(2):538-46. doi: 10.2337/diacare.27.2.538.

30. Esmailzadeh A, Mirmiran P, Azizi F. Whole-grain consumption and the metabolic syndrome: a favorable association in Tehranian adults. *Eur J Clin Nutr* 2005;59(3):353-62. doi: 10.1038/sj.ejcn.1602080.
31. Steffen LM, Jacobs DR, Stevens J, Shahar E, Carithers T, Folsom AR. Associations of whole-grain, refined-grain, and fruit and vegetable consumption with risks of all-cause mortality and incident coronary artery disease and ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) study. *Am J Clin Nutr* 2003;78(3):383-90.
32. Kant AK, Schatzkin A, Graubard BI, Schairer C. A prospective study of diet quality and mortality in women. *JAMA-J Am Med Assoc* 2000;283(16):2109-15. doi: 10.1001/jama.283.16.2109.
33. Wu YH, Qian YF, Pan YW, Li PW, Yang J, Ye XH, Xu G. Association between dietary fiber intake and risk of coronary heart disease: A meta-analysis. *Clin Nutr* 2015;34(4):603-11. doi: 10.1016/j.clnu.2014.05.009.
34. Zhu SK, Wang ZM, Heshka S, Heo MS, Faith MS, Heymsfield SB. Waist circumference and obesity-associated risk factors among whites in the third National Health and Nutrition Examination Survey: clinical action thresholds. *Am J Clin Nutr* 2002;76(4):743-9.
35. Yao BD, Fang H, Xu WH, Yan YJ, Xu HL, Liu YN, Mo M, Zhang H, Zhao YP. Dietary fiber intake and risk of type 2 diabetes: a dose-response analysis of prospective studies. *Eur J Epidemiol* 2014;29(2):79-88. doi: 10.1007/s10654-013-9876-x.
36. Andersson A, Tengblad S, Karlstrom B, Kamal-Eldin A, Landberg R, Basu S, Aman P, Vessby B. Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. *J Nutr* 2007;137(6):1401-7.
37. Chen CYO, Milbury PE, Collins FW, Blumberg JB. Avenanthramides are bioavailable and have antioxidant activity in humans after acute consumption of an enriched mixture from oats. *J Nutr* 2007;137(6):1375-82.
38. Wang Q, Han PH, Zhang MW, Xia M, Zhu HL, Ma J, Hou MJ, Tang ZH, Ling WH. Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. *Asia Pac J Clin Nutr* 2007;16:295-301.

39. Parikka K, Rowland IR, Welch RW, Wahala K. In vitro antioxidant activity and antigenotoxicity of 5-n-alkylresorcinols. *J Agric Food Chem* 2006;54(5):1646-50. doi: 10.1021/jf052486e.
40. Labaj J, Slamenova D, Lazarova M, Kosikova M. Lignin-stimulated reduction of oxidative DNA lesions in testicular cells and lymphocytes of Sprague-Dawley rats in vitro and ex vivo. *Nutr Cancer* 2004;50(2):198-205. doi: 10.1207/s15327914nc5002_10.
41. Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, Sharbaugh CO, Trabulsi J, Runswick S, Ballard-Barbash R, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: The OPEN Study. *Am J Epidemiol* 2003;158(1):1-13. doi: 10.1093/aje/kwg092.
42. Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, Bingham S, Schoeller DA, Schatzkin A, Carroll RJ. Structure of dietary measurement error: Results of the OPEN biomarker study. *Am J Epidemiol* 2003;158(1):14-21. doi: 10.1093/aje/kwg091.
43. Ross AB, Kristensen M, Seal CJ, Jacques P, McKeown NM. Recommendations for reporting whole-grain intake in observational and intervention studies. *Am J Clin Nutr* 2015;101(5):903-7. doi: 10.3945/ajcn.114.098046.
44. Black AE, Cole TJ. Biased over- or under-reporting is characteristic of individuals whether over time or by different assessment methods. *J Am Diet Assoc* 2001;101(1):70-80. doi: 10.1016/s0002-8223(01)00018-9.
45. Freedman LS, Carroll RJ, Wax Y. ESTIMATING THE RELATION BETWEEN DIETARY-INTAKE OBTAINED FROM A FOOD FREQUENCY QUESTIONNAIRE AND TRUE AVERAGE INTAKE. *Am J Epidemiol* 1991;134(3):310-20.
46. Schoeller DA, Ravussin E, Schutz Y, Acheson KJ, Baertschi P, Jequier E. ENERGY-EXPENDITURE BY DOUBLY LABELED WATER - VALIDATION IN HUMANS AND PROPOSED CALCULATION. *Am J Physiol* 1986;250(5):R823-R30.
47. Rossato SL, Fuchs SC. Handling random errors and biases in methods used for short-term dietary assessment. *Revista De Saude Publica* 2014;48(5):845-50. doi: 10.1590/s0034-8910.2014048005154.
48. Rosner B, Willett WC, Spiegelman D. CORRECTION OF LOGISTIC-REGRESSION RELATIVE RISK ESTIMATES AND CONFIDENCE-

INTERVALS FOR SYSTEMATIC WITHIN-PERSON MEASUREMENT ERROR. *Stat Med* 1989;8(9):1051-69.

49. Kipnis V, Carroll RJ, Freedman LS, Li L. Implications of a new dietary measurement error model for estimation of relative risk: Application to four calibration studies. *Am J Epidemiol* 1999;150(6):642-51.
50. Wacholder S. WHEN MEASUREMENT ERRORS CORRELATE WITH TRUTH - SURPRISING EFFECTS OF NONDIFFERENTIAL MISCLASSIFICATION. *Epidemiology* 1995;6(2):157-61. doi: 10.1097/00001648-199503000-00012.
51. Lichtman SW, Pisarska K, Berman ER, Pestone M, Dowling H, Offenbacher E, Weisel H, Heshka S, Matthews DE, Heymsfield SB. DISCREPANCY BETWEEN SELF-REPORTED AND ACTUAL CALORIC-INTAKE AND EXERCISE IN OBESE SUBJECTS. *New Engl J Med* 1992;327(27):1893-8. doi: 10.1056/nejm199212313272701.
52. Dietary Reference Intakes Research Synthesis: Workshop Summary. Washington, DC: National Academies Press, 2007.
53. Hardin DS. Validating Dietary Intake with Biochemical Markers. *J Am Diet Assoc* 2009;109(10):1698-9. doi: 10.1016/j.jada.2009.07.014.
54. Kaaks RJ. Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: Conceptual issues. *Am J Clin Nutr* 1997;65(4):1232-9.
55. Potischman N. Biologic and methodologic issues for nutritional biomarkers. *J Nutr* 2003;133(3):875S-80S.
56. Kaaks R, Ferrari P, Ciampi A, Plummer M, Riboli E. Uses and limitations of statistical accounting for random error correlations, in the validation of dietary questionnaire assessments. *Public Health Nutr* 2002;5(6A):969-76. doi: 10.1079/phn2002380.
57. Tasevska N, Runswick SA, Bingham SA. Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. *J Nutr* 2006;136(5):1334-40.
58. Bingham SA. Urine nitrogen as a biomarker for the validation of dietary protein intake. *J Nutr* 2003;133(3):921S-4S.
59. Landberg R, Aman 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 (vol 89, pg 290, 2009). *Am J Clin Nutr* 2009;89(5):1478-. doi: 10.3945/ajcn.2009.27667.

60. Andersson A, Marklund M, Diana M, Landberg R. Plasma Alkylresorcinol Concentrations Correlate with Whole Grain Wheat and Rye Intake and Show Moderate Reproducibility over a 2-to 3-Month Period in Free-Living Swedish Adults. *J Nutr* 2011;141(9):1712-8. doi: 10.3945/jn.111.139238.
61. 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. *Am J Clin Nutr* 2008;87(4):832-8.
62. Chen Y, Ross AB, Aman P, Kamal-Eldin A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. *J Agric Food Chem* 2004;52(26):8242-6. doi: 10.1021/jf049726v.
63. Ross AB, Kamal-Eldin A, Lundin EA, Zhang JX, Hallmans G, Aman P. Cereal alkylresorcinols are absorbed by humans. *J Nutr* 2003;133(7):2222-4.
64. Ross AB, Shepherd MJ, Knudsen KEB, Glitz LV, Bowey E, Phillips J, Rowland I, Guo ZX, Massy DJR, Aman P, et al. Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats. *Br J Nutr* 2003;90(4):787-94. doi: 10.1079/bjn2003965.
65. Rigotti A. Absorption, transport, and tissue delivery of vitamin E. *Mol Asp Med* 2007;28(5-6):423-36. doi: 10.1016/j.mam.2007.01.002.
66. Marklund M, McKeown NM, Blumberg JB, Chen CYO. Hepatic biotransformation of alkylresorcinols is mediated via cytochrome P450 and beta-oxidation: A proof of concept study. *Food Chem* 2013;139(1-4):925-30. doi: 10.1016/j.foodchem.2013.01.122.
67. Aubertin-Leheudre M, Koskela A, Samaletdin A, Adlercreutz H. Responsiveness of urinary and plasma alkylresorcinol metabolites to rye intake in finnish women. *Cancers* 2010;2(2):513-22. doi: 10.3390/cancers2020513.
68. Guyman LA, Adlercreutz H, Koskela A, Li L, Beresford SAA, Lampe JW. Urinary 3-(3,5-dihydroxyphenyl)-1-propanoic acid, an alkylresorcinol metabolite, is a potential biomarker of whole-grain intake in a US population. *J Nutr* 2008;138(10):1957-62.
69. Marklund M, Landberg R, Andersson A, Aman P, Kamal-Eldin A. Alkylresorcinol metabolites in urine correlate with the intake of whole grains and cereal fibre in free-living Swedish adults. *Br J Nutr* 2013;109(1):129-36. doi: 10.1017/s0007114512000621.

70. Linko AM, Ross AB, Kamal-Eldin A, Serena A, Kjaer AKB, Jorgensen H, Penalvo JL, Adlercreutz H, Aman P, Knudsen KEB. Kinetics of the appearance of cereal alkylresorcinols in pig plasma. *Br J Nutr* 2006;95(2):282-7. doi: 10.1079/bjn20051642.
71. Landberg R, Linko AM, Kamal-Eldin A, Vessby B, Adlercreutz H, Aman P. Human plasma kinetics and relative bioavailability of alkylresorcinols after intake of rye bran. *J Nutr* 2006;136(11):2760-5.
72. Soderholm PP, Koskela AH, Lundin JE, Tikkanen MJ, Adlercreutz HC. Plasma pharmacokinetics of alkylresorcinol metabolites: new candidate biomarkers for whole-grain rye and wheat intake. *Am J Clin Nutr* 2009;90(5):1167-71. doi: 10.3945/ajcn.2009.28290.
73. Marklund M, Stromberg EA, Laerke HN, Knudsen KEB, Kamal-Eldin A, Hooker AC, Landberg R. Simultaneous Pharmacokinetic Modeling of Alkylresorcinols and Their Main Metabolites Indicates Dual Absorption Mechanisms and Enterohepatic Elimination in Humans. *J Nutr* 2014;144(11):1674-80. doi: 10.3945/jn.114.196220.
74. Zhu YD, Shurlknight KL, Chen XX, Sang SM. Identification and Pharmacokinetics of Novel Alkylresorcinol Metabolites in Human Urine, New Candidate Biomarkers for Whole-Grain Wheat and Rye Intake. *J Nutr* 2014;144(2):114-22. doi: 10.3945/jn.113.184663.
75. Borel P. Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clin Chem Lab Med* 2003;41(8):979-94. doi: 10.1515/cclm.2003.151.
76. Ross AB, Chen Y, Frank J, Swanson JE, Parker RS, Kozubek A, Lundh T, Vessby B, Aman P, Kamal-Eldin A. Cereal alkylresorcinols elevate gamma-tocopherol levels in rats and inhibit gamma-tocopherol metabolism in vitro. *J Nutr* 2004;134(3):506-10.
77. Andersson U, Dey ES, Holm C, Degerman E. Rye bran alkylresorcinols suppress adipocyte lipolysis and hormone-sensitive lipase activity. *Mol Nutr Food Res* 2011;55:S290-S3. doi: 10.1002/mnfr.201100231.
78. Jansson E, Landberg R, Kamal-Eldin A, Wolk A, Vessby B, Aman P. Presence of alkylresorcinols, potential whole grain biomarkers, in human adipose tissue. *Br J Nutr* 2010;104(5):633-6. doi: 10.1017/s0007114510001169.
79. Birringer M, Drozan D, Brigelius-Flohe R. Tocopherols are metabolized in HepG2 cells by side chain omega-oxidation and consecutive beta-oxidation.

- Free Radic Biol Med 2001;31(2):226-32. doi: 10.1016/s0891-5849(01)00574-3.
80. Ross AB, Aman P, Kamal-Eldin A. Identification of cereal alkylresorcinol metabolites in human urine - potential biomarkers of wholegrain wheat and rye intake. *J Chromatogr B* 2004;809(1):125-30. doi: 10.1016/j.jchromb.2004.06.015.
 81. Marklund M, Landberg R, Aman P, Kamal-Eldin A. Determination of alkylresorcinol metabolites in human urine by gas chromatography-mass spectrometry. *J Chromatogr B* 2010;878(11-12):888-94. doi: 10.1016/j.jchromb.2010.02.007.
 82. Landberg R, Kamal-Eldin A, Andersson SO, Johansson JE, Zhang JX, Hallmans G, Aman P. Reproducibility of Plasma Alkylresorcinols during a 6-Week Rye Intervention Study in Men with Prostate Cancer. *J Nutr* 2009;139(5):975-80. doi: 10.3945/jn.108.099952.
 83. Paalanen L, Mannisto S, Virtanen MJ, Knekt P, Rasanen L, Montonen J, Pietinen P. Validity of a food frequency questionnaire varied by age and body mass index. *J Clin Epidemiol* 2006;59(9):994-1001. doi: 10.1016/j.jclinepi.2006.01.002.
 84. Braam L, Ocke MC, Bueno-de-Mesquita HB, Seidell JC. Determinants of obesity-related underreporting of energy intake. *Am J Epidemiol* 1998;147(11):1081-6.
 85. Aubertin-Leheudre M, Koskela A, Marjamaa A, Adlercreutz H. Plasma alkylresorcinols and urinary alkylresorcinol metabolites as biomarkers of cereal fiber intake in Finnish women. *Cancer Epidemiol Biomarkers Prev* 2008;17(9):2244-8. doi: 10.1158/1055-9965.epi-08-0215.
 86. Ross AB, Pineau N, Kochhar S, Bourgeois A, Beaumont M, Decarli B. Validation of a FFQ for estimating whole-grain cereal food intake. *Br J Nutr* 2009;102(11):1547-51. doi: 10.1017/s0007114509990845.
 87. Landberg R, Kamal-Eldin A, Aman P, Christensen J, Overvad K, Tjønneland A, Olsen A. Determinants of plasma alkylresorcinol concentration in Danish post-menopausal women. *Eur J Clin Nutr* 2011;65(1):94-101. doi: 10.1038/ejcn.2010.193.
 88. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Akesson B, Onning G, Jonsdottir SE, Rosqvist F, Schwab U, Herzig KH, et al. Plasma Alkylresorcinols Reflect Important Whole-Grain Components of a Healthy Nordic Diet. *J Nutr* 2013;143(9):1383-90. doi: 10.3945/jn.113.175588.

89. Linko AM, Juntunen KS, Mykkanen HM, Adlercreutz H. Whole-grain rye bread consumption by women correlates with plasma alkylresorcinols and increases their concentration compared with low-fiber wheat bread. *J Nutr* 2005;135(3):580-3.
90. Albertson AM, Reicks M, Joshi N, Gugger CK. Whole grain consumption trends and associations with body weight measures in the United States: results from the cross sectional National Health and Nutrition Examination Survey 2001-2012. *Nutr J* 2016;15:14. doi: 10.1186/s12937-016-0126-4.
91. Ross AB, Becker W, Chen Y, Kamal-Eldin A, Aman P. Intake of alkylresorcinols from wheat and rye in the United Kingdom and Sweden. *Br J Nutr* 2005;94(4):496-9. doi: 10.1079/bjn20051511.
92. Marklund M, Landberg R, Andersson R, Aman P, Kamal-Eldin A. Alkylresorcinol Metabolism in Swedish Adults Is Affected by Factors Other Than Intake of Whole-Grain Wheat and Rye. *J Nutr* 2012;142(8):1479-86. doi: 10.3945/jn.112.159244.
93. Magnusdottir OK, Landberg R, Gunnarsdottir I, Cloetens L, Akesson B, Rosqvist F, Schwab U, Herzig KH, Hukkanen J, Savolainen MJ, et al. Whole Grain Rye Intake, Reflected by a Biomarker, Is Associated with Favorable Blood Lipid Outcomes in Subjects with the Metabolic Syndrome - A Randomized Study. *PLoS One* 2014;9(10):8. doi: 10.1371/journal.pone.0110827.
94. Uusitupa M, Hermansen K, Savolainen MJ, Schwab U, Kolehmainen M, Brader L, Mortensen LS, Cloetens L, Johansson-Persson A, Oumlnning G, et al. Effects of an isocaloric healthy Nordic diet on insulin sensitivity, lipid profile and inflammation markers in metabolic syndrome - a randomized study (SYSDIET). *J Int Med* 2013;274(1):52-66. doi: 10.1111/joim.12044.
95. Montonen J, Landberg R, Kamal-Eldin A, Aman P, Knueppel S, Boeing H, Pischon T. Reliability of fasting plasma alkylresorcinol concentrations measured 4 months apart. *Eur J Clin Nutr* 2010;64(7):698-703. doi: 10.1038/ejcn.2010.71.