

**MOST Ancillary Study 12-01 (AS12-01)**  
**“Obesity and Knee Osteoarthritis: Understanding the Link” (Devyani Misra)**

**TABLE OF CONTENTS**

1. Dataset description and Analyst Notes.....	2
2. Selection plan .....	3
3. Appendix A. Assay documentation provided by laboratory .....	4
4. Appendix B. Quality Control report for serum Leptin results by gender.....	5
5. Appendix C. Additional information based on the data .....	6

## 1. Dataset description and Analyst Notes

Dataset: AS1201\_bioassay.sas7bdat

Observations: 691 records (691 participants, 1 assay, 2 visits)

Documentation:

- VariableGuide\_AS1201\_bioassay.pdf
- Distributions\_AS1201\_bioassay.pdf

AS1201\_bioassay dataset contains 691 records (one record per participant) and includes one assay result (Leptin). Assay was performed at the University of Vermont Laboratory for Clinical Biochemistry Research on baseline and 30m serum:

- 691 records from baseline serum blood sample
- 73 records from 30m serum blood sample

Table 1: Participant eligibility and records included in the dataset

Bio-specimen sample Time Point	Baseline serum (50uL/ppt)	30m serum (50uL/ppt)	Total Vials
Only BL serum (excluding paired analysis)	618	-	618
Paired samples (baseline and 30m)	73	73	146
Total serum vials	<b>691</b>	73	<b>764</b>

Note: the laboratory performing the assay was blinded if there was two or more vials for the same participant from different visits and those vials may be processed in separate batches.

### Analyst Notes:

- Leptin assays result variables (V0LEPTIN, V2LEPTIN) include a prefix that indicates the visit when blood was collected: V0 for baseline and V2 for 30-months.

- When assay results were not obtained, special missing values were used:

.L=below low detection level

.H=above high detection level

Values (.L and .H) can be used in the categorical analysis only. Alternatively, analyst can assign the special value above detection or below detection if requested by investigator.

Each record is marked by variable AnalysisType (values: BL only, BL+30m) to indicate what type of analysis can be performed.

Note: the laboratory performing the assay was blinded if there was two or more vials for the same participant from different visits and those vials may be processed in separate batches.

- One participant selected for AnalysisType "BL+30m" does not contain BL assay result - vial was missing.
- If results were missing for all time points due to insufficient volume or some other reason assay could not be performed, the record was dropped from the analytical dataset.

## 2. Selection plan

Enrolled in MOST: N=3026 participants / 6052 knees

Select BL and 30m serum samples as outlined in Table 2 using time point and outcome to classify participants:

- A. No OA on either knee at baseline and 30m (or 60m) follow up x-ray reading complete (at least one knee)
- B. No OA on either knee at baseline and 30m and 60m follow up x-ray reading complete (at least one knee)

**Table 2: Biospecimen Sample and Time Point Selection**

Biospecimen sample Time	Baseline serum (250uL/ppt)	30m serum (250uL/ppt)	Total Vials
Only BL serum (excluding paired analysis)	618 (Table A)	-	<b>618</b>
Paired samples (baseline and 30m)	73 (Table B)	73 (Table B)	146
Total serum vials	<b>691</b>	<b>73</b>	<b>764</b>

### Detailed Inclusion/Exclusion Criteria for Each Group:

**A. BL SERUM and no OA:** Participants with BL serum available, no OA on either knee at baseline, have x-ray reading status non-missing at 30m or 60m.

Inclusion	Excluded
Participants with no OA status on either knee at baseline with outcome assessment at 30 month available at least in 1 knee <b>N=1268</b>	TFROA at baseline: 1607 30m missing: 151
<b>543 participants can be included in analysis (AS0501)</b>	Baseline and 30 month serum in AS0501: <b>N=543</b>
<b>73 participants can be included in analysis (group B)</b>	Baseline and 30 month serum Selected in group B: <b>=73</b>
	Baseline serum not available <b>N=32</b>
<b>620</b> selected	2 missing assay results

**B. BL and 30M paired SERUM and no OA at baseline and 30m:** Participants with BL and 30m serum available, no OA on either knee at baseline and 30m, have x-ray reading status non-missing at 60m on at least one knee.

Inclusion	Excluded
Participants with no OA status on either knee at baseline and 30m with outcome assessment at 60 month available at least in 1 knee <b>N=946</b>	1130 no TF ROA at 30m (and BL) 60m missing: 184
<b>414 participants can be included in analysis (AS0501)</b>	30 month serum in AS0501: <b>N=414</b>
	30m serum not available <b>N=449</b>
	BL serum not available <b>N=10</b>
<b>73</b> selected	

### 3. Appendix A. Assay documentation provided by laboratory

(1) Leptin assay performed in 2015.

Assay	Manufacturer	Method	Catalog#	Kit Lot	Volume	Sample Type	Range of Standard*	Dilution	Low Detection	High Detection	Estimated Normal Range**
Leptin (Serum)	R&D Systems	Elisa	DLP00	LOT# 326589	15	Serum	15.6 - 1000 pg/mL	100	~1500	~110,000	Males: 2205 - 11,149 Females: 3877 - 77,273

\*The standard curve range is different from the detectable range (low and high) based on the dilution factor of the sample in the assay (in this case 1:100). Assay values can be detected from approximately 1500 pg/mL to 110,000 to 120,000 pg/mL at the 1:100 dilution. Most people just want to know the detectable range of the assay.

\*\*The normal range are values from assay run on a small representation of healthy donors.

(2) Inter- and Intra-CV provided, by assay:

Assay	Inter-Assay CVs	Intra-assay CV
Leptin (Serum) 20145	from 9% to 14%	n/a

#### 4. Appendix B. Quality Control report for serum Leptin results by gender

Coordinating center performed QC report for the study results. Note: results marked as special values (below or above detection level) are missing and therefore not included in this report.

Table 3. WOMEN only results: Leptin by visit  
*Assay=Leptin pg/ml Normal min=3877 Normal max=77273*

Assay	visit	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
Leptin pg/ml	V0	430	37022.5	34411.6	1361.2	114870.9	407	94.20%	3	0.70%	20	4.60%
	V2	54	49495.4	45484.3	1890.3	117936.7	42	71.20%	2	3.40%	10	16.90%
Leptin pg/ml		484	38414.1	34676.7	1361.2	117936.7	449	91.40%	5	1.00%	30	6.10%

Table 4. MEN only results: Leptin by visit  
*Assay=Leptin pg/ml Normal min=2205 Normal max=11149*

Assay	visit	N obs	Mean	Median	Min	Max	N within Normal range	% within Normal range	N below normal	% below normal	N above normal	% above normal
Leptin pg/ml	V0	254	15099.1	10938.4	1338.9	104062.2	118	45.60%	14	5.40%	122	<b>47.10%</b>
	V2	13	15960.5	11135.1	3801.5	35407.3	7	50.00%	0	0.00%	6	<b>42.90%</b>
Leptin pg/ml		267	15141	10949.4	1338.9	104062.2	125	45.80%	14	5.10%	128	46.90%

## 5. Appendix C. Additional information based on the data

The AS12-01 proposal indicates the following: serum results obtained for AS05-01 (Lewis) will be used to supplement the available sample of this study for participants eligible for one or more selection groups. Coordinating Center identified 579 records (from baseline and 30m serum) with results obtained in eligible participants for this study.

Please note plasma results are not recommended to use in conjunction with serum leptin results.

Before combining the results from these two studies, please note that the manufacturer and method provided as part of the assay documentation can vary even when running the same assay from the same blood type. It is recommended to perform batch-specific sensitivity analysis.

Table 5. Comparison of manufacturer/method and blood type used for the study

Study and Year	Assay	Manufacturer	Method	Catalog #	Kit Lot	Volume	Sample Type	Range of Standard*	Dilution	Low Detection	High Detection	Estimated Normal Range**
AS05-01 pilot, 2008-2009	Leptin (Plasma)	Millipore	Bio-Rad Luminex Flow Cytometry	HADK2-61K-B		75	EDTA	16 - 250,000 pg/mL	1	16	250000	Male: 2205 - 11149 pg/ml: Females: 3877 - 77273 pg/mL
AS05-01 Lewis, 2010	Leptin (Serum)	R&D Systems	Elisa	DLP00	279515	15	Serum	15.6 - 1000 pg/mL	100	~1300	~120,000	
AS10-07 Neogi, 2014	Leptin (Plasma)	R&D Systems	Elisa	DLP00	317538	15 uLs	EDTA	15.6 - 1000 pg/mL	100	~1500	~120,000	
AS12-01 Misra, 2015	Leptin (Serum)	R&D Systems	Elisa	DLP00	LOT# 326589	15	Serum	15.6 - 1000 pg/mL	100	~1500	~110,000	

\*The standard curve range is different from the detectable range (low and high) based on the dilution factor of the sample in the assay (in this case 1:100). Assay values can be detected from approximately 1500 pg/mL to 110,000 to 120,000 pg/mL at the 1:100 dilution. Most people just want to know the detectable range of the assay.

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