MOST Baseline Nutritional Analysis Dataset
V0NUTR

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1. Dataset description and Analyst Notes

Dataset: V0NUTR_bioassay.sas7bdat
Observations: 1826 records (1826 participants, 7 assays, 1 visit)
Documentation:

- VariableGuide_V0NUTR_bioassay.pdf
- Distributions_V0NUTR_bioassay.pdf

The Nutritional Analysis Dataset (V0NUTR.SAS7BDAT) includes data from the nine nutritional study batches, which consisted of fasting blood specimens from 1826 participants. Assays for the core MOST analytes and two ancillary studies were performed at the Tufts University Human Nutrition Research Center by Dr. Paul Jacques.

Participants whose specimens were used for the nutritional analysis were selected based on several sets of selection criteria (variable "SELECTION" is included in the dataset): N's below refer to number of participants in whom assay measurements were performed.

- **1=579 ppts** with BL knee ROA had their knee MRIs read (dataset V02WORMS_BLROA);
- **2 and 3=277 ppts** had their knee MRIs read (dataset V01WORMS) as an incident knee symptom case at 15m (N=77) or control at 15m (N=200);
- **4 and 5=358 ppts** had their knee MRIs read (dataset V02WORMS_INCIDENTROA) or developed incidence WK ROA (MRI not available). Total incident ROA cases at 30m (N=145); selected controls at 30m (N=213);
- **8=177 ppts** participants with 1.5T MRI obtained at 30m as part of Laxity and/or Gadolinium ancillary studies, which each had specific selection criteria);
- **9=435 ppts** met inclusion criteria for ancillary study AS06-01 (J. Curtis; Ethnic differences and OA) – sample enriched by race minorities.

If participant met more than one set of inclusion criteria, the biospecimen assay was done only once (in order of selection priority). Selection was based on knee status at the time of selection, which may change status at a later time. The V01235XRAY dataset contains all final and adjudicated x-ray readings; some knees may be included in the V01WORMS or V02WORMS datasets based on the preliminary x-ray readings available at time of selection.

The dataset V0NUTR includes concentrations for N=1826 of the five MOST core analytes related to the study specific aims: Vitamin C (plasma supernatant), Vitamin A (serum), Vitamin E (serum), Vitamin D (plasma), and PTH (serum). In addition, the ancillary study AS05-06 (T. Neogi) requested the vitamin K assay be performed from the residual of serum sample; ancillary study AS06-01 (J. Curtis) selected eligible participants for additional vitamin D and PTH measures.

Investigator and analyst should strategize approach on which statistical method to use to combine samples selected from different groups.

Table 1. Assay inclusion in the dataset V0NUTR by selection status.

	Total	Vitamin A (ug/dl) Vitamin E (ug/dl)		Total Vitamin C[(1)] (mg/dl)		25(OH) Vitamin D (ng/mL)		Intact PTH (pg/mL)		Vitamin K[(2)](nM/L)	
		missing	value	missing	value	missing	value	missing	value	missing	value
1:random BLROA sample	579	3	576	3	576	2	577	3	576	105	474
2:SxOA incidence case at 15m	77		77	27	50		77		77	25	52
3:SxOA control at 15m	200	1	199	107	93		200		200	68	132
4:WKROA case at 30m	145	11	134	55	90		145	.	145	12	133
5:WKROA control at 30m	213	2	211	1	212		213	2	211	1	212
8:additional sample (1.5T MRI)	177	2	175	131	46		177	3	174	1	176
9:AS0601 additional sample [(3)]	435	435	0	435	0	1	434	0	435	435	0
Total	1826	454	**1372**	759	**1067**	3	**1823**	8	**1818**	647	**1179**

[(1)]Vitamin C results were not provided for some batches (missing 759 ppts) as laboratory reported results were inconsistent/invalid.
[(2)]Ancillary study AS05-06 requested Vitamin K to be processed from residual serum sample.
[(3)]Ancillary study AS06-01 requested Vitamin D and PTH to be processed for selected list of participants.

2. References:

1. Chaganti RK, Tolstykh I, Javaid MK, Neogi T, Torner J, Curtis J, Jacques P, Felson D, Lane NE, Nevitt MC. High plasma levels of Vitamin C and E are associated with incident radiographic knee osteoarthritis. Osteoarthritis Cartilage. 2014 Feb;22(2):190-6. doi: 10.1016/j.joca.2013.11.008. Epub 2013 Nov 28. PMCID: 3933364 http://www.ncbi.nlm.nih.gov/pubmed/?term=24291351

2. Chaganti RK, Lane NE, Nevitt MC. Response to Letter to the Editor: "Food frequency questionnaire is an effective method for measuring micronutrient intake." Osteoarthritis and Cartilage. 2014 Nov;22(11):1949-50. doi: 10.1016/j.joca.2014.08.007. Epub 2014 Aug 26. PMID: 25168364 http://www.ncbi.nlm.nih.gov/pubmed/25168364

3. Wright NC, Chen L, Niu J, Neogi T, Javaid K, Nevitt MC, Lewis CE, Curtis JR. Defining physiologically "normal" vitamin D in African Americans. Osteporos Int. 2012 Sep;23(9):2283-91. Epub 2011 Dec 22. PMCID: 3677509 http://www.ncbi.nlm.nih.gov/pubmed/22189572

4. Misra D, Booth SL, Tolstykh I, Felson DT, Nevitt MC, Lewis CE, Torner J, Neogi T. Vitamin K deficiency is associated with incident knee osteoarthritis.
Am J Med. 2013 Mar;126(3):243-8. doi: 10.1016/j.amjmed.2012.10.011. PMCID: 3641753 http://www.ncbi.nlm.nih.gov/pubmed/23410565

Additional publications:

5. Felson DT, Niu J, Guermazi A, Roemer F, Aliabadi P, Clancy M, Torner JC, Lewis CE, Nevitt MC. Correlation of the development of knee pain with enlarging bone marrow lesions on magnetic resonance imaging. Arthritis Rheum. 2007 Sep;56(9):2986-92. PMID: 17763427 http://www.ncbi.nlm.nih.gov/pubmed/17763427

6. Englund M, Niu J, Guermazi A, Roemer FW, Hunter DJ, Lynch JA, Lewis CE, Torner JC, Nevitt MC, Zhang YQ, Felson DT. Effect of meniscal damage on the development of frequent knee pain, aching, or stiffness. Arthritis Rheum. 2007 Dec;56(12):4048-54. PMID: 18050201 http://www.ncbi.nlm.nih.gov/pubmed/18050201

7. Englund M, Guermazi A, Roemer FW, Aliabadi P, Yang M, Lewis CE, Torner JC, Nevitt MC, Sack B, Felson DT. Meniscal tear in knees without surgery and the development of radiographic osteoarthritis among middle-aged and elderly persons: The Multicenter Osteoarthritis Study.
Arthritis Rheum. 2009 Mar 60(3):831-9. PMCID: 2758243
http://www.ncbi.nlm.nih.gov/pubmed/19248082

8. Crema MD, Roemer FW, Marra MD, Niu J, Lynch JA, Felson DT, Guermazi A. Contrast-enhanced MRI of subchondral cysts in patients with or at risk for knee osteoarthritis: the MOST study.
Eur J Radiol. 2010 Jul;75(1):e92-6. Epub 2009 Sep 19. PMCID: 2891222
http://www.ncbi.nlm.nih.gov/pubmed/19767165

9. Baker K, Grainger A, Niu J, Clancy M, Guermazi A, Crema M, Hughes L, Buckwalter J, Wooley A, Nevitt MC, Felson DT. Relation of synovitis to knee pain using contrast-enhanced MRIs.
Ann Rheum Dis. 2010 Oct;69(10):1779-83. Epub 2010 May 14. PMCID: 3885343
http://www.ncbi.nlm.nih.gov/pubmed/20472593

3. Appendix A. Documentation about assays provided by the laboratory.

The reference ranges for the analytes are listed below:

	Tot.vit.C mg/dl	Vit.A ug/dl	Vit.E ug/dl	25(OH) Vit D ng/mL	Intact PTH pg/mL
Reference Range	0.4 - 2.2	30 - 90	500 - 1800	9 - 38	10 - 69

The assay information provided by the Tufts University HNRC is listed below:

25-Hydroxy Vitamin D

Matrix:	EDTA Plasma, Serum
Reaction type:	Rapid extraction of 25-OH-D with acetonitrile ^{125}I Radioimmunoassay, Competitive Binding
POA:	Commercial Kit Packard Cobra II Gamma Counter
Controls:	Supplied by manufacturer
Normal Range:	8-38 ng/mL
CV%	Intra Assay 11.7, 10.5, 8.6, 12.5 Inter Assay 9.4, 8.2, 9.1, 11.0

Reference:

Document: 2004. Catalog: 68100E. DiaSorin Inc, 1951 Northwestern Ave, PO Box 285, Stillwater, MN 55082.

Intact PTH

Matrix:	Serum
Reaction type:	Solid-phase, two-site chemiluminescent immunometric assay
POA:	Commercial Kit IMMULITE 1000
Controls:	DPC PTH Bi-Level Control Module
Normal Range:	10-69 pg/mL
CV%	Intra 5.5-6.6 % Inter 7.9-8.6 %
Conversion Factor	pg/mL x 0.1053 = pmol/L

Reference:

1. Document: IMMULITE /IMMULITE 1000 Intact PTH (PILKPP-9, 2004-02-24), Diagnostic Products Corporation (DCP) Los Angeles, CA 90045-5597.
2. Babson, AL. The DPC Cirrus IMMULITE automated immunoassay system. J Clin Immunoassay 1991; 14:83-8.
3. Babson AL, Olson DR, Palmieri T, Ross AD, Becker DM, Mulqueen PJ. The IMMULITE assay tube: a new approach to heterogeneous ligand assay. Clin Chem 1991; 37:1521-2.
4. Chan DW, editor. Immunoassay: a pratical guide. New York. Academic Press, 1987.

Ascorbate, Total

Matrix:	Plasma, Tissue, Food; protein free supernatant
Reaction type:	Isocratic reverse HPLC, amperometric
POA:	Waters Associates, Inc. HPLC system with Millennium 32 software, Waters 515 pump & Waters 717 auto sampler Bioanalytical Systems Inc., BAS EC-5 Electrochemical detector with amperometric detection
Controls:	In-house PCA precipitated Pool, Hi/Lo Spike BSA
Normal Range:	0.40 - 2.20 mg/dL
CV%	6%

Reference:

Behrens, W.A. and Madere, L. (1987). A Highly Sensitive High Performance Liquid Chromatography Method for the Estimation of Ascorbic and Dehydroascorbic Acid in Tissues, Biological fluids, and Foods. *Anal. Biochem*. **165**, 102-107.

Tocopherol

Matrix:	Plasma or Serum, light protected
Reaction type:	HPLC, Reverse Phase
POA:	Waters Associates HPLC Millennium 32 “Network” WISP 717plus Pump 510 Detector: Waters 490 Programmable Multiwavelength Detector
Normal Range:	500 - 1800 ug/dL
CV%	5.6%

Reference:

Bieri, J.G., Tolliver, T.J., Catignani, G.L. (1979). Simultaneous Determination of Alpha-tocopherol and Retinol in Plasma or Red Cells by High Pressure Liquid Chromatography. *Amer J Clin Nutr.* **32**, 2143-2149.

Retinol

Matrix:	Plasma or Serum, light protected
Reaction type:	HPLC, Reverse Phase
POA:	Waters Associates HPLC, Millennium 32 “Network” WISP 717plus, Pump 510, Detector: Waters 490 Programmable Multiwavelength Detector
Normal Range:	30 - 90 ug/dL
CV%	5%

Reference:

Bieri, J.G., Tolliver, T.J., Catignani, G.L. (1979). Simultaneous Determination of Alpha-tocopherol and Retinol in Plasma or Red Cells by High Pressure Liquid Chromatography. *Amer J Clin Nutr.* **32**, 2143-2149.

4. Appendix B. QC report by selection status.

Coordinating Center performed QC report by selection for each assay.

Analyte=V0PTH Normal range min=10 Normal range max=69											
Selection	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
1:random BLROA sample	576	48.5	43.5	3	345	485	84.20%	6	1.04%	85	14.76%
2:SxOA incidence case at 15m	77	45.49	44	9	196	71	92.21%	1	1.30%	5	6.49%
3:SxOA control at 15m	200	45.91	43	4	168	178	89.00%	2	1.00%	20	10.00%
4:WKROA case at 30m	145	51.95	48	10	151	122	84.14%	0	0.00%	23	15.86%
5:WKROA control at 30m	211	50.97	47	5	188	172	81.52%	1	0.47%	38	18.01%
8:additional sample	174	48.59	45	2	212	152	87.36%	1	0.57%	21	12.07%
9:AS0601 additional sample	435	61.44	51	3	1161	317	72.87%	2	0.46%	116	**26.67%**
V0PTH= Intact PTH (pg/mL)	1818	51.75	47	2	1161	1497	82.34%	13	0.72%	308	16.94%

Analyte=V0VITD Normal range min=8 Normal range max=38											
Selection	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
1:random BLROA sample	577	21.08	20	3	150	557	96.53%	10	1.73%	10	1.73%
2:SxOA incidence case at 15m	77	22.25	21	5	51	73	94.81%	1	1.30%	3	3.90%
3:SxOA control at 15m	200	22.16	22.5	6	45	196	98.00%	2	1.00%	2	1.00%
4:WKROA case at 30m	145	19.54	19	5	38	142	97.93%	3	2.07%	0	0.00%
5:WKROA control at 30m	213	19.49	19	3	36	207	97.18%	6	2.82%	0	0.00%
8:additional sample	177	21.35	21	6	41	173	97.74%	1	0.56%	3	1.69%
9:AS0601 additional sample	434	15.74	14	3	50	386	88.94%	44	**10.14%**	4	0.92%
V0VITD= 25(OH) Vitamin D (ng/mL)	1823	19.69	19	3	150	1734	95.12%	67	3.68%	22	1.21%

Analyte=V0VITA Normal range min=30 Normal range max=90											
Selection	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
1:random BLROA sample	576	74.42	72.010851	26.187998	143.5	464	80.56%	2	0.35%	110	19.10%
2:SxOA incidence case at 15m	77	71.94	70	38	125	68	88.31%	0	0.00%	9	11.69%
3:SxOA control at 15m	199	71.85	70.004223	34	170	170	85.43%	0	0.00%	29	14.57%
4:WKROA case at 30m	134	71.27	69	25	121.79523	112	83.58%	1	0.75%	21	15.67%
5:WKROA control at 30m	211	73.59	71	33.5	147.5	171	81.04%	0	0.00%	40	18.96%
8:additional sample	175	71.87	70.529762	27	117	148	84.57%	1	0.57%	26	14.86%
V0VITA= Vitamin A (ug/dl)	1372	73.15	71.104323	25	170	1133	82.58%	4	0.29%	235	17.13%

Analyte=V0VITE Normal range min=500 Normal range max=1800											
Selection	**N obs**	**Mean**	**Median**	**Min**	**Max**	**N within Normal range**	**% within Normal range**	**N below normal**	**% below normal**	**N above normal**	**% above normal**
1:random BLROA sample	576	1755.69	1578.0478	569.05527	9221.5	376	65.28%	0	0.00%	200	**34.72%**
2:SxOA incidence case at 15m	77	1739.15	1599	640.58889	4046	50	64.94%	0	0.00%	27	**35.06%**
3:SxOA control at 15m	199	1569.12	1384.5	695	4760	146	73.37%	0	0.00%	53	**26.63%**
4:WKROA case at 30m	134	1677.92	1549.25	575.71475	4852.8766	82	61.19%	0	0.00%	52	**38.81%**
5:WKROA control at 30m	211	1734.16	1516	333	4504.5	124	58.77%	1	0.47%	86	**40.76%**
8:additional sample	175	1695.46	1592	623.5	4897.5	110	62.86%	0	0.00%	65	**37.14%**
V0VITE= Vitamin E (ug/dl)	1372	1709.11	1540.8665	333	9221.5	888	64.72%	1	0.07%	483	35.20%

Analyte=V0VITC Normal range min=0.4 Normal range max=2.2											
Selection	**N obs**	**Mean**	**Median**	**Min**	**Max**	**N within Normal range**	**% within Normal range**	**N below normal**	**% below normal**	**N above normal**	**% above normal**
1:random BLROA sample	576	1.31	1.308	0.096	3.276	507	88.02%	34	5.90%	35	6.08%
2:SxOA incidence case at 15m	50	1.4	1.338	0.324	3.69	45	90.00%	1	2.00%	4	8.00%
3:SxOA control at 15m	93	1.34	1.29	0.228	3.168	83	89.25%	5	5.38%	5	5.38%
4:WKROA case at 30m	90	1.35	1.299	0.096	3.33	80	88.89%	4	4.44%	6	6.67%
5:WKROA control at 30m	212	1.33	1.308	0.162	3.276	197	92.92%	5	2.36%	10	4.72%
8:additional sample	46	1.33	1.245	0.114	3.18	42	91.30%	2	4.35%	2	4.35%
V0VITC= Total Vitamin C (mg/dl)	1067	1.33	1.302	0.096	3.69	954	89.41%	51	4.78%	62	5.81%

5. Appendix C. measurement of vitamin K for AS05-06

Measurement of Biochemical Vitamin K: Phylloquinone

Fasting plasma or serum phylloquinone was measured from a stored frozen aliquot by Dr. Sarah Booth at the Human Nutrition Research Center on Aging at Tufts University and a world leader in vitamin K research. The assay, a reversed-phase high-performance liquid chromatography method using post-column, solid phase chemical reduction of phylloquinone to its hydroquinone, followed by fluorometric detection, has been developed by her laboratory and applied to population and metabolic studies of vitamin K nutritional status.

Dr. Booth is a colleague and collaborator of Dr. Paul Jacques', who is performing other MOST blood specimen analyses (vitamins D, C, A/E, and PTH) at the same institution. Vitamins D and K can be measured from the same sample as they are both very stable and can be performed on serum or plasma (S. Booth, P. Jacques, personal communication). The laboratory at which Dr. Jacques performed the analyses, the N(utritional) E(valuation) L(aboratory), is the same clinical laboratory as Dr. Booth's. Thus, it was efficient for Dr. Jacques to provide the sample to Dr. Booth's laboratory from their aliquots. The volume required for measuring phylloquinone was 0.6mL and underwent a single freeze-thaw cycle prior to measurement.

QC report for Vit K1 assay (by selection batch) performed by Coordinating Center.

Analyte=V0VITK1 Normal range min=0.1 Normal range max=2.2

Selection	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
1:random BLROA sample	474	1.83	1.3	0	13.6	363	76.58%	3	0.63%	108	**22.78%**
2:SxOA incidence case at 15m	52	2.45	1.2	0.4	22.2	39	75.00%	0	0.00%	13	**25.00%**
3:SxOA control at 15m	132	1.36	1.1	0	7.4	112	84.85%	3	2.27%	17	12.88%
4:WKROA case at 30m	133	2.18	1.2	0	41.7	107	80.45%	1	0.75%	25	18.80%
5:WKROA control at 30m	212	1.86	1.3	0.3	16.1	170	80.19%	0	0.00%	42	19.81%
8:additional sample	176	1.58	1.2	0.2	7.8	143	81.25%	0	0.00%	33	18.75%
V0VITK1= Vitamin K1(nM/L)	1179	1.81	1.2	0	41.7	934	79.22%	7	0.59%	238	20.19%

Reference:

Misra D, Booth SL, Tolstykh I, Felson DT, Nevitt MC, Lewis CE, Torner J, Neogi T. Vitamin K deficiency is associated with incident knee osteoarthritis.
Am J Med. 2013 Mar;126(3):243-8. doi: 10.1016/j.amjmed.2012.10.011. PMCID: 3641753
http://www.ncbi.nlm.nih.gov/pubmed/23410565

6. Appendix D. MOST Study Design and Selection Comments

Recommendations for analyses using MOST data sets

- Assess the effects of study-design related covariates and take clinic into account in analyses as appropriate in sensitivity analyses.

- Clinic site. All study procedures and equipment were standardized between the two study clinic sites. However, there remains the potential for systematic differences between clinics in data collection, equipment performance, racial/ethnic distribution, etc.. Differences in associations by clinic should be explored and incorporated into analyses as appropriate. The clinic populations differ substantially be race/ethnicity. In analyses in which race/ethnicity is expected to be a factor, a race by clinic interaction should be explored. Note that the subject and knee selection for case-control and nested case-control substudies in MOST were usually matched by clinic.

- Sex. Consider performing sex-specific analyses to determine if associations are similar between men and women before combining the data and adjusting for sex.

- Readers for image assessments and assay batches. Although all readings and assay measurements across different batches were standardized to the extent possible, systematic differences by reader and batch may still exist and should be explored. Information on readers and assay batches are available for most measurements.

- A number of specific measurements were obtained in multiple nested case-control studies and substudies. These data can be combined to increase the number of records available for analysis. However, it is important that investigators understand the different selection criteria used for defining samples (e.g. the same exposure measurements in case-control studies related to different case definitions/outcomes – see Table 1 above for nutritional assay example) and do exploratory analyses to evaluated potential selection bias effects in the combined samples.

  VERY IMPORTANT: Investigators need to exercise caution when combining subsamples to address a research question.
  A) Using observations from nested case-control samples in MOST in order to study a predictor of an outcome other than the one defining the original case and control samples. When there is an association between the new outcome variable and case–control status the analysis can give biased results if it does not take the original case–control sampling design into account.
  B) Combining observations from additional subsamples (knees or subjects) with the observations from a case-control sample in order to study a predictor of the original case-control outcome. When the selection criteria for the additional subsample are associated with case-control status and are effects of the predictor, an analysis that does not take the complex sampling design into account can give biased results. These situations have in common that observations come from subsamples selected using criteria that are related to the outcome under investigation. It is essential to seek guidance from a statistician when considering or attempting such analyses.

  For additional information about this type of situation:
  Lee AJ, McMurchy L, Scott AJ. Re-using data from case-control studies. Stat Med. 1997 Jun 30;16(12):1377-89. PMID: 9232759.
  Scott A, Wild C. Case-Control Studies with Complex Sampling. J R Stat Soc Ser C Appl Stat. 2001;50(3):389-401.

- For analyses of knee- and joint- based outcomes, use a statistical method that accounts for the correlation between knee/joints, such as generalized estimating equations (GEE) models, in order to get accurate estimates of confidence intervals and p-values.