MOST Ancillary Study 14-06 (AS14-06)
"Fat and Fiber" (D. Felson, D. Misra)

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1. Dataset description and Analyst Notes

Dataset: AS1406_bioassay.sas7bdat
Observations: 994 records (994 participants, 55 assays, 6 selection groups)
Documentation:

- VariableGuide_ AS1406_bioassay.pdf
- Distributions_ AS1406_bioassay.pdf

AS1406_bioassay dataset contains 994 records (one record per participant) with Lipid panel, Magnesium, Fatty Acids and Alkylresorcinol results performed at the Cardiovascular Nutrition Laboratory Tufts University laboratory on baseline serum.
Note, for this study, the laboratory was blinded to clinical data (case/control selection) and demographic characteristics of the participants.

ANALYST NOTES:

- If there were insufficient volume or other reason and all assay values were missing, the record is not included in the analytical dataset.
- Variables #5 to #10 are indicator for the selected groups described below in details (see section – Selection plan).
- When assay results were not obtained, special missing value were used:
  .L = below low detection level
  .H = above high detection level
  Values .L and .H can be used in categorical analysis only. Alternatively, the analyst can assign the special value above detection or below detection if requested by investigator.
- There are 7 samples with missing values (variables #61 to 65) for the In Alkylresorcinol assay results. These samples were all marked as non-detectable in the laboratory report. Analyst and investigator should strategize if the value=0 would be acceptable for these samples. Note that the summary of the five assay values (variable #66 Sum) is provided assuming that the missing values are equal to zero for these 7 samples.

2. References.

Schwager JL, Nevitt MC, Torner J, Lewis CE, Matthan NR, Wang N, Sun X, Lichtenstein AH, Felson D; Multicenter Osteoarthritis Study Group. Association of serum low density lipoprotein, high density lipoprotein or total cholesterol with development of knee osteoarthritis; Arthritis Care Res (Hoboken). 2022;74(2):274-280. Epub 2020/09/23. doi: 10.1002/acr.24455. PMID:32961029; PMCID: PMC8054264

Felson DT, Misra D, LaValley M, Clancy M, Chen X, Lichtenstein A, Matthan N, Torner J, Lewis CE, Nevitt MC; Fatty acids and osteoarthritis: The MOST study. Osteoarthritis Cartilage. 2021 Jul;29(7):973-978. doi:10.1016/j.joca.2021.03.006. Epub 2021 Mar 20.
PMID: 33757857; PMCID: PMC8217156.

J-P Zertuche, G Rabasa, A H Lichtenstein, N R Matthan, M Nevitt, J Torner, C E Lewis, D Misra, D Felson; Alkylresorcinol, a biomarker for whole grain intake, and its association with osteoarthritis: the MOST study. Osteoarthritis Cartilage. 2022 Jul 19;S1063-4584(22)00796-8. doi: 10.1016/j.joca.2022.07.004. PMCID: PMC9554937

3. Selection plan

Participants enrolled in MOST: N=3026 subjects / 6052 knees

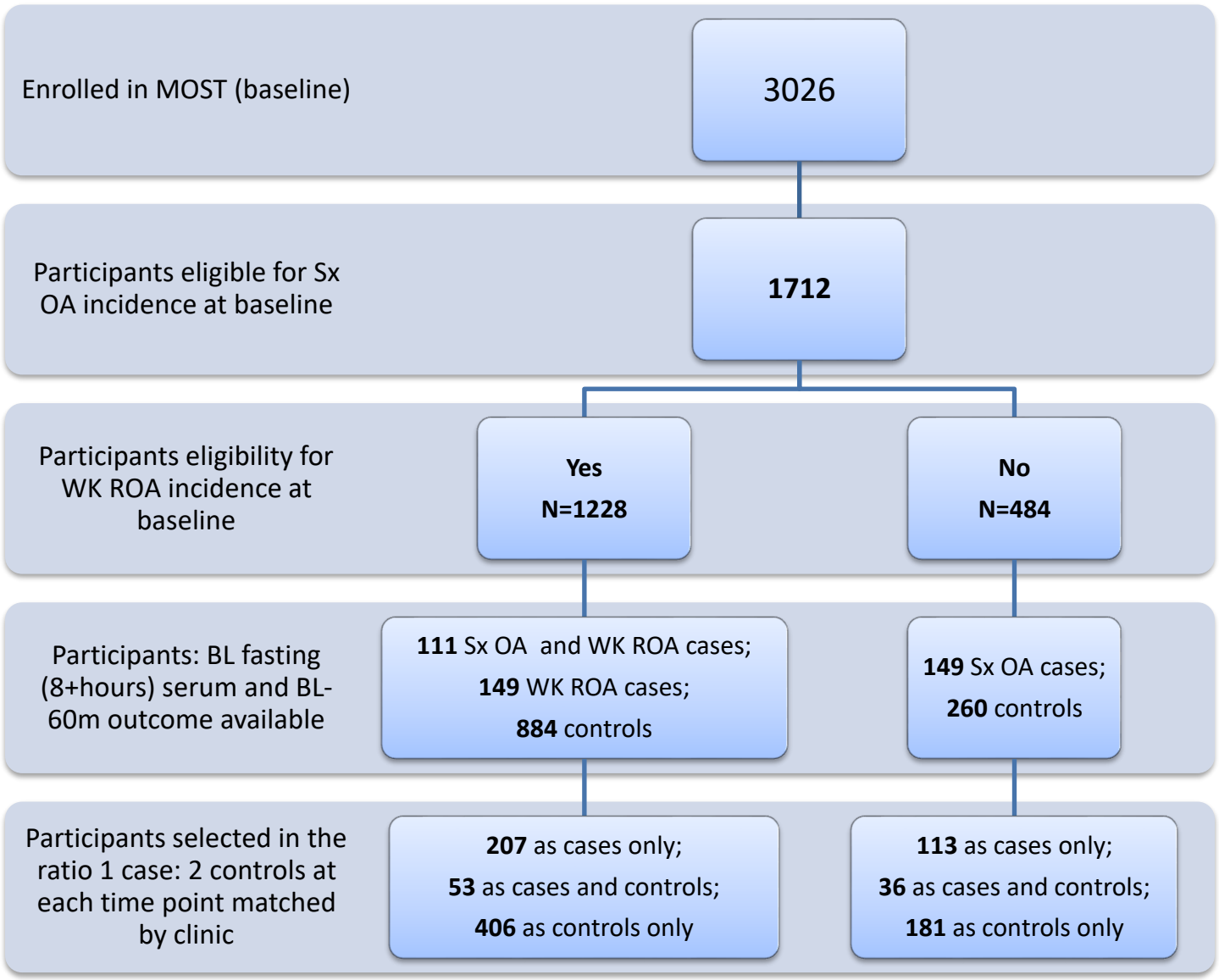
Total N = 996 (463 UAB + 533 UI) participants selected for assay - 2 outcomes for 3 time points; cases and controls frequency matched by clinic site as 1 case: 2 controls for each outcome (Sx OA and WK ROA) at each time point (15m/30m/60m)

N=320 selected only as a case for at least one of the outcomes;

N=89 selected as a case for at least one outcome and as a control for different outcome or different time point;

N=587 selected only as a control for at least one of the outcomes.

Figure 1 – FLOW chart for study selection.



Outcome of interest – any time between baseline and 60m (15m, 30m or 60m follow-up).

4. SECTION 1. Sx OA definition

Eligible for Sx OA incidence:
Participants with both knees without (TF ROA or PF ROA) or without frequent knee pain at baseline (V0XSXOAk=0). Participants with baseline KR or other x-ray exclusion (RA, amputation, missing patella, necrosis) are not included. Additionally - knees with whole knee radiographic OA but with pain at ONLY one time - either phone call or clinic are NOT eligible for incident SxOA. For participant to be eligible for incidence, both knees need to be eligible.

Variables and code:

```
if v0xRTFROA=0 and V0xRPFROA=0 then right_eligSx=1; else
if 0<=v0xRKL<=4 and v0r_fkp in ("NN","ND","DN") then right_eligSx=1; else
if (v0xRTFROA>0 or V0xRPFROA>0) and index(v0r_fkp,"Y")>0 then right_eligSx=0;
if v0xlTFROA=0 and V0xlPFROA=0 then left_eligSx=1; else
if 0<=v0xLKL<=4 and v0l_fkp in ("NN","ND","DN")  then left_eligSx=1; else
if (v0xlTFROA>0 or V0xlPFROA>0) and index(v0l_fkp,"Y")>0 then left_eligSx=0;
if right_eligsx=1 and left_eligsx=1 then elig_sx=1;
```

Sx OA incidence case between BL and 60 months **N=245+30=275**
Participants who met both conditions: presence of WK ROA and frequent knee pain at 15/30/60m on at least one knee or eligible participants with KR reported at 15/30/60m.

Variables and code: elig_sx=1 and (VxXSXOAk>0 or VxR_TKR=1 or VxL_TKR=1)
Note: to be included as a case, participants have to complete the clinic visit with knee pain questions answered as Yes during phone interview and Yes during clinic interview.
If TF ROA/PF ROA was not present at baseline, participants were required to have x-ray obtained and read (PA view, left and right lateral view) and TF ROA or PF ROA condition determined in at least one knee.
If TF ROA/PF ROA was present at any visit, this information was projected to consecutive follow up status determination.

Sx OA incidence clean controls **N=1373**:
Participants who did not developed Sx OA between baseline and 60m.
Variables and code: VxXSXOAk=0 for at least one time point.
Note: to be included as a control, participants have to complete at least one follow up visit with x-ray obtained and read (PA view, left and right lateral view) and neither TF ROA nor PF ROA condition determined in both knees or have to have knee pain questions answered as No at least one time (by phone or during the clinic interview) during contact. The TF ROA or PF ROA status determined at any visit was projected to consecutive follow up visit with or without x-ray confirmation.
Note 2. Cases in the prior time point cannot be counted as controls; cases at later time point could be selected as controls by random selection.

Table 1. Incidence Sx OA rate by category

Eligibility and OA status at baseline	N participants	Sx OA cases (rate%)	Sx OA controls	Without BL to 60m outcome
Strata 1. Without OA at baseline	1228	**119 (9.8%)**	**1094**	15
Strata 2. With OA at baseline				
eligible for TF ROA progression	391	134 (35.5%)	243	14
bilateral TF ROA end stage	5	3 (60%)	2	0
unilateral TF ROA end stage	10	5 (50%)	5	0
No TF ROA, but PF ROA	39	14 (36%)	25	0
No TF ROA, PF ROA status unknown	39	0 (0%)	4	35
Sub-total	484	**156 (36%)**	**279**	49
Total eligible for Sx OA incidence	**1712**	**275 (16.7%)**	**1373**	**64**

Note – highlighted cells are reported in detail in Table 2 and Table 3 by visit.

Table 2. Cumulative Sx OA outcomes by visit – also eligible for WKROA incidence; participants without OA at baseline.

Sx OA status	**time: for cases – first occurrence; for controls – last known contact**			
	15m	**30m**	**60m**	**Total**
control	7	170	917	**1094**
case	12	35	61	**108**
KR case	2	1	8	**11**
Total	21	206	986	1213

Table 3. Cumulative Sx OA outcomes by visit – not eligible for WKROA incidence; participants with OA at baseline.

Sx OA status	**time: for cases – first occurrence; for controls – last known contact**			
	15m	**30m**	**60m**	**Total**
control	5	46	228	**279**
case	41	52	44	**137**
KR case	3	3	13	**19**
Total	49	101	285	435

5. SECTION 2. Sx OA selection for participants without OA at baseline

Table 4. Baseline fasting (8+ hours) serum available for risk set selection Sx OA outcomes by visit – Eligible for WK ROA incidence (participants without OA at baseline)

Sx OA status	time: for cases – first occurrence; for controls – last known contact			
	15m	30m	60m	Total
Controls available for selection	257	1068	867	n/a
Control censored at the time point	5	161	867	1033
Case or KR case	14	35	62	111
Total	19	196	929	1144

Table 5. Selection for this strata and outcome – by visit

Sx OA status	Visit			Total
	15m	30m	60m	
Case or KR case	14	35	62	111
Controls available for selection at time point	257	1068	867	1033*
Controls selected matched by clinic	28	70	124	159*
Total selected	42	105	186	270*

*Controls for the last part of the table are clean controls only (if case at later time was selected as a control for the previous time, it was not counted twice in this section of the table)

Table 6. Sx OA selection for all time points – Cross tab of selected participants

Table 1 of ppts selection by SITE				Legend:
				0 = means selected as controls
				1=means selected as case
Controlling for strata=1 (no OA at BL)				*x= means not selected (or not eligible)*
				position 1 - 15m selection
Selection type	SITE			*position 3 - 30m selection*
	Site=1	Site=2	Total	*position 5 - 60m selection*
0_x_0	1	1	2	control at 15m and 60m
0_x_1	1	2	3	control at 15m; case at 60m
0_x_x	12	11	23	control at 15m
1_x_x	7	7	14	case at 15m
x_0_0	34	17	51	control at 30m and 60m
x_0_1	4	3	7	control at 30m; case at 60m
x_0_x	6	6	12	control at 30m
x_1_x	22	13	35	case at 30m
x_x_0	27	44	71	control at 60m
x_x_1	26	26	52	case at 60m
x_x_x	172	224	396	not selected for Sx OA; but selected for ROA (see details below in Appendix 2)
Total	312	354	666	

6. SECTION 3. Sx OA selection for participants with OA at baseline

Table 7. Baseline fasting (8+ hours) serum available for risk set selection Sx OA outcomes by visit – Not eligible for WK ROA incidence (participants with OA at baseline) only

Sx OA status	time: for cases – first occurrence; for controls – last known contact			
	15m	30m	60m	Total
Controls available for selection	101	298	212	n/a
Control censored at the time point	5	43	212	260
Case or KR case	44	52	53	149
Total	49	95	265	409

Table 8. Selection for this strata and outcome – by visit

Sx OA status	Visit			Total
	15m	30m	60m	
Case or KR case	44	52	53	149
Controls available for selection at time point	101	298	212	260*
Controls selected matched by clinic	88	104	106	181*
Total selected	132	156	159	330*

*Controls for the last part of the table are clean controls only (if case at later time was selected as a control for the previous time, it was not counted twice in this section of the table).

Table 9. Sx OA selection for all time points – Cross tab for selected participants

Table 2 of ppts selection by SITE				*Legend:*
Controlling for strata=2 (OA at BL)				*position 1 – 15m selection*
Selection type	SITE			*position 3 – 30m selection*
	Site=1	Site=2	Total	*position 5 – 60m selection*
0_0_0	2	5	7	control at 15m, 30m and 60m
0_0_1	3	3	6	control at 15m and 30m; case at 60m
0_0_x	7	6	13	control at 15m and 30m
0_1_x	6	3	9	control at 15m; case at 30m
0_x_0	6	7	13	control at 15m and 60m
0_x_1	3	6	9	control at 15m; case at 60m
0_x_x	13	18	31	control at 15m
1_x_x	20	24	44	case at 15m
x_0_0	18	17	35	control at 30m and 60m
x_0_1	6	6	12	control at 30m; case at 60m
x_0_x	14	17	31	control at 30m
x_1_x	19	24	43	case at 30m
x_x_0	22	29	51	control at 60m
x_x_1	12	14	26	case at 60m
Total	151	179	330	

7. SECTION 4. WK ROA incidence definition and selection

Eligible for the WK ROA incidence:
Participant status: both knees without TF ROA or PF ROA at baseline.
Variables and code: V0XWKROAk=0

WK ROA incidence case **N=266+8=274**
Participants who developed WK ROA or KR at 15/30/60m.
Variables and code: V0XWKROAk=0 and (VxXWKROAk>0 or VxR_TKR=1 or VxL_TKR=1)
Note: to be included as a case, participants have to complete the 30m visit with x-ray obtained and read (PA view, left and right lateral view) and TF ROA or PF ROA condition determined in at least one knee. Those eligible and developed KR could be with or without follow up clinic visit (could be self-reported by phone).

WK ROA incidence control **N=939**
Participants who completed follow up visit with x-ray and did not developed WK ROA or KR at 15/30/60m.
Variables and code: V0XWKROAk=0 and VxXWKROAk=0
Note: to be included as a control, participants have to have completed at least one follow up visit with x-ray obtained and read (PA view, left and right lateral view) and neither TF ROA nor PF ROA condition determined in both knees.

Table 10. Cumulative WK ROA outcomes by visit

WK ROA status	time: for cases – first occurrence of ROA; for controls – last known contact			
	15m	30m	60m	Total
control	8	167	764	939
case	14	114	138	266
KR case	2	1	5	8
Total	24	282	907	1213

Table 11. Baseline fasting (8+ hours) serum available for cumulative WKROA outcomes by visit

WK ROA status	time: for cases – first occurrence of ROA; for controls – last known contact			
	15m	30m	60m	Total
control	6	158	720	884
case	14	107	131	252
KR case	2	1	5	8
Total	22	266	856	1144

Table 12. N=260 WK ROA cases – Baseline fasting (8+ hours) serum available for Sx OA outcomes by visit

WK ROA cases by Sx OA status	time: for cases – first occurrence of Sx OA; for controls – last known contact			
	15m	30m	60m	Total
WKROA case and Sx control	0	8	141	149
WKROA case and Sx case	12	34	55	101
KR case	2	1	7	10
Total	14	43	203	260

Note – the Sx case and control status could be on the later time.

Table 13. Selection for this outcome – by visit

WK ROA status	Visit			Total
	15m	30m	60m	
Case or KR case	16	108	136	260
Controls available for selection at time point	257	990	720	884*
Controls selected matched by clinic	32	216	272	362*
Total selected	48	324	408	622*

*Controls for the last part of the table are clean controls only (if case at later time was selected as a control for the previous time, it was not counted twice in this section of the table).

Table 14. WK ROA selection for all time points – Cross tab for selected participants

Table 1 of ppts selection by SITE				*Legend:*
Controlling for strata=1				*position 1 – 15m selection*
Selection type	SITE			*position 3 – 30m selection*
	Site=1	Site=2	Total	*position 5 – 60m selection*
0_0_0	3	1	4	control at 15m, 30m and 60m
0_0_1	0	1	1	control at 15m and 30m; case at 60m
0_0_x	0	1	1	control at 15m and 30m
0_1_x	0	1	1	control at 15m; case at 30m
0_x_0	2	4	6	control at 15m and 60m
0_x_1	4	4	8	control at 15m; case at 60m
0_x_x	9	2	11	control at 15m
1_x_x	9	7	16	case at 15m
x_0_0	55	48	103	control at 30m and 60m
x_0_1	14	15	29	control at 30m; case at 60m
x_0_x	38	40	78	control at 30m
x_1_x	55	52	107	case at 30m
x_x_0	56	103	159	control at 60m
x_x_1	40	58	98	case at 60m
x_x_x	27	17	44	not selected for ROA; but selected for Sx OA (see details below in Appendix 2)
Total	312	354	666	

8. SECTION 5. Summary of selection for both outcomes

Table 15. Summary participants available for selection and selected for WK ROA and Sx OA outcome: baseline fasting (8+ hours) serum available; by clinic site

Sx OA status	WK ROA status	Both clinics	Site=1	Site=2	Selected
Participants without OA at BL – ratio Controls : Cases		3.4:1	3.6:1	3.2:1	
Sx OA control	ROA control	**884**	441	443	**406**
Sx OA control	ROA case or KR case	**149**	62	87	**149**
Sx OA case or KR case	ROA case or KR case	**111**	60	51	**111**
Participants with OA at BL – ratio Controls : Cases		1.74:1	1.68:1	1.80:1	
Sx OA control	- (n/a)	**260**	116	144	**181**
Sx OA case or KR case	- (n/a)	**149**	69	80	**149**

Note 1. All the identified cases were selected (red font in Table 5).
Note 2. Due to funding constrains, the group of controls were selected as a risk sample (2 controls at each time point for 1 case) matched by clinic.
Note 3. 2 samples have missing all assays due to insufficient volume of serum sample and therefore their records are NOT included in the analytical dataset. Final dataset contains 994 records.

9. Appendix 1. Magnesium Assay Documentation Provided by Laboratory

Magnesium	
Manufacturer	BeckmanCoulter
Instrument	AU480 Clinical Chemistry Analyzer
Method (as specified in procedural insert)	colorimetric, endpoint
Additional Reference	Mann et al
Catalog Number	OSR6189
Kit Lot (Reagent Lot)	#2569
Sample Size/Minimum Volume Required for Analysis	2 uL/175uL
Units of Measure	mEq/L
Low Detection Threshold	0.4 mEq/L
High Detection Threshold	6.6 mEq/L
Calibration Factor	2.6 mEq/L
Range of Standard (Range of QC Material Used)	1.4-4.29
Estimated Normal Range	1.3-2.6 mEq/L
Intra-assay Coefficient of Variance	2.2%
Inter-assay Coefficient of Variance	3.0%
References:	
Mann, C.K. and Yoe, J.H., Anal Chem, 28: 202-205, 1956.	

Notes about missing assays parameters:
There were four samples that had clots and therefore we were unable to provide magnesium levels.
Three of these samples correspond to the same samples with clots as the lipid measures.
The fourth sample is the same sample where there was insufficient volume for the triglyceride measure.
There was also a fifth sample with insufficient volume remaining after the lipid assays were performed.

10. Appendix 2. Cholesterol, HDL and Triglycerides Assay Documentation Provided by Laboratory

	Cholesterol	HDL	Triglycerides	LDL-d (N=11)
Manufacturer	BeckmanCoulter	BeckmanCoulter	BeckmanCoulter	BeckmanCoulter
Instrument	AU480 Clinical Chemistry Analyzer	AU480 Clinical Chemistry Analyzer	AU480 Clinical Chemistry Analyzer	AU480 Clinical Chemistry Analyzer
Method (as specified in procedural insert)	enzymatic procedure	two-phase reaction with colorimetric endpoint detection	coupled enzymatic reaction	two reagent colorimetric enzymatic homogenous system
Additional Reference	Allain et al[1] and Rieschlau et al[2]		Trinder[3] and Bucolo et al[4]	
Catalog Number	OSR6116	OSR6187	OSR6033	OSR6196
Kit Lot (Reagent Lot)	#2528	#2405	#2534	#2298
Sample Size/Minimum Volume Required for Analysis	2 uL/200 uL	2 uL/200 uL	2 uL/200 uL	2 uL/175 uL
Units of Measure	mg/dL	mg/dL	mg/dL	mg/dL
Low Detection Threshold	25 mg/dL	2.5 mg/dL	10 mg/dL	7.0 mg/dL
High Detection Threshold	700 mg/dL	200 mg/dL	1000 mg/dL	400 mg/dL
Calibration Factor	228 mg/dL	57 mg/dL	259 mg/dL	116 mg/dL
Range of Standard (Range of QC Material Used)	78-267 mg/dL	21.6-83.4 mg/dL	76.2-224 mg/dL	33.4-166 mg/dL
Estimated Normal Range	130-230 mg/dL	30-85 mg/dL	41-212 mg/dL	60-187 mg/dL
Intra-assay Coefficient of Variance	2.0%	3.0%	2.0%	2.4%
Inter-assay Coefficient of Variance	2.8%	4.0%	3.4%	3.6%

References:

[1]Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C., Enzymatic determination of total Serum Cholesterol. Clin Chem, 1974:20:470-475.

[2]Rieschlau, P., Bernt, E. and Gruber, W., Z, Enzymatic determination of total cholesterol in serum. Klin Chem Klin Biochem, 1974: Sep:12(9): 403-407.

[3]Trinder, P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem, 1969:6:24-25.

[4]Bucolo, G. and David. H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem, 1973 May;19(5):476

CHOL	Total cholesterol
HDL-C	High density lipoprotein -cholesterol
TG	Triglyceride
VLDL-C	Very low density lipoprotein-cholesterol
	(calculated as TG/5)
LDL-C*	low density lipoprotein -cholesterol
	(calculated using the Friedewald formula)

*LDL-C was calculated using the Friedwald equation, except when triglycerides were above 400mg/dl. For these 11 samples, a direct LDL kit was use, therefore the calculated VLDL and LDL values are not valid and have been removed.

Notes about missing assays parameters:
There were three samples that had clots and therefore we were unable to provide lipid levels. There was also one sample with insufficient sample volume for repeat triglyceride level, also resulting in no calculated VLDL or LDL level.

11. Appendix 3. Quality Control Report for Serum Results (Mg and Lipid panel) by Laboratory Batch.

The Coordinating Center performed QC procedures on the study results and generated this report.
Note: the difference between “N obs” (column 2) and “N” (column 3) is due to missing assay results.

Table 1. Magnesium Assay by Laboratory Running Batch.

Analysis Variable : Magnesium									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	1.83	0.17	1.23	1.73	1.83	1.95	2.19
2	**81**	80	1.83	0.13	1.51	1.76	1.84	1.9	2.2
3	**81**	80	1.93	0.2	1.33	1.84	1.93	2.05	2.4
4	**81**	81	1.91	0.17	1.53	1.75	1.93	2.04	2.23
5	**81**	81	1.92	0.24	1.3	1.76	1.9	2.11	2.39
6	**81**	81	1.99	0.16	1.65	1.88	1.98	2.09	2.35
7	**81**	79	1.9	0.2	1.26	1.81	1.89	2.02	2.45
8	**81**	81	1.99	0.17	1.6	1.88	2	2.1	2.36
9	**81**	81	1.87	0.17	1.32	1.78	1.87	1.98	2.42
10	**81**	81	1.87	0.15	1.54	1.78	1.89	1.97	2.31
11	**81**	81	1.78	0.16	1.34	1.67	1.76	1.88	2.39
12	**103**	102	1.83	0.18	1.32	1.74	1.82	1.93	2.37

Table 2. Total Cholesterol Assay by Laboratory Running Batch.

Analysis Variable : CHOL									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	220.67	47.79	110	190	215	250	356
2	**81**	81	216.6	42.26	126	183	212	246	391
3	**81**	81	233.17	45.3	139	195	235	267	335
4	**81**	81	228.23	40.16	131	203	224	245	361
5	**81**	81	226.41	52.35	135	189	226	262	419
6	**81**	81	228.79	41.48	130	202	225	258	318
7	**81**	79	230.15	57.55	112	198	221	261	404
8	**81**	81	235.09	51.37	124	201	234	271	378
9	**81**	81	247.25	56.12	141	206	243	281	374
10	**81**	81	226.12	50	133	190	226	258	434
11	**81**	81	217.51	49.18	136	185	213	240	355
12	**103**	102	221.53	36.02	142	196	218.5	248	325

Table 3. Triglyceride (mg/dl) Assay by Laboratory Running Batch.

Analysis Variable : TG									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	140.83	105.61	51	91	125	173	981
2	**81**	80	141.86	54.45	47	97	137.5	176.5	262
3	**81**	81	157.01	66.93	58	100	148	206	332
4	**81**	81	147.75	82.05	46	92	124	194	413
5	**81**	81	147.67	58.13	50	111	142	181	300
6	**81**	81	140.95	63.33	50	97	126	169	367
7	**81**	79	154.63	85.18	45	86	136	189	428
8	**81**	81	154.15	75.12	51	94	142	199	395
9	**81**	81	149.04	76.79	44	96	130	185	408
10	**81**	81	167.84	93.12	57	96	145	211	508
11	**81**	81	161.22	86.96	53	104	137	206	579
12	**103**	102	158.24	86.86	36	97	140	186	523

Table 4. High density lipoprotein-cholesterol Assay by Laboratory Running Batch.

Analysis Variable : HDL_C									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	60.43	14.05	33.8	50.2	60.9	68.6	102.9
2	**81**	81	58.65	14.57	37.6	47.4	56.8	65.7	101.8
3	**81**	81	59.27	17.43	35.4	44.8	56.1	70	119.8
4	**81**	81	59.57	15.63	36	46.8	57.6	69.4	98.1
5	**81**	81	59.67	16.39	30.7	48.9	56.1	69.7	115.5
6	**81**	81	61.33	14.76	32.9	49.7	59.9	71.2	101.9
7	**81**	79	64.47	16.06	34.5	52.8	61.8	76.9	128
8	**81**	81	68.85	18.61	40.3	54.4	65.8	81.4	113.9
9	**81**	81	67.72	20.16	30.5	54.2	65.7	76.1	125.6
10	**81**	81	64.14	19.87	29	50.4	61.6	73.7	117.4
11	**81**	81	58.23	15.93	34.3	47.6	55.2	69.3	104.7
12	**103**	102	60.12	15.9	34.2	47.9	57.05	66.9	105.9

Table 5. Very low density lipoprotein-cholesterol (calculated as TG/5) Assay by Laboratory Running Batch.

Analysis Variable : VLDL_C									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	28.17	21.12	10.2	18.2	25	34.6	196.2
2	**81**	80	28.37	10.89	9.4	19.4	27.5	35.3	52.4
3	**81**	81	31.4	13.39	11.6	20	29.6	41.2	66.4
4	**81**	81	29.55	16.41	9.2	18.4	24.8	38.8	82.6
5	**81**	81	29.53	11.63	10	22.2	28.4	36.2	60
6	**81**	81	28.19	12.67	10	19.4	25.2	33.8	73.4
7	**81**	79	30.93	17.04	9	17.2	27.2	37.8	85.6
8	**81**	81	30.83	15.02	10.2	18.8	28.4	39.8	79
9	**81**	81	29.81	15.36	8.8	19.2	26	37	81.6
10	**81**	81	33.57	18.62	11.4	19.2	29	42.2	101.6
11	**81**	81	32.24	17.39	10.6	20.8	27.4	41.2	115.8
12	**103**	102	31.65	17.37	7.2	19.4	28	37.2	104.6

Table 6. Low density lipoprotein -cholesterol (calculated using the Friedewald formula, except when triglycerides were above 400mg/dl) Assay by Laboratory Running Batch.

Analysis Variable : LDL_C									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	133.29	40.04	52.7	106.5	131	154.2	258.3
2	**81**	80	129.2	34.55	63.9	105.5	126.9	152.2	280.6
3	**81**	81	142.46	39.49	66	110	140	175	238
4	**81**	81	139.67	32.42	69	123	137	158	235
5	**81**	81	137.26	42.13	64.8	109.1	134	162.1	302.9
6	**81**	81	139.27	34.61	53.3	113.8	138.3	163	221.3
7	**81**	79	135.08	45.55	35.3	111.5	127.3	153.8	261.3
8	**81**	81	135.4	42.98	35.2	107.4	131.2	159.7	251.6
9	**81**	81	149.6	46.72	73.4	114.9	146.4	178.8	279.9
10	**81**	81	129.25	37.66	58	104.6	127.3	149.6	306.6
11	**81**	81	127.47	40.85	45.9	101.3	120.8	151.8	233.7
12	**103**	102	130.37	31.74	41.4	110.9	131.8	148.5	207.8

12. Appendix 4. Assay Documentation (Fatty Acids) provided by Laboratory

Information from Laboratory				SAS dataset variables	
Class name	**Fatty Acid Abbreviations**	**Trivial name**	**Systematic Name (IUPAC) - counting from the carboxyl acid end**	**Variable name**	**Variable label**
SATURATED FATTY ACIDS (SFA)	10:0	Capric	Decanoic	SFA10_0	SFA10:0
	12:0	Lauric	Dodecanoic	SFA12_0	SFA12:0
	14:0	Myristic	Tetradecanoic	SFA14_0	SFA14:0
	15:0	Pentadecylic	Pentadecanoic	SFA15_0	SFA15:0
	16:0	Palmitic	Hexadecanoic	SFA16_0	SFA16:0
	18:0	Stearic	Octadecanoic	SFA18_0	SFA18:0
	20:0	Arachidic	Eicosanoic	SFA20_0	SFA20:0
	22:0	Behenic	Docosanoic	SFA22_0	SFA22:0
	24:0	Lignoceric	Tetracosanoic	SFA24_0	SFA24:0
	Summary			SFA	SFA
cis MONOUNSATURATED FATTY ACIDS	14:1n-5	Myristoleic	cis-9-tetradecenoic	MFA14_1n_5	MFA14:1n-5
	16:1n-9		cis-7-hexadecenoic	MFA16_1n_9	MFA16:1n-9
	16:1n-7	Palmitoleic	cis-9-hexadecenoic	MFA16_1n_7	MFA16:1n-7
	18:1n-9	Oleic	cis-9-octadecenoic	MFA18_1n_9	MFA18:1n-9
	18:1n-7	cis-Vaccenic	cis-11-octadecenoic	MFA18_1n_7	MFA18:1n-7
	20:1n-9	Gondoic	cis-11-eicosenoic	MFA20_1n_9	MFA20:1n-9
	22:1n-9	Erucic	cis-13-docosenoic	MFA22_1n_9	MFA22:1n-9
	24:1n-9	Nervonic	cis-15-tetracosenoic	MFA24_1n_9	MFA24:1n-9
	Summary			MUFA	MUFA
POLYUNSATURATED FATTY ACIDS (n-6)	18:2n-6	Linoleic	cis-9,cis-12-octadecadienoic	PFA18_2n_6	PFA18:2n-6
	18:3n-6	Gamma-Linolenic	cis-6,9,12-octadecatrienoic	PFA18_3n_6	PFA18:3n-6
	20:2n-6	Dihomolinoleic	cis-9,12-eicosadienoic	PFA20_2n_6	PFA20:2n-6
	20:3n-6	Dihomo-Gamma-Linolenic	cis-8,11,14-eicosatrienoic	PFA20_3n_6	PFA20:3n-6
	20:4n-6	Arachidonic	cis-5,8,11,14-eicosatetraenoic	PFA20_4n_6	PFA20:4n-6
	22:2n-6	Docosadienoic	cis-13,16-docosadienoic	PFA22_2n_6	PFA22:2n-6
	22:4n-6	Adrenic	cis-7,10,13,16-docosatetraenoic	PFA22_4n_6	PFA22:4n-6
	22:5n-6	Docosapentaenoic	cis-4,7,10,13,16-docosapentanoic	PFA22_5n_6	PFA22:5n-6
	Summary			PUFA_n_6	PUFA n-6
POLYUNSATURATED FATTY ACIDS (n-3)	18:3n-3	Alpha Linolenic (ALA)	cis-9,12,15-octadecatrienoic	PFA18_3n_3	PFA18:3n-3
	18:4n-3	Stearidonic (SDA)	cis-6,9,12,15-octadecatetraenoic	PFA18_4n_3	PFA18:4n-3
	20:3n-3	Eicosatrienoic (ETE)	cis-11,14,17-eicosatrienoic	PFA20_3n_3	PFA20:3n-3
	20:5n-3	Eicosapentaenoic (EPA)	cis-5,8,11,14,17-eicosapentaenoic	PFA20_5n_3	PFA20:5n-3
	22:5n-3	Docosapentaenoic (DPA)	cis-7,10,13,16,19-docosapentaenoic	PFA22_5n_3	PFA22:5n-3
	22:6n-3	Docosahexaenoic (DHA)	cis-4,7,10,13,16,19-decosahexaenoic	PFA22_6n_3	PFA22:6n-3
	Summary			PUFA_n_6	PUFA n-6
TRANS FATTY ACIDS	16:1n-9T		trans-7-hexadecenoic	TFA16_1n_9T	TFA16:1n-9T
	16:1n-7T	Palmitelaidic	trans-9-hexadecenoic	TFA16_1n_7T	TFA16:1n-7T
	18:1n-10 to 12T*	Petroselinic	trans-6-octadecenoic	TFA18_1n10_12T	TFA18:1n10-12T
	18:1n-9T	Elaidic	trans-9-octadecenoic	TFA18_1n_9T	TFA18:1n-9T
	18:1n-7T	trans-vaccenic	trans-11-octadecenoic	TFA18_1n_7T	TFA18:1n-7T
	18:2T**	Linelaidic	trans-9,trans-12-octadecadienoic	TFA18_2T	TFA18:2T
	18:2-CLA	Conjugated Linoleic	cis-9, trans-11-octadecadienoic	TFA18_2CLA	TFA18:2CLA
	Summary			Total_trans	Total trans

*Sum of 18:2 n-10, n-11 and n-12 trans isomers | **Sum of all 18:2 trans isomers

13. Appendix 5. Fatty Acids Assay Documentation Provided by Laboratory

METHODOLOGY
Serum Phospholipid Fatty Acid Analysis by Gas Chromatography with Flame Ionizing Dectector (GC-FID)

REFERENCES
Matthan NR, Ooi EM, Van Horn L, Neuhouser ML, Woodman R, Lichtenstein AH.
Plasma phospholipid fatty acid biomarkers of dietary fat quality and endogenous metabolism predict coronary heart disease risk: a nested case-control study within the Women's Health Initiative observational study.

J Am Heart Assoc. 2014; 3 (4). pii: e000764. doi: 10.1161/JAHA.113.000764.

Matthan NR, Ip B, Resteghini N, Ausman LM, Lichtenstein AH.
Long-term fatty acid stability in human serum cholesteryl ester, triglyceride, and phospholipid fractions.

J Lipid Res. 2010;51:2826-32.

UNIT OF MEASURE molar percentage (mol%)
SAMPLES RECEIVED 996
SAMPLES ANALYZED 996

QUALITY CONTROL MEASURES
INTERNAL STANDARD (added into every sample)

Fatty Acid	Ideal Concentration (ug/uL)	Mean concentration (ug/uL)	Recovery
1,2 diheptadecanoyl-Glycero-3-phosphocholine	2.00	1.98	97.5%

POOLED and SPIKED CONTROLS (run at the beginning and end of every box of samples)

Fatty Acid concentration in control (mol%)	CV % Range	Ideal CV % range
>5.0	1.3 - 2.4	<5.0
1.0-5.0	1.9 - 3.5	<5.0
0.05-<1.0	1.4 -11.8	<10.0
<0.05	8.2 - 12.2	<15.0

EXTERNAL STANDARD (run with every batch of samples)

Fatty Acids	CV%
10:0	4.8
12:0	4.1
14:0	3.1
15:0	2.5
16:0	2.0
18:0	1.5
20:0	2.7
22:0	4.2
24:0	4.3
14:1n-5	3.2
16:1n-7	1.7
18:1n-9	0.9
18:1n-7	1.3
20:1n-9	5.0
22:1n-9	1.8
24:1n-9	3.7
18:2n-6	0.9
18:3n-6	1.2
20:2n-6	2.4
20:3n-6	2.3
20:4n-6	2.2
22:2n-6	4.0
22:4n-6	4.8
22:5n-6	3.6
18:3n-3	4.0
18:4n-3	4.0
20:3n-3	1.8
20:5n-3	2.5
22:5n-3	3.7
22:6n-3	3.7
16:1n-7T	2.1
18:1n-9T	1.6
18:1n-7T	2.1
18:2T	1.9
18:2CLA	1.9

14. Appendix 6 - Quality Control Report for Serum Results (Fatty Acids) by Shipping Box and Sex.

The Coordinating Center performed QC procedures on the study results and generated this report.

Table 1. SFA summary by shipping Box and Sex.

Analysis Variable : SFA									
box	N Obs	N	Mean	Std Dev	Minimum	25th Pctl	50th Pctl	75th Pctl	Maximum
Box 1	**81**	81	38.42	1.66	34.18	37.49	38.32	39.45	43.23
Box 2	**81**	81	38.35	1.57	35.62	37.25	38.19	39.65	43.9
Box 3	**81**	81	38.43	1.45	34.59	37.75	38.3	39.51	41.14
Box 4	**81**	81	38.13	1.46	34.5	37.23	38.04	39.02	41.46
Box 5	**81**	81	37.95	1.64	33.41	36.9	37.81	38.86	41.43
Box 6	**81**	81	37.8	1.42	33.48	36.96	37.65	38.76	41.45
Box 7	**81**	81	37.79	1.73	33.72	36.52	37.7	38.95	41.66
Box 8	**81**	81	37.62	1.5	34.36	36.67	37.11	38.65	42.37
Box 9	**81**	81	37.36	1.6	33.88	36.22	37.26	38.37	42.19
Box 10	**81**	81	36.71	1.41	33.7	35.85	36.66	37.46	41.28
Box 11	**81**	81	38.14	1.28	34.01	37.39	38.16	38.88	41.75
Box 12	**81**	81	37.98	1.62	34.21	36.88	37.95	39.27	42.32
Box 13	**24**	24	38.02	1.65	34.83	36.92	37.9	38.71	42.06

Analysis Variable : SFA									
Sex/Gender	N Obs	N	Mean	Std Dev	Minimum	25th Pctl	50th Pctl	75th Pctl	Maximum
Female	**589**	589	37.84	1.57	33.41	36.85	37.83	38.81	43.9
Male	**407**	407	37.96	1.63	33.7	36.86	37.93	39.07	41.76

Table 2. MUFA summary by shipping Box and Sex.

Analysis Variable : MUFA									
box	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Box 1	**81**	81	12.83	1.25	9.46	12.09	12.72	13.51	15.69
Box 2	**81**	81	12.95	1.33	10.34	12.19	12.78	13.63	17.98
Box 3	**81**	81	12.85	1.38	9.79	11.77	12.89	13.67	16.16
Box 4	**81**	81	13.22	1.4	9.97	12.33	13.22	14.07	17.8
Box 5	**81**	81	12.83	1.29	9.76	11.99	12.91	13.64	16.03
Box 6	**81**	81	13.32	1.43	10.87	12.27	13.08	13.88	18.43
Box 7	**81**	81	12.72	1.49	9.63	11.95	12.8	13.24	19.38
Box 8	**81**	81	12.37	1.47	8.92	11.52	12.11	13.2	17.67
Box 9	**81**	81	12.84	1.43	9.76	11.9	12.78	13.51	17.5
Box 10	**81**	81	12.69	1.35	9.64	11.69	12.71	13.65	15.23
Box 11	**81**	81	13.07	1.2	9.8	12.29	13.13	13.79	16.55
Box 12	**81**	81	12.69	1.19	10.26	11.85	12.73	13.49	15.54
Box 13	**24**	24	12.84	1.61	9.5	11.85	13.02	13.73	16.48

Analysis Variable : MUFA									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	589	12.94	1.38	8.92	12.01	12.97	13.73	18.43
Male	**407**	407	12.76	1.36	9.46	11.9	12.68	13.41	19.38

Table 3. PUFA n-6 summary by shipping Box and Sex.

Analysis Variable : PUFA_n_6 PUFA n-6									
box	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Box 1	**81**	81	41.33	2.32	35.61	39.94	41.4	43.04	46.21
Box 2	**81**	81	41.62	2.57	33.57	40.17	41.86	43.33	48.62
Box 3	**81**	81	41.35	2.37	33.67	40.21	41.76	42.67	47.1
Box 4	**81**	81	41.21	2.22	35.18	39.39	41.24	42.75	45.99
Box 5	**81**	81	42.06	2.24	35.03	40.92	42.47	43.37	48.51
Box 6	**81**	81	41.49	2.5	33.36	40	41.98	42.94	47.16
Box 7	**81**	81	42.05	2.41	34.45	40.87	42.32	43.54	47.35
Box 8	**81**	81	42.36	2.21	35.24	41.11	42.33	43.97	46.82
Box 9	**81**	81	42.02	2.46	35.07	40.57	42.12	43.6	46.85
Box 10	**81**	81	42.86	2.23	35.78	41.35	43.12	44.15	47.55
Box 11	**81**	81	41.14	1.88	35.83	40.19	41.23	42.25	44.99
Box 12	**81**	81	41.75	2.35	35.87	40.41	41.65	43.36	48.19
Box 13	**24**	24	42.07	2.23	37.46	40.46	42.06	43.91	46.26

Analysis Variable : PUFA_n_6 PUFA n-6									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	589	41.71	2.26	33.36	40.31	41.85	43.22	48.62
Male	**407**	407	41.87	2.49	33.77	40.41	42.16	43.57	47.35

Table 4. PUFA n-3 summary by shipping Box and Sex.

Analysis Variable : PUFA_n_3 PUFA n-3									
box	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Box 1	**81**	81	5.08	1.03	3.16	4.29	4.91	5.62	8.03
Box 2	**81**	81	4.95	1.3	3.11	4.07	4.69	5.52	9.76
Box 3	**81**	81	5.01	1.36	3.12	4.2	4.7	5.34	10.04
Box 4	**81**	81	5.12	1.41	2.82	4.13	4.78	5.68	8.87
Box 5	**81**	81	4.84	0.93	3.01	4.18	4.66	5.18	7.93
Box 6	**81**	81	5.08	1.27	3.19	4.2	4.96	5.66	9.97
Box 7	**81**	81	5.16	1.15	3.03	4.39	4.92	5.84	10.1
Box 8	**81**	81	5.34	1.27	3.25	4.48	4.93	5.94	9.03
Box 9	**81**	81	5.39	1.35	3.54	4.47	5.08	6.08	10.8
Box 10	**81**	81	5.43	1.25	3.39	4.51	5.18	6.22	9.27
Box 11	**81**	81	5.33	1.45	2.77	4.54	5	5.56	10.94
Box 12	**81**	81	5.3	1.43	3.12	4.3	5.06	5.76	11.05
Box 13	**24**	24	4.87	1.13	3.1	3.88	4.65	5.81	7.35

Analysis Variable : PUFA_n_3 PUFA n-3									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	589	5.16	1.29	2.77	4.31	4.92	5.7	11.05
Male	**407**	407	5.16	1.27	3.03	4.3	4.84	5.65	10.1

Table 5. Total trans summary by shipping Box and Sex.

Analysis Variable : Total_trans Total trans									
box	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Box 1	**81**	81	2.35	0.74	1.12	1.76	2.32	2.7	4.14
Box 2	**81**	81	2.13	0.67	0.64	1.62	2.15	2.6	3.72
Box 3	**81**	81	2.36	0.75	0.92	1.89	2.28	2.71	4.28
Box 4	**81**	81	2.32	0.69	0.97	1.91	2.21	2.66	4.27
Box 5	**81**	81	2.32	0.76	1.04	1.83	2.15	2.8	4.71
Box 6	**81**	81	2.31	0.68	1.12	1.86	2.23	2.67	4.16
Box 7	**81**	81	2.28	0.72	0.91	1.74	2.27	2.72	4.14
Box 8	**81**	81	2.31	0.8	0.99	1.72	2.16	2.71	4.95
Box 9	**81**	81	2.39	0.87	0.63	1.79	2.27	2.83	5.76
Box 10	**81**	81	2.31	0.68	1.15	1.82	2.23	2.81	4.23
Box 11	**81**	81	2.33	0.69	0.85	1.89	2.22	2.67	4.96
Box 12	**81**	81	2.28	0.77	0.89	1.68	2.09	2.73	4.34
Box 13	**24**	24	2.2	0.63	1.18	1.81	2.18	2.55	3.99

Analysis Variable : Total_trans Total trans									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	589	2.34	0.71	0.64	1.83	2.28	2.77	5.76
Male	**407**	407	2.25	0.76	0.63	1.72	2.14	2.66	4.95

15. Appendix 7. Assay Documentation for Alkylresorcinol results provided by Laboratory

Alkylresorcinol	**Abbreviation**
5-Heptadecylresorcinol	AR C17_0
5-nonadecylresorcinol	AR C19_0
5-heneicosylresorcinol	AR C21_0
5-tricosylresorcinol	AR C23_0
5-pentacosylresorcinol	AR C25_0
SUM	AR C17_0+C19_0+C21_0+C23_0+C25_0)

Deuterated Standard	**Abbreviation**	***Ideal Concentration (nmol/L)***	***Mean concentration (nmol/L)***	***Recovery (%)***
5-nonadecylresorcinol-deuterated	AR C19-D4	65.7	62.6	95.2
5-heneicosylresorcinol-deuterated	AR C21-D4	65.7	59.9	91.2
5-tricosylresorcinol-deuterated	AR C23-D4	65.7	61.4	93.5
5-pentacosylresorcinol-deuterated	AR C25-D4	65.7	63.4	96.5

EXTERNAL STANDARDS (run with every batch of samples to control for matrix effect)

Alkylresorcinol (nmol/L)	***CV (%)***
AR C17_0	8.7
AR C19_0	7.8
AR C21_0	8.4
AR C23_0	9.5
AR C25_0	9.9

POOLED CONTROL SAMPLES (run with every box of samples)

Alkylresorcinol (nmol/L)	***CV range***	***Observed CV (%)****
AR C17_0	20 to 30	25.3
AR C19_0	10 to 15	13.2
AR C21_0	10 to 15	12.1
AR C23_0	10 to 15	13.6
AR C25_0	10 to 15	14.9
Sum	<10	6.9

* Average for 13 boxes

16. Appendix 8. Method Details and Assay Documentation Provided by Laboratory

Manufacturer:

Instrument:

UHPLC-QToF-MS: Agilent 6550 QTOF mass spectrometer with an ion funnel and dual atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) sources.
QTOF column: Agilent ZORBAX RRHD Eclipse Plus C18 column (2.1 x 50mm, 1.8 µm; Cat # 959757-902).

Solvents:

mobile phase A: LC-MS grade methanol (VWR Cat# BDH85800.100E)

mobile phase B: LC-MS grade water (Fischer Cat # 11332)
mobile phase C: 10% of dichloromethane in methanol (v/v) (HPLC grade from Millipore Sigma Cat #: 2008389)

Chemicals:

All alkylresorcinol (AR) homologues were purchased from ReseaChem GmbH, Burgdorf, Switzerland.

AR C17_0: 5-Heptadecylresorcinol (>95%); Cat #: 41442-57-2

AR C19_0: 5-nonadecylresorcinol (>98%); Cat #: 35176-46-6

AR C19_0-d4: 5-nonadecylresorcinol-d4 (>98%); Cat # 1108148-95-3

AR C21_0: 5-heneicosylresorcinol (>98%); Cat #: 70110-59-7

AR C21_0-d4: 5-heneicosylresorcinol-d4 (>98%); Cat #: RCG-491

AR C23_0: 5-tricosylresorcinol (>98%); Cat #: 70110-60-0

AR C23_0-d4: 5-tricosylresorcinol-d4 (>98%); Cat #: RCG-492

AR C25_0: 5-pentacosylresorcinol (>98%); Cat #: 70110-61-1

AR C25_0-d4: 5-pentacosylresorcinol-d4 (>98%); Cat #: RCG-493

Catalog number:	Listed in manufacturer section.
Kit Lot:	Custom made, one batch
Minimum volume required:	50 µL of plasma/serum
Low detection threshold:	0.0013 nmol/L
High detection threshold:	525 nmol/L
Range of calibration standard:	0.013 ~ 525 nmol/L. R2: all > 0.99
Estimated normal range:	Not available

17. Appendix 9 - Quality Control Report for Serum Results by Batch (Shipping Box) and Sex.

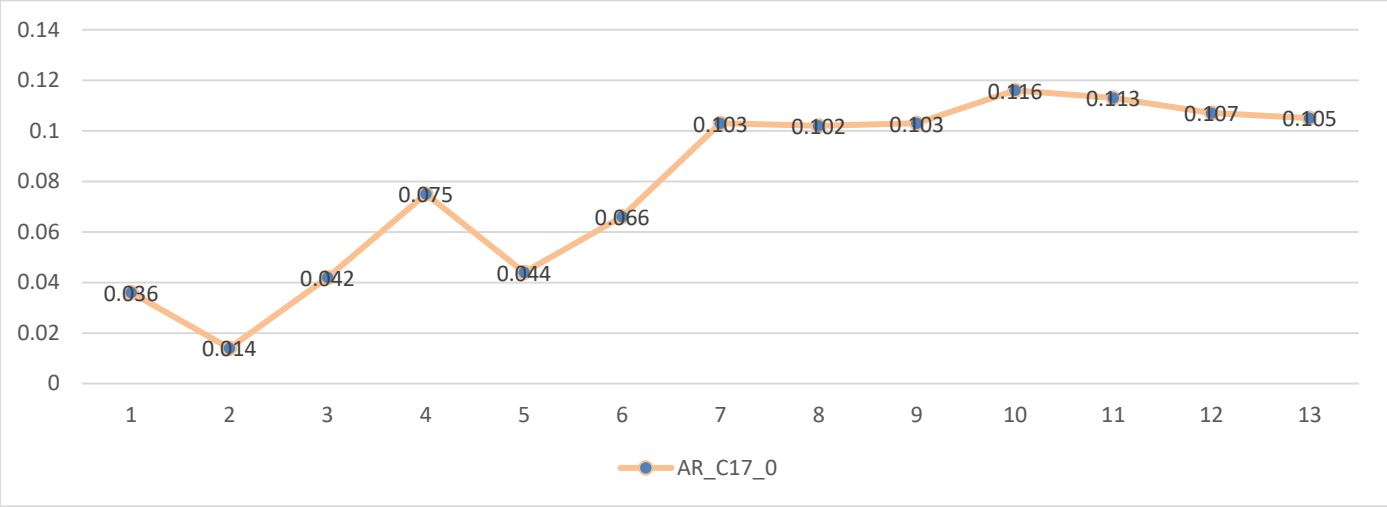
The Coordinating Center performed QC procedures on the study results and generated this report.

Table 1A. AR_C17_0 summary by Batch.

Analysis Variable : AR_C17_0									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	0.036	0.026	0.003	0.019	0.029	0.048	0.14
2	**81**	81	0.014	0.01	0.003	0.007	0.01	0.019	0.07
3	**81**	81	0.042	0.022	0.008	0.024	0.037	0.054	0.111
4	**81**	80*	0.075	0.05	0.018	0.044	0.058	0.092	0.283
5	**81**	81	0.044	0.031	0.002	0.021	0.035	0.057	0.131
6	**81**	81	0.066	0.035	0.008	0.039	0.056	0.087	0.167
7	**81**	81	0.103	0.054	0.011	0.067	0.092	0.127	0.289
8	**81**	81	0.102	0.045	0.017	0.061	0.105	0.136	0.201
9	**81**	81	0.103	0.052	0.023	0.064	0.094	0.133	0.268
10	**81**	81	0.116	0.056	0.026	0.072	0.107	0.147	0.323
11	**81**	81	0.113	0.059	0.021	0.07	0.098	0.144	0.28
12	**81**	78*	0.107	0.055	0.009	0.072	0.095	0.143	0.302
13	**24**	22*	0.105	0.035	0.057	0.077	0.102	0.138	0.172

*Note: 1 sample from Batch 4, 3 samples from Batch 12 and 2 samples from Batch 13 are marked as non-detectable (missing).

Graph 1. AR_C17_0 mean value by batch



Note: significant trend upward by batch

Table 1B. AR_C17_0 summary by Sex.

Analysis Variable : AR_C17_0									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	585	0.076	0.052	0.002	0.037	0.065	0.104	0.302
Male	**407**	405	0.079	0.059	0.003	0.032	0.068	0.118	0.323

Table 2A. AR_C19_0 summary by Batch.

Analysis Variable : AR_C19_0									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	0.767	0.726	0.031	0.245	0.518	1.003	3.94
2	**81**	81	0.636	0.662	0.021	0.215	0.397	0.898	3.748
3	**81**	81	1.241	1.019	0.066	0.428	0.828	1.914	3.867
4	**81**	81	0.959	0.977	0.057	0.238	0.678	1.277	4.645
5	**81**	81	0.644	0.767	0.018	0.131	0.394	0.824	3.568
6	**81**	81	0.8	0.817	0.013	0.187	0.445	1.166	4.074
7	**81**	81	1.023	1.082	0.052	0.328	0.622	1.195	5.305
8	**81**	81	1.21	1.098	0.061	0.423	0.945	1.61	5.584
9	**81**	81	1.223	1.294	0.053	0.383	0.759	1.527	5.845
10	**81**	81	0.894	0.964	0.027	0.21	0.542	1.197	5.629
11	**81**	81	0.272	0.439	0.029	0.069	0.127	0.218	2.367
12	**81**	79*	0.485	0.608	0.027	0.093	0.136	0.873	2.631
13	**24**	22*	0.327	0.378	0.096	0.155	0.236	0.304	1.896

*Note: 2 samples from Batch 12 and 2 samples from Batch 13 are marked as non-detectable (missing).

Graph 2. AR_C19_0 mean value by batch

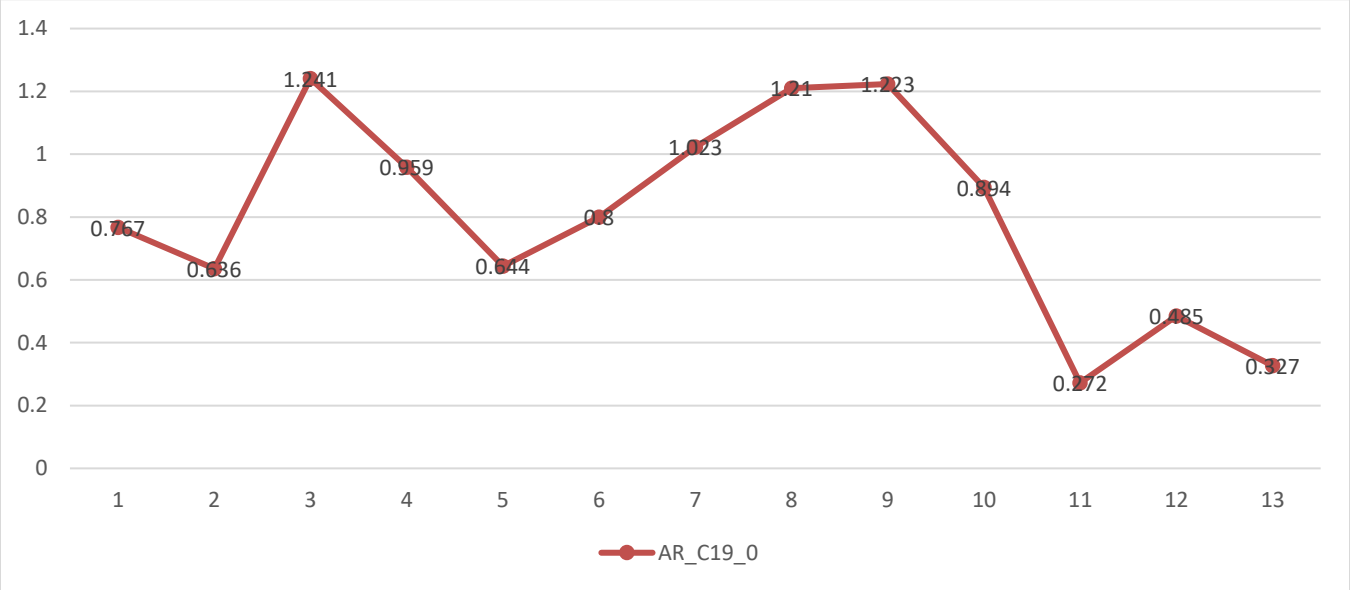


Table 2B. AR_C19_0 summary by Sex.

Analysis Variable : AR_C19_0									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	586	0.693	0.788	0.013	0.16	0.409	0.949	5.584
Male	**407**	406	1.041	1.089	0.018	0.255	0.657	1.397	5.845

Note: significant difference by sex.

Table 3A. AR_C21_0 summary by Batch.

Analysis Variable : AR_C21_0									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	4.937	4.577	0.146	2.164	3.627	6.203	23.572
2	**81**	81	4.385	4.542	0.179	1.542	3.014	5.43	21.761
3	**81**	81	6.12	4.355	0.36	3.071	5.417	7.399	20.102
4	**81**	81	4.887	5.501	0.359	1.224	3.166	6.539	34.792
5	**81**	81	5.862	7.259	0.18	1.953	3.49	6.427	41.074
6	**81**	81	3.881	3.744	0.102	1.003	3.417	5.291	23.399
7	**81**	81	4.578	5.286	0.23	1.404	2.452	5.033	26.823
8	**81**	81	2.978	2.392	0.212	1.194	2.244	4.161	12.063
9	**81**	81	3.47	4.133	0.11	1.146	2.036	3.907	22.651
10	**81**	81	3.422	4.884	0.144	0.898	2.025	4.676	39.224
11	**81**	81	3.588	3.653	0.221	0.945	2.086	5.5	17.74
12	**81**	80*	5.652	4.298	0.325	1.929	4.861	8.489	18.437
13	**24**	23*	4.689	6.751	0.337	1.111	2.94	4.1	28.662

*Note: 1 sample from Batch 12 and 1 sample from Batch 13 are marked as non-detectable (missing).

Graph 3. AR_C21_0 mean value by batch

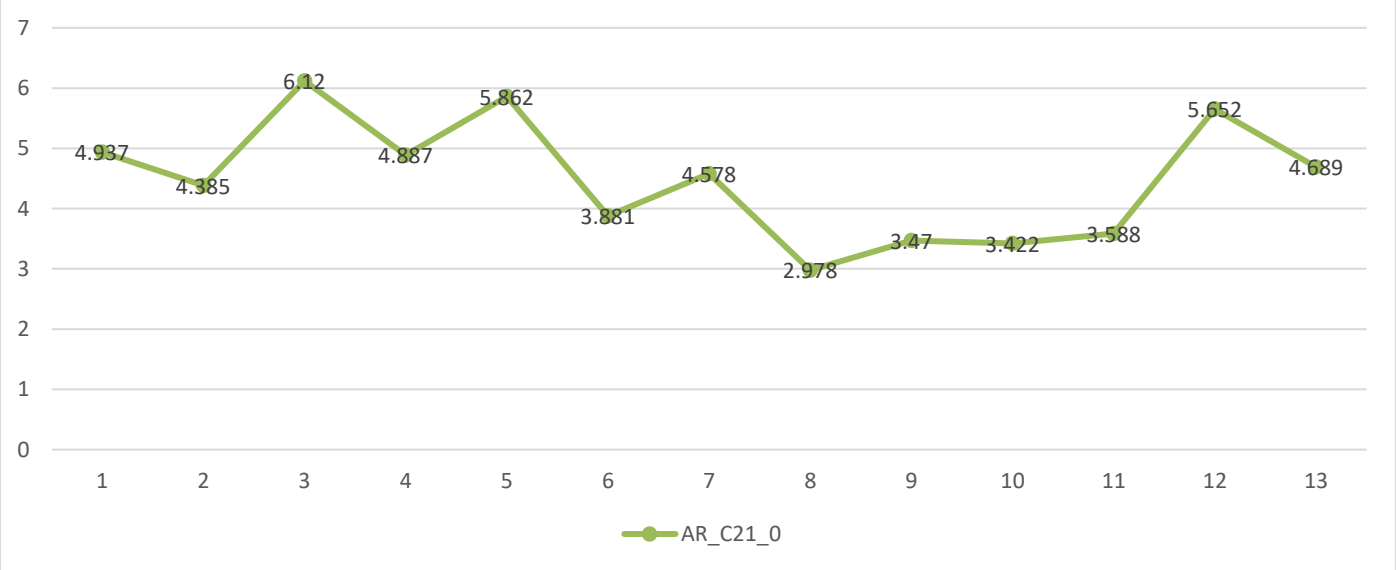


Table 3B. AR_C21_0 summary by Sex.

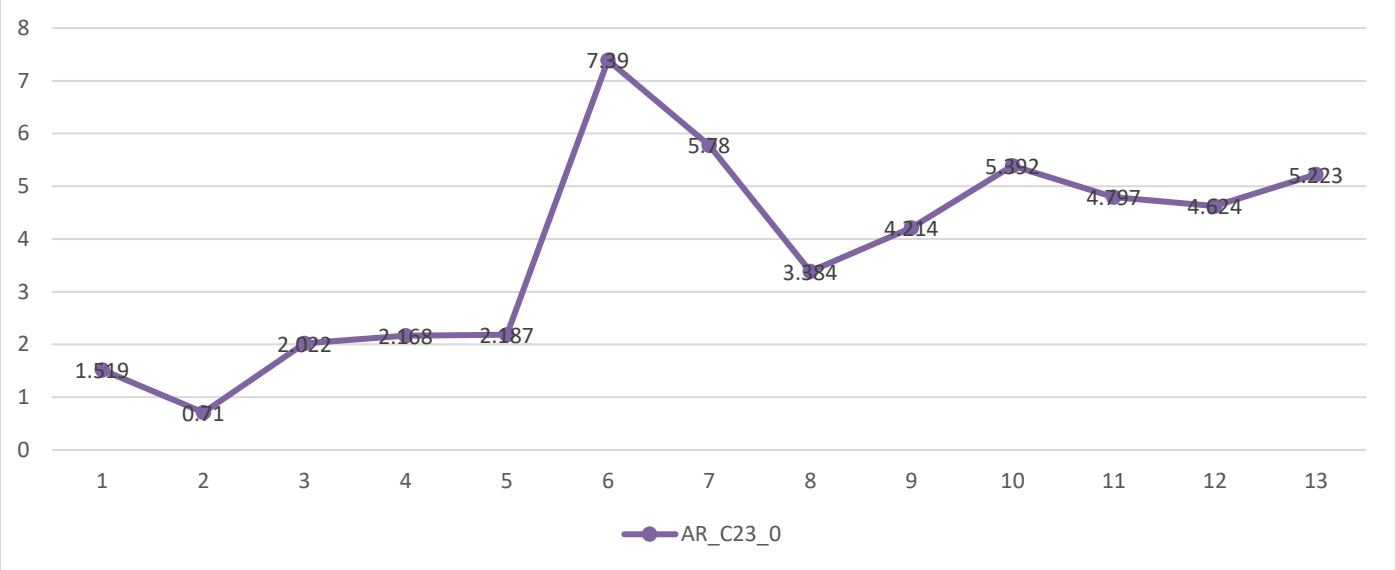
Analysis Variable : AR_C21_0									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	588	3.914	4.194	0.102	1.165	2.641	5.053	31.011
Male	**407**	406	5.309	5.505	0.221	1.771	3.536	6.798	41.074

Note: significant difference by sex.

Table 4A. AR_C23_0 summary by Batch.

Analysis Variable : AR_C23_0									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	1.519	1.061	0.224	0.843	1.194	1.95	6.058
2	**81**	81	0.71	0.691	0.061	0.307	0.456	0.833	4.314
3	**81**	81	2.022	1.479	0.145	0.976	1.531	2.655	7.322
4	**81**	81	2.168	2.569	0.24	0.962	1.319	2.212	13.953
5	**81**	81	2.187	1.981	0.308	0.757	1.228	3.306	8.67
6	**81**	81	7.39	4.964	0.486	1.49	8.128	10.549	17.358
7	**81**	81	5.78	4.328	0.842	1.645	6.422	8.575	18.578
8	**81**	81	3.384	2.214	0.95	1.953	2.826	3.999	12.277
9	**81**	81	4.214	3.587	0.241	1.899	2.71	5.483	16.672
10	**81**	81	5.392	3.861	0.582	2.643	4.315	6.916	16.301
11	**81**	81	4.797	2.142	1.191	3.345	4.336	6.232	11.771
12	**81**	81	4.624	2.602	1.288	2.642	3.693	6.175	11.989
13	**24**	24	5.223	3.279	0.406	3.281	4.917	7.272	13.645

Graph 4. AR_C23_0 mean value by batch



Note: significant trend upward by batch

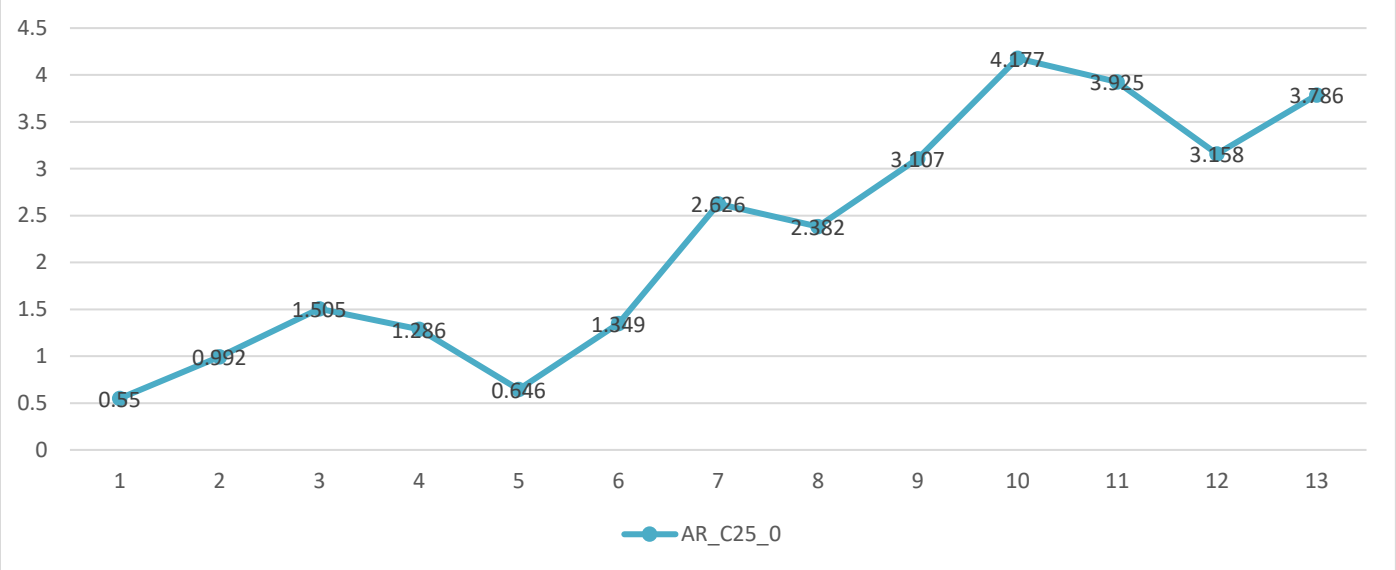
Table 4B. AR_C23_0 summary by Sex.

Analysis Variable : AR_C23_0									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	589	3.561	3.188	0.092	1.228	2.418	4.894	17.358
Male	**407**	407	3.948	3.852	0.061	1.188	2.573	5.649	18.578

Table 5A. AR_C25_0 summary by Batch.

Analysis Variable : AR_C25_0									
Batch	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
1	**81**	81	0.55	0.405	0.061	0.245	0.438	0.751	2.15
2	**81**	81	0.992	0.911	0.043	0.375	0.622	1.291	4.358
3	**81**	81	1.505	1.838	0.117	0.52	0.7	1.511	9.6
4	**81**	81	1.286	1.269	0.285	0.657	0.84	1.325	7.773
5	**81**	81	0.646	1.362	0.027	0.104	0.187	0.374	9.292
6	**81**	81	1.349	0.798	0.237	0.818	1.12	1.667	5.208
7	**81**	81	2.626	1.79	0.88	1.637	2.034	2.845	9.979
8	**81**	81	2.382	1.59	0.608	1.234	1.751	3.059	6.737
9	**81**	81	3.107	0.903	1.468	2.447	2.972	3.631	6.494
10	**81**	81	4.177	2.576	1.592	2.623	3.379	4.77	19.45
11	**81**	81	3.925	2.01	1.23	2.551	3.263	4.643	11.853
12	**81**	81	3.158	1.499	1.097	2.127	2.615	4.2	7.064
13	**24**	24	3.786	2.228	0.301	2.416	3.129	4.774	8.497

Graph 5. AR_C25_0 mean value by batch



Note: significant trend upward by batch

Table 5B. AR_C25_0 summary by Sex.

Analysis Variable : AR_C25_0									
Sex/Gender	**N Obs**	**N**	**Mean**	**Std Dev**	**Minimum**	**25th Pctl**	**50th Pctl**	**75th Pctl**	**Maximum**
Female	**589**	589	2.143	1.807	0.043	0.751	1.72	2.96	10.17
Male	**407**	407	2.237	2.166	0.027	0.642	1.659	3.128	19.45

Comments from Cardiovascular Nutrition Laboratory at Tufts University about QA report:

This sample subset would fall in an overall low fiber intake category based on our data from clinical studies where the values correspond to our low fiber intervention groups.

Documentation on any factors that influence the results: ARs are relative new biomarkers of whole grain wheat, rye and barley intake and we have very limited data on this. Also, we have no cut-off points or acceptable ranges because there are individuals who are naturally low absorbers/fast metabolizers of AR. Below is a summary of the findings thus far.

- Two studies have found differences in concentrations between males and females [1, 2]
- No consistent effects have been found for age or BMI [1-3, 5].
- Plasma lipids are correlated with plasma AR, though it is debatable whether adjusting for total plasma lipids or triglycerides will affect correlations with AR intake or other measures [2, 5].
- There is a wide interindividual variation of plasma AR concentrations with similar intakes [4, 5].
- AR values typically need to be log transformed during analysis.
- Total AR and the distribution of AR homologues varies greatly by country, with rye (high in AR 17) being the primary source in European countries, while wheat (low in AR 17 and higher in AR 21, AR 19 and AR 23) predominates in the US. Barley intake tends to increase AR 25. There are other minor contributors in the US (quinoa etc.)

Additional references

1. Montonen J, Landberg R, Kamal-Eldin A, et al. Reliability of fasting plasma alkylresorcinol concentrations measured 4 months apart. *European Journal of Clinical Nutrition*. 2010;64(7):698–703.

2. Ross AB, Bourgeois A, Macharia HN, et al. Plasma alkylresorcinols as a biomarker of whole grain food consumption in a large population: Results From the WHOLEheart Intervention Study. *American Journal of Clinical Nutrition*. 2012;95(1):204–211.

3. Landberg R, Kamal-Eldin A, Åman P, et al. Determinants of plasma alkylresorcinol concentration in Danish post-menopausal women. *European Journal of Clinical Nutrition*. 2011;65:94–101.

4. Rodríguez-Morató J, Jayawardene S, Huang NK, Dolnikowski GG, Galluccio J, Lichtenstein AH, Matthan NR. Simplified method for the measurement of plasma alkylresorcinols: Biomarker of whole grain intake. *Rapid Communications in Mass Spectrometry.* 2020 Apr 16:e8805. doi: 10.1002/rcm.8805.

5. Ma J, Ross A.B, Shea M.K, Bruce S.J, Jacques P.F, Saltzman E, Lichtenstein A.H, Booth S.L, McKeown, N.M. Plasma alkylresorcinols, biomarkers of whole-grain intake, are related to lower BMI in older adults. *Journal of Nutrition*. 2012;142: 1859–1864.