DOCUMENTATION
for

MIDJA 2
CLINIC VISIT
DATA COLLECTION
PROTOCOLS

University of Wisconsin ♦ Institute on Aging
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INTRODUCTION

This document provides an overview of the data collection protocols implemented at the Clinic Visit for the MIDJA 2 Biomarker Protocol. It describes the protocols for assessment of vital signs and morphometrics, collecting and processing tissue samples, and collecting medication data. It also provides information about the creation and usage of related administrative and constructed variables. In addition, it contains descriptions of the biomarker assay methods including details about assay ranges, reference ranges, and inter-, and intra- assay covariation.

Data users are also encouraged to review the MIDJA 2 Biomarker DataFile Notes.doc. This document provides information about naming conventions, administrative and filter variables, and the order in which variables appear in the data file. It also includes information about how we handled missing values and other issues that arose over the course of the study.

This document will be periodically revised and updated as more information is gathered, and researchers continue to work with the MIDJA 2 Biomarker data. If there are suggestions or comments, please contact midus_help@aging.wisc.edu.

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SECTION A

OVERVIEW OF CLINIC DATA AND COLLECTION PROTOCOLS

OVERVIEW OF CLINIC DATA AND COLLECTION PROTOCOLS

The MIDJA 2 Biomarker clinic visit included collection of:

- Vital signs and morphometrics:
  - blood pressure
  - height, weight, waist and hip
- Time Preference (*new*)
- Blood samples for biomarker assay
- Medication data

As described in "MIDJA Biomarker 2 Data File Notes", the naming convention organizes variables according to the data type or method used for data collection. The variable names for the Clinic Visit data, unless otherwise noted below, begin with the unique 3 character set "K2C".

The remainder of this section provides some administrative details as well as an overview of the following sections.

Vital Signs & Morphometrics

A copy of the form completed during the clinic visit appears in Section B. This section also includes detailed instructions for obtaining vital signs and morphometrics according to the same protocols for collecting the corresponding data in the MIDUS Biomarker study.

Time Preference

English and Japanese versions of this 4 page questionnaire are available as standalone PDFs. Details about the items and scale construction can be found in the MIDJA 2 Biomarker Documentation of Scales and Constructed Variables.

Blood Assays

The blood samples allow for assessment of multiple indicators within the following major systems as follows (**bolded** measures are new at MIDJA 2):

- *Cardiovascular:* Cholesterol Panel (Total, HDL, **LDL, Triglycerides**), Glycosylated Hemoglobin (HA1c)
- *Neuroendocrine:* DHEA, DHEA-S, Creatinine
- *Inflammatory:* Fibrinogen, CRP(C-Reactive Protein), Fibrinogen, IL6 (InterLeukin 6), sIL6r (soluble IL-6 receptor), **IL8 (Interleukin 8), IL10 (Interleuken 10), and TNF α (Tumor Necrosis Factor alpha)**
- Renal System: Creatinine, **estimated Glomerular Filtration Rate (eGFR)**

Blood samples are non-fasting samples that were collected and processed into fresh and frozen aliquots using standardized protocols. Details about these protocols appear in Section C. The aliquots were then sent to Syowa Medical Service Co. LTD, in Tokyo, Japan (SMS) for further processing and some assays as follows:

- Fresh aliquots are assayed by SMS for:
  - Glycosylated hemoglobin (HA1c) – this MIDUS biomarker requires whole blood that is fresh or has been refrigerated for just a few days thus it is conducted in Japan.
  - Life Style Assessments – these are routine tests performed as a part of clinic visits in Japan and include: tests of cholesterol, HDL and LDL cholesterol, tryglycerides, liver function (GPT, gamma GPT, GOT), kidney function (BUN, uric acid), blood glucose, and anemia (e.g. red cell count, hematocrit).

- Frozen aliquots were packaged and shipped to the MIDUS BioCore lab at the University of Wisconsin. Samples were shipped to the U.S. so they could be assayed at the same labs that used for MIDUS biomarker assays.

Details about tissue assay methods samples appear in Section D. This section includes details about assay method and sensitivity for inclusion in manuscripts, as well as information about any changes in assay method and subsequent corrections to the data. Assays corresponding to MIDUS measures are listed first followed by the Lifestyle assays done at the Syowa Laboratory in Tokyo. A table summarizing assay ranges, reference ranges, and intra- and inter-assay co-variation appears at the end of this section.
Please also note the following:

- Variable names for assays performed in the U.S begin with the unique 3 character set "K2B".
- Although saliva samples are collected at home details about the saliva cortisol assay and sensitivity are also include in Section D.
- Variable names for the saliva data begin with the unique 3 character set "K2S".

Medication Data

Participants are instructed to bring medications or information about medications to the clinic visit. At the end of the visit staff record medication names, dosage, route of administration, frequency, how long the participant has been taking the medication and why they think they are taking it. A copy of the form used to collect this information and additional details about data collection are included in Section E along with information about the structure of the data and basic processing.

MIDJA medication data are also processed (i.e. reasons for taking medications are coded, therapeutic and pharmacologic class codes are added) according to a standardized set of procedures developed with the MIDUS Biomarker Medication data. These protocols are described in a separate document that applies to data from both studies. (See Documentation for MIDUS and MIDJA Medication Data).

SECTION B

CLINIC VISIT FORM AND INSTRUCTIONS

CLINIC VISIT RECORD FORM

#ID_____ DATE_____

NAME_____

◆**Record of Last Meal**：
When did you have a meal last time?

DATE:_____ TIME:_____

◆**Record of Body Measurement**：

HEIGHT（cm）	WEIGHT（kg）

WAIST（cm）	ABDOMINAL GIRTH（cm）	HIP（cm）

BLOOD PRESSURE 1 (high/low)	BLOOD PRESSURE 2 (high/low)	BLOOD PRESSURE 3 (high/low)

◆**Blood Sample**：

①Took blood → □Yes □No
（Reason： ）

②Date and time of taking blood→ Date:_____, Time:_____

Data Collection Protocols: Vital Signs and Morphometrics

The following provides a general overview of the steps completed by project staff to obtain the indicated measurements. More detailed instructions are provided at item B, below as appropriate.

A. **General Instructions**:

1. *Hip and Waist*: measured using a Gulik II Tape measure according to the detailed according to the instructions below
2. *Weight and Height*:
    a. For weight measurement, participants should take off their shoes and empty their pockets.
    b. They do not have to take off any other clothing.
    c. The amount of clothing is defined at 500g
3. *Blood Pressure*:
    a. Have respondent sit quietly for 5 minutes
    b. Measure blood pressure 3 times allowing a maximum of 30 seconds between each measurement.

B. **Detailed Instructions**: Hip & Waist Measurement

Project staff use a Gulik II Tape Measure to obtain hip and measurements. These measurements are recorded to 1 decimal place. The following standard is used for 'rounding':

If less than 0.05 (centimeters) round down
If greater than or equal to 0.05 (centimeters) round up

Measurements should be made directly on skin or over a single layer of clothing.
If, for some reason, these measurements have to be done over loose clothing, make sure to smooth the clothing as flat as possible DO NOT BUNCH the material.

Have participant stand erect with feet placed shoulder width apart and toes pointing forward.

1. Waist Circumference is measured directly on skin or over a single layer of clothing if the garment is a camisole or undershirt. <u>DO NOT measure over a loose fitting blouse or shirt</u>):
    a. Place tape around narrowest point between ribs and the iliac crest (tips of the large bones of the pelvis).
    b. Be sure the tape goes around evenly (parallel to the floor).
    c. Record measurement to the nearest millimeter (1 decimal place).

2. Hip Circumferences is measured over a single layer of clothing, typically subject's underwear:
    a. <u>1st circumference (Iliac Crest, Abdominal Girth):</u>
        - Ask subject to point to their hipbone.
        - Place the tape measure at the iliac crest and wrap around the body.
        - Make sure the measure is parallel to the floor.
        - Record measurement to the nearest millimeter (1 decimal place).

    b. <u>2nd circumference (Maximum Extension, Hip):</u>
        - Place the tape measure at the maximum diameter of the buttocks and wrap it around the body.
        - Stand to the side of the participant to see that the tape is placed at the point of maximum buttock extension.
        - Record measurement to the nearest millimeter (1 decimal place).

SECTION C

TISSUE SAMPLE COLLECTION AND PROCESSING PROTOCOLS

BLOOD SAMPLES

Blood Collection Protocol

Blood samples are collected in the order described below. Collect blood samples and process as follows:

a. Preparation for blood draw
   i. Use non-dominant arm if possible.
   ii. Make reasonable number of attempts to get needed samples (e.g., 3 or so tries is generally sufficient – if unable to obtain samples, indicate this on Clinic Visit form, above)
   iii. Subject should avoid strenuous activity before blood draw.

b. Fill tubes in the following order and then process as indicated:
   i. Three 8.5-ml SST (Serum Separator Tube) tubes, gently invert tube 3-5 times after draw.
   ii. Two 1.8ml citrated tubes.
   iii. One 2.0-ml FNa tube (whole blood for HA1c), gently invert tube 3-5 times after draw and cool at 4 degree.
   iv. One 2.0-ml EDTA2K tube (for blood counts for lifestyle assessment), gently invert tube 3-5 times after draw and cool at 4 degrees.

c. Complete the Clinic Visit Record Form
   i. Record the time that the participate last ate, using 24-hour clock
   ii. Record date and time blood draw began using 24-hour clock.
   iii. Indicate if the blood draw was completed or not.
   If no blood was drawn record the reason it was not collected.
   iv. Record any problems encountered or other information regarding aspects of the collection that are not standard (per protocol).

Blood Sample Processing Protocol

Blood samples were processed by study staff on the University of Tokyo (UT) campus at the time of the clinic visit and then sent to Syowa Medical services for HbAlc testing, lifestyle assessments, and storage until shipment to the Biocore lab in Madison WI, USA, as indicated below.

1. FNa tube containing fresh blood is cooled at 4°C and tested for HA1c at Syowa.
2. EDTA2K tube containing fresh blood is cooled at 4°C and sent to Syowa for blood counts.
3. Two 1.8 ml Citrate tubes:
   a. Centrifuge as soon as possible to separate plasma
      i. Use refrigerated centrifuge (4°C).
      ii. Centrifuge samples for 15 minutes.
      iii. Set speed at 2000-3000 rpm.
      iv. On form, record time centrifuge begun.
   b. From two citrate tube, aliquot all plasma into two tubes, with 1.0ml plasma in each tube, apply blue label.
   c. On form, indicate amount of plasma aliquoted into vial.
   d. Store in a MIDJA freezer in designated freezer (-60 to -80 degrees) for delivery to Syowa for shipment to the US.
   e. Record the time the specimens were stored in freezer on the form.
4. Three 8.5-ml SST tubes (Serum Separator Tube).
   a. Let stand for 15-30 minutes after draw (maximum of 2 hours allowed between draw and centrifuging).
   b. Centrifuge to separate sera at UT.
      i. Use refrigerated centrifuge (4°C)
      ii. Centrifuge samples for 10 minutes.
      iii. Set speed at 2000-3000 rpm.

iv. Record the time centrifugation was begun on the form.

c. From three SST tubes, aliquot sera into two 6.5 ml vials with red label (4 ml sera in each tube) for shipment to UW.
d. Aliquot the remaining 2 or more ml of sera into a 6.5 ml vial with colorless label for Syowa.
e. On form, indicate number of aliquots filled of each type.
f. If none were filled or fewer than number specified were filled, please indicate why.
g. Store two red capped tubes in MIDJA storage box in designated freezer (-60 to -80 degrees) for delivery to Syowa for shipment to the US. The remaining tube is kept at 4 degree and sent to Syowa for measurement of biochemical indicators at Syowa.
h. Record the time specimens were stored in freezer on the form.

SECTION D

BLOOD ASSAY DOCUMENTATION

Blood Assay Descriptions
Appendix A: Assay Sensitivity Summary Table
Appendix B: Regression Equations

MIDJA 2 BIOMARKER ASSAY DESCRIPTIONS

Overview

The following provides information about laboratory assays of tissue samples collected in 2013-2014 as part of the MIDJA 2 Biomarker Protocol, including details about assay method and sensitivity for inclusion in manuscripts. If the assay method changed during the data collection period, information about the change and any corrections to the data are also provided. Assays are listed first by tissue source (blood, saliva), and then by physiological system assessed (cardiovascular, inflammatory etc). Each section begins with a brief overview of sample collection and processing. Information about the assays was provided by the lab performing the assay or was developed by Dr. Chris Coe's lab (i.e. MIDUS Biocore lab). The MIDJA 2 protocol included a set of blood assessments (assays and cell counts), performed at Showa Medical Science, that were reported back to Japanese patients as part of a standard physical exam. A few of these assessments replicate MIDUS blood assays. Where appropriate, information about assays done in both locations is included below. A summary table listing all the assays along with the specimen type, assay type and sensitivity appears in Appendix A beginning at page D-10

Blood samples were obtained from 326 of 328 individuals who completed a MIDJA 2 clinic visit. Unless otherwise note samples from this set of 326 individuals were sent for all assays described below.

Information about corresponding assays in the MIDUS II Project 4 (Biomarker) protocol can be found in the "BloodUrineSaliva.pdf" documentation file in the public archive at ICPSR http://www.icpsr.umich.edu/icpsrweb/NACDA/studies/29282/documentation

I. Blood Assays

A. Sample Collection:
Blood samples were collected from each participant at the clinic visit, which could occur at any time during the day. Participants were encouraged not to eat at least one hour before the visit. The last time they ate was recorded prior to the blood draw. To ensure consistency, all samples were collected and processed using standardized procedures. Frozen samples were stored in a -60^{o} C to -80^{o} C freezer until shipped on dry ice to the MIDUS Biocore Lab. Samples were subsequently stored in a -65^{o} C freezer until assayed.

- *Whole blood* samples (sodium fluoride-anticoagulated) as well as freshly-separated *sera,* were refrigerated and delivered to the Showa Lab in Tokyo Japan within 24 hours of collection for
  - Complete blood count (CBC) and HA1c determination
  - Serum chemistry: Cholesterol (Total, HDL, LDL) Triglycerides, Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), γ-Glutamyl transpeptidase (GGTP), Blood urea nitrogen (BUN), Uric acid (UA).

- *Frozen serum and plasma* samples were shipped to the MIDUS Biocore Lab monthly for the following biomarker assays:
  - Cardiovascular markers – Cholesterol, HDL Cholesterol, Triglycerides
  - Hormone markers - DHEA and DHEA-S

- Inflammation markers - IL6, IL8, IL10, TNFa, soluble receptors for IL6 (s-IL6-r), C-Reactive protein (CRP), Fibrinogen
- Serum Creatinine

B1. Assay Details - Cardiovascular Markers

Glycosylated Hemoglobin (HA1c):

HA1c assays were performed at the Showa lab in Tokyo Japan. Glycosylated hemoglobin (HA1c) was measured using antibodies attached to latex particles, which specifically bind HA1c. Remaining free antibodies are agglutinated with a synthetic polymer. The change in turbidity is inversely related to the quantity of bound glycopeptides and is measured turbidometrically. Hemoglobin concentration in blood is determined by adding sodium lauryl sulfate (SLS) to anticoagulated blood to lyse the cells, thus releasing their hemoglobin into the plasma so that it can be measured colorimetrically. Percent HA1c is determined by dividing the concentration of HA1c by the total hemoglobin concentration

Comparability with MIDUS values: The original MIDJA HA1c value can be used in analyses of MIDJA-only data, or for comparison to HA1c values from other Japanese studies. For comparison with U.S. data (MIDUS), however, two issues must be addressed: 1) MIDJA values tended to be lower than the MIDUS values and 2) the U.S. HA1c assay at Meriter labs changed twice over the course of MIDUS II data collection and once since then.

To address the first issue, a small experiment was conducted to determine if the lower MIDJA values reflected differences in laboratory assay methods, or physiological variation. EDTA-anticoagulated blood was drawn from 7 individuals in April, 2014. Each sample was divided into two aliquots, one of which was assayed immediately for HA1c at the Showa lab, and one of which was sent to Madison and assayed within 1-2 days at Meriter Labs. From the two sets of data obtained on these ten samples, a scatterplot (Appendix B) was made and a linear regression line generated[1]:

D =0. 9207*K + 0.9415

where K is the value obtained in Tokyo and D is the value obtained in Madison. The coefficient of determination for this regression line is 0.9269.

To address the second issue, equations were obtained from the in-house comparisons at Meriter Labs to adjust their reported values to account for shifts occurring on May 22, 2006; February 29, 2007; and April 17, 2010:[2,3,4]

- These three equations were combined to obtain the equation below to convert the 2010 Meriter-reported values (D) to bring them in line with the values in the MIDUS II data file (A) [5]:

  A = 1.3849 * D -2.1438

  where D represents the values reported by Meriter after April 17, 2010 and A represents the values reported by Meriter before May 22, 2006

- These MIDUS-adjusted values were plotted against the Tokyo values, and a second scatterplot was obtained. From this plot, a second equation was obtained, which was used to convert the Tokyo values (K) to pre-5/22/06 Meriter values[1,5]:

  A = 1.2751*K – 0.8399

The data file includes the original value (K2CHBA1C) obtained from the Showa lab and the final MIDUS-adjusted value.

Cholesterol Panel (Lipid Assays):

Biocore Assays: Total Cholesterol, HDL Cholesterol, and Triglyceride assays were performed at Meriter Labs (Madison, WI) using a Cobas Integra® analyzer (Roche Diagnostics, Indianapolis, IN).

Cholesterol: The instrument uses the enzyme cholesterol esterase to cleave cholesterol esters into free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, this hydrogen peroxide effects the coupling of phenol and 4-aminoantipyrine to form a red quinone-imine dye. The color intensity of the dye is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.[6]

HDL-cholesterol: Serum is first mixed with synthetic polyanions, which adsorb to the surfaces of the other lipoproteins (LDL, VLDL, and chylomicrons); they are thereby transformed into detergent-resistant forms, whereas HDL is not. After solubilizing the HDL with detergent, it is measured as described above for total cholesterol.[7]

*T*he HDL assay was re-standardized at Meriter on April 3, 2007 while the MIDUS II data collection was ongoing.[8] Using data obtained from 8 samples assayed by both the old and new reagents, the following equation was obtained (Appendix B) [9]:

Adjusted value = 1.1423(new value) - 0.9028

This equation was used to adjust all subsequent HDL values reported by Meriter for both MIDUS and MIDJA, to bring them in line with the earlier MIDUS values.

Triglycerides: Triglycerides are hydrolyzed by the enzyme lipoprotein lipase to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase. The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide effects the oxidative coupling of 4-chlorophenol and 4-aminophenazone to form a red-colored quinoneimine dye, which is measured at 512 nm. The increase in absorbance is directly proportional to the concentration of triglycerides in the sample.[10]

LDL-cholesterol: An estimation of LDL-cholesterol level is made using the Fridewald formula from direct measurements of total cholesterol (TC), triglycerides (TG), and HDL-cholesterol. The Fridewald formula is as follows[11]:

LDL = TC - HDL - TG/5

The Fridewald formula begins to be unreliable when triglycerides are elevated (≥350 mg/dl). Meriter adopts a conservative approach and will not report it if the subject's triglycerides level is ≥350 mg/dl. Therefore, when the triglycerides level is ≥350 mg/dl the Biocore lab will calculate LDL using the above formula.

If the Subject's triglyceride level is >400 mg/dl, then the Biocore lab will use 400 mg/dl as the upper limit for triglycerides when calculating LDL-cholesterol.[11]

The mathematical adjustment of HDL values after 2007 (described above) also made it necessary to generate a second set of values for LDL, and for the Ratio of Total/HDL Cholesterol.

Showa Medical Science Assays: Cholesterol (Total, HDL, LDL) and Triglyceride assays were performed in Tokyo for Life Style Assessment reports that were sent to study participants. These values can be used in analysis of MIDJA-only data or for comparison to values from other Japanese studies.

Total serum cholesterol: This procedure employs the enzyme cholesterol esterase (CE) to cleave cholesterol esters into free cholesterol and fatty acids. Cholesterol oxidase (COD) then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase (POD), the hydrogen peroxide effects the oxidation of a dye, causing a color change which can be measured colorimetrically.

HDL- and LDL- cholesterol: Each of these is isolated from the other lipoproteins, and then measured as described above for total cholesterol.

Triglycerides: Triglycerides are hydrolyzed to glycerol and fatty acids. Free glycerol is then phosphorylated to glycerol-3-phosphate, which in turn is oxidized by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of the enzyme peroxidase (POD), the hydrogen peroxide oxidizes a dye, causing a color change which can be measured colorimetrically.

Comparability with MIDUS values-
Lipid profiles were also run on sera from all 326 MIDJA participants at the Showa lab in Tokyo. This was done so assay results could be sent in a timely way to study participants. Frozen sera from these same participants were sent to Madison, where assays of HDL, total cholesterol, and triglycerides were performed at Meriter Labs; LDL and the cholesterol/HDL ratio were calculated by the Coe lab from these. These measurements were used to generate the scatter plots and linear regression lines shown in Appendix B. Across all five charts,"x" values were obtained in Tokyo, while"y" values represent values obtained in the US. The figures for HDL Cholesterol (Figure 3), LDL Cholesterol, and the Ratio of Total/HDL Cholesterol (Figure 4) each include two scatter plots: one comparing the Tokyo values to the US values reported by Meriter (US-MIDJA), and one comparing the Tokyo values to the MIDUS-adjusted values.

For more information about corresponding assays in the MIDUS II Biomarker (P4) project see the MIDUS BloodUrineSaliva.pdf in the public archive at:
http://www.icpsr.umich.edu/icpsrweb/NACDA/studies/29282/documentation

Measure	Variable names		
	Meriter Lab Value	MIDUS Adjusted Value	Showa Lab Value
Total Cholesterol	K2BCHOL	Not applicable	K2CTCHOL
HDL Cholesterol	K2BHDL	K2BHDLA	K2CHDL
Triglycerides	K2BTRIGLY	Not applicable	K2CTRIG
LDL Cholesterol	K2BLDL	K2BLDLA	K2CLDL
Ratio Total/HDL Cholesterol	K2BRTHDL	K2BRTHDA	K2CRTHDL

Investigators are encouraged to use the MIDUS-adjusted values when comparing MIDJA 1 & 2 data to MIDUS 2 data, for the sake of consistency.

B2. Assay Details – Hormone Markers

DHEA and DHEA-S assays are performed at the Associated Regional & University Pathologists (ARUP) laboratory (Salt Lake City, UT).

DHEA:

ARUP uses LC-MS/MS (liquid chromatography tandem mass spectrometry) to assay DHEA[12] Early in the MIDUS II and MIDJA I projects, it had used radioimmunoassay. For comparison, 14 MIDUS samples were assayed for DHEA using both methods and used to generate the following equation (Appendix B) [13].

Adjusted value = 1.6145(new value) + 0.4668

DHEA values obtained since May 18, 2009 have been adjusted using this equation, to bring the new values in line with the older data.

DHEA-Sulfate:

This assay was performed with a Roche Modular Analytics E170 analyzer, using an Elecsys® kit (Roche Diagnostics, Indianapolis, IN).

By incubating the serum with a DHEA-S specific biotinylated antibody, an immunocomplex is formed, the amount of which is dependent upon the DHEA-S concentration in the specimen. After addition of strepavidin-coated microparticles and a DHEA-S derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into a measuring cell where the microparticles are magnetically captured onto the surface of an electrode. Unbound substances are removed. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier.[14]

B3. Assay Details – Inflammation Markers

Cytokines and IL6sr were assayed in the MIDUS Biocore Laboratory (University of Wisconsin, Madison, WI). The fibrinogen and CRP assays are performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT).

IL6, IL8, IL10, and TNFα by Immunoelectrochemiluminescence:

These cytokines were measured using a V-plex Custom Human Cytokine Kit (catalog #K151A0H-2) manufactured by Meso Scale Diagnostics, Rockville, MD. This technology employs a 96-well multispot plate, with each spot precoated with a capture antibody for a particular cytokine. These spots are connected to an electrode surface at the bottom of the plate. Serum samples are pipetted into wells, where the cytokines to be measured are allowed to adhere to their corresponding antibody spots. After washing off nonadherent samples, a solution containing detection antibodies tagged with ruthenium(II) tris-bipyridine-(4-methylsulfonate) N-hydroxysuccinimide ester (MSD Sulfo-tag™) is pipetted into the wells and allowed to adhere to the immobilized cytokines. After washing off unbound antibodies, a special buffer is added, and the plate is loaded into an imager (Meso Scale Discovery Sector Imager Model #HTS24), where a voltage applied to the plate electrodes causes the captured Sulfo-tag™ to emit light. The instrument measures the intensity of the emitted light to provide a quantitative measure of analytes in each sample.[15]

IL6 by ELISA:

IL6 was measured using the Quantikine® High-sensitivity ELISA kit #HS600B (R & D Systems, Minneapolis, MN). This is a sandwich ELISA using a microplate precoated with a monoclonal antibody specific for IL6. Standards and samples are pipetted into the wells, and any IL6 present is bound by the immobilized antibody. After washing away the unbound substances, an alkaline phosphatase-labeled detection antibody specific for IL6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate (NADPH) is added, which is converted to NADH. After an incubation period, an amplifier solution containing two additional enzymes whose actions are coupled to the cycling of NADH and NAD+ (diaphorase and alcohol dehydrogenase) and their respective substrates (iodonitrotetrazolium-violet and ethanol) are added. The diaphorase converts the iodonitrotetrazolium-violet to formazan, a red-colored product. The reaction is stopped at a predetermined endpoint with sulfuric acid, and the absorbance, which is proportional to the concentration of IL6 in the sample, is read at 490 nm using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA).[16]

Soluble Receptors for IL6 (IL6-sr):

Concentration of IL6-sr was measured using the Quantikine® ELISA kit #DR600 (R & D Systems, Minneapolis, MN). This is a sandwich ELISA using a microplate precoated with a monoclonal antibody specific for IL6-sr. Standards and samples are pipetted into the wells, and any IL6-sr is bound by the immobilized antibody. After washing away the unbound substances, a horseradish peroxidase-labeled detection antibody specific for IL6-sr is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate (tetramethylbenzidine) is added to the wells, and a blue-colored product develops. This reaction is stopped at a predetermined endpoint using sulfuric acid, which changes the color to yellow. Absorbance is read at 450 nm using a Dynex MRXe plate reader (Magellan Biosciences, Chantilly, VA), and provides a quantitative measure of the concentration of IL6-sr in the samples.[17]

Fibrinogen:

Fibrinogen was measured using kit #10446313 for the BNII nephelometer (Dade Behring Inc., Deerfield, IL).[18] The amount of fibrinogen present in the sample is quantitatively determined by immunochemical reaction. Complexes formed between antigen and antibody molecules scatter light passing through the sample. The intensity of the scattered light is proportional to the concentration of the antigen (fibrinogen) in the sample.[19]

C-reactive protein (CRP):

CRP was measured using kit #10446091 for the BNII nephelometer (Dade Behring).[18] This is a particle-enhanced immunonepholometric assay. Polystyrene particles are coated with monoclonal antibodies to CRP, which, in the presence of antigen (CRP) agglutinate to cause an increase in the intensity of scattered light. The increase in scattered light is proportional to the amount of CRP in the sample.[19]

Samples falling below the assay range for CRP by this method were re-assayed using a high-sensitivity assay kit (Meso Scale Diagnostics catalog #K151STD) by immunoelectrochemiluminescence.[18] For comparison, 7 MIDJA samples were assayed using both assays, and the following equation was generated (Appendix B):[20]

$$\text{nephCRP} = 10^{0.914 \times \log(\text{MSDCRP}) - 0.016}$$

CRP values obtained using the MSD high-sensitivity assay were adjusted, using this equation, to bring them in line with the rest of the data.

B4. Assay Details – Serum Creatinine

Serum Creatinine was measured using a Cobas Integra® analyzer (Roche Diagnostics, Indianapolis, IN).

This assay employs the enzymes creatininase, creatinase, and sarcosine oxidase which react with creatinine and produce hydrogen peroxide as one of the end products. This reacts with aminophenazone and HT1B to form a quinine imine chromogen as an end product. The absorbance of this colored end product is measured at 552 nm and is directly proportional to the creatinine concentration in the serum.

Estimated glomerular filtration rate (eGFR):

Meriter uses serum creatinine to estimate glomerular filtration rate (GFR). They use the IDMS-traceable MDRD formula found at http://www.kidney.org/professionals/kdoqi/gfr_calculator, and report eGFR in mL/min/1.73 m^2.

B5. Assay Details – Japanese Life Style Assessments

In Japan clinic visits routinely include collection of blood samples for assessment of life-style related diseases. Serum samples, refrigerated never frozen, were sent to Showa Medical Sciences for these assessments and a report of these results were sent to study participants. These results are also included in the MIDJA Biomarker data file and can be used with other MIDJA data and for comparison to other Japanese samples. In addition to the HA1c, Cholesterol, and Triglyceride assays described above, the following measurements of liver and kidney function were obtained along with a complete blood count:

Liver Function: Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), γ-Glutamyl transpeptidase (GGTP)

Kidney Function: Blood Urea Nitrogen (BUN), Uric Acid (UA)

Liver Function Assays:

Glutamic oxaloacetic transaminase (GOT): In this assay, aspartate and α-ketoglutarate are mixed with serum, and the GOT in the serum catalyzes the conversion of these substrates to glutamate and oxaloacetate. The oxaloacetate is then converted to malic acid by the enzyme malate dehydrogenase; this reaction consumes NADH, whose disappearance can be monitored by measuring UV absorbance at 340 nm.

Glutamic pyruvic transaminase (GPT): Alanine and α-ketoglutarate are mixed with serum, and the GPT in the serum catalyzes the conversion of these substrates to glutamate and pyruvate. The pyruvate is then converted to lactate by the enzyme lactate dehydrogenase; this reaction consumes NADH, whose disappearance can be monitored by measuring UV absorbance at 340 nm.

γ-Glutamyl transpeptidase (GGTP): A donor substrate, (γ-L-Glutamyl)-3-carboxy-4-nitroanilide, and an acceptor substrate are mixed with serum; the GGTP in the serum catalyzes the formation of 5-amino-2-nitrobenzoic acid, whose appearance can be monitored by measuring light absorbance at 410 nm.

Kidney Function Assays:

Blood urea nitrogen: This is a coupled enzymatic assay employing urease to convert urea to ammonium carbonate, and glutamate dehydrogenase (GLDH) and α-ketoglutarate to convert the ammonium ion to glutamic acid. The latter reaction consumes NADH, whose disappearance can be monitored by UV absorbance at 340 nm.

Uric acid: The enzyme uricase converts uric acid to allantoin, carbon dioxide, and hydrogen peroxide. The hydrogen peroxide product is reduced to water by the enzyme peroxidase (POD), and this reaction is coupled to an indicator reaction which can be monitored photometrically.

Complete Blood Count (CBC):

Hematocrit, Hemoglobin, Red blood cells, Mean cell volume, Mean cell hemoglobin, Mean cell hemoglobin concentration, White blood cells, Platelets: These measurements were automated.

II. Saliva Assays

A. Sample Collection:

Saliva samples were collected by participants in their homes over a 3 day period following the clinic visit. The samples for this assay were collected on cotton swabs in salivettes (Sarstedt Cat. #51.1534) and frozen. Four samples (upon awakening, 30 minutes after waking, midday, and night) were collected each day. Samples were then frozen and shipped to the MIDUS Biocore lab for storage and subsequent cortisol assay. [21]

B1. Assay Details – Saliva Cortisol

Salivary Cortisol:

Saliva samples were assayed at the Core Lab for the UW Institute for Clinical and Translational Research. At the time of assay, they were thawed and centrifuged at 3000 rpm for 5 min, resulting in a particulate-free, clear fluid of low viscosity. Concentrations of free cortisol (the only type found in saliva) were determined using radioimmunoassay (catalog # 07-221106 from MP Biomedicals, Solon, OH). [23]

In order to compare data from this assay to MIDJA 1 (and MIDUS 2) data from the immunoluminescence assay (kit #RE62011 manufactured by IBL International, Hamburg, Germany) used by the Dresden LabService in Germany, 73 pairs of saliva samples were assayed at the ICTR using both types of assays. From this, the following linear regression equation was generated (Appendix B):[25]

IBL value = 0.7629 (RIA value) – 0.0544 R^2= 0.9178

This equation was used to adjust the RIA data to bring it in line with the data in the earlier studies.

APPENDIX A: MIDJA ASSAY SENSITIVITY SUMMARY TABLE

A. MIDJA Bioassays from blood samples.

Assay	Assay Type	Performed at:	Assay Range	Variability	Reference Range
Hb(blood)	SLS-Hb method	Showa	0-25 g/dL	inter-assay CV: 1.0%	Male:13.5-17.6 Female::11.3-15.2
HA1c(blood)	Latex agglutination assay	Showa	3.0-13.0%	inter-assay CV: 10%	4.3-5.8 %
Total Cholesterol(serum)	Enzymatic colorimetric	Meriter	0-800 mg/dL[6]	inter-assay CV: 1.8%[24] intra-assay CV: 0.5-0.8%[6]	<200 mg/dL[22]
Total Cholesterol(serum)	Enzymatic colorimetric(CE-COD-POD)	Showa	2-700 mg/dL	inter-assay CV: 3.0%	120-219 mg/dL
HDL-cholesterol (serum)	Enzymatic colorimetric	Meriter	0-155 mg/dL[7]	inter-assay CV: 7.8%[24] intra-assay CV: 1.1-1.4%[7]	≥40 mg/dL[22]
HDL-cholesterol(serum)	Enzymatic colorimetric	Showa	2-150 mg/dL	inter-assay CV: 5.0%	Male 40-70 mg/dL Female 40-75 mg/dL
LDL-cholesterol (serum)	Calculated	Univ. of Wisconsin (Coe Lab)	N/A	N/A	<129 mg/dL[22]
LDL-cholesterol(serum)	Enzymatic colorimetric	Showa	1-450 mg/dL	inter-assay CV:5.0%	70-139 mg/dL
Triglycerides(serum)	Enzymatic colorimetric	Meriter	0-875 mg/dL[10]	inter-assay CV: 1.4%[24] intra-assay CV: 1.6%[10]	<150 mg/dL[22]
Triglycerides(serum)	Enzyme assay(Gpo-POD/free glycerol elimination technique)	Showa	2-600 mg/dL	inter-assay CV: 3.0%	35-149 mg/dL
DHEA-sulfate (serum)	Immunoelectro-chemiluminescent	Associated Regional & University Pathologists (ARUP)	min <1 μg/dL[26]	inter-assay CV: 4.7%[24] intra-assay CV: 0.8-3.8%[27]	Age Male Female[28] 30-39 120-520 45-270 40-49 95-530 32-240 50-59 70-310 26-200 60-69 42-290 13-130 ≥ 70 28-275 10-90
DHEA (serum)	LC-MS/MS (Liquid Chromatography tandem Mass Spectrometry)	ARUP	0.05-9 ng/mL[29]	inter-assay CV: 5.5%[24] intra-assay CV: <7.4%[29]	18-39 yr: 1.33-7.78[28] ≥ 40 yr: 0.63-4.70
Creatinine (serum)	Enzymatic colorimetric	Meriter	0-30.5 mg/dL[21]	inter-assay CV: 2.8%[24]	0.5-1.2 mg/dL[21]
IL6, IL8, IL10, and TNFα	Immunoelectro-chemiluminescent	Univ. of Wisconsin (Coe Lab)	0.06-488 pg/mL IL6[15] 0.04-375 pg/mL IL8 0.03-233 pg/mL IL10	inter-assay CV: 4-17%[30] intra-assay CV: 3-6 %[31]	0.16-27.2 pg/mL IL6[15] 1.48-1720 pg/mL IL8

Assay	Assay Type	Performed at:	Assay Range	Variability	Reference Range
			0.04-248 pg/mL TNFα		0.06-3.08 pg/mL IL10 0.1-1.75 pg/mL TNFα
IL-6 (serum)	ELISA	Univ. of Wisconsin (Coe Lab)	0.156-10 pg/mL[16]	inter-assay CV: 3.8%[30] intra-assay CV: 3.8%[32]	0.45-9.96 pg/mL[16]
IL6-sr (serum)	ELISA	University of Wisconsin (Coe Lab)	31.3-2000 pg/mL[17]	inter-assay CV: 9.0%[30] intra-assay CV: 1.9%[32]	13500-45000 pg/mL[17]
Fibrinogen (citrated plasma)	Immunonephelometric	Univ. of Vermont (Tracy Lab)	56-904 mg/dL[18]	CV: 3.2-3.8%[33]	180-350 mg/dL[19]
CRP (citrated plasma)	Immunonephelometric	Univ. of Vermont (Tracy Lab)	0.17-800 ug/mL[18]	CV: 3.4-5.0%[33]	≤3 ug/mL[19]
High-sensitivity CRP (citrated plasma)	Immunoelectro-chemiluminescent	Univ. of Vermont (Tracy Lab)	0.00024-200 ug/mL[18]	CV: 7.8-11.0[33]	<3 ug/mL[19]
Glutamic Oxaloacetic Transaminase (GOT)	UV(JSCC*)	Showa	0-1500 U/L	inter-assay CV: 5.0%	10-40 IU/L
Glutamic Pyruvic Transaminase (GPT)	UV(JSCC*)	Showa	0-1500 U/L	inter-assay CV: 5.0%	5-45 IU/L
γ- Glutamyl Transpeptidase (GGTP)	5-amino-2-nitrobenzoic acid (JSCC*)	Showa	0-2000 U/L	inter-assay CV: 5.0%	Male 0-70 IU/L Female 0-35 IU/L
Blood Urea Nitrogen (BUN)	Urease/GLDH method (ammonia elimination)	Showa	2.25-200 mg/dL	inter-assay CV: 3.0%	8-23 mg/dL
Uric Acid	Enzyme Assay（Uricase/POD）	Showa	0.05-80 mg/dL	inter-assay CV: 5.0%	Male 3.4-7.0 mg/dL Female 2.4-7.0 mg/dL

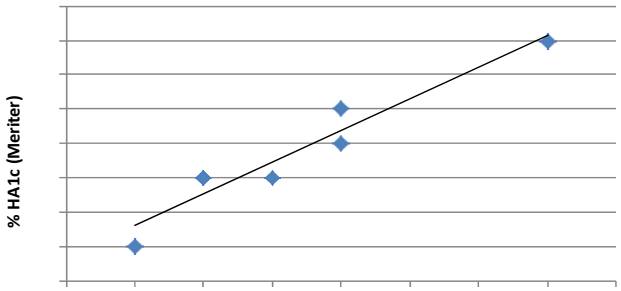
* Japan Society of Clinical Chemistry (JSCC) recommended method

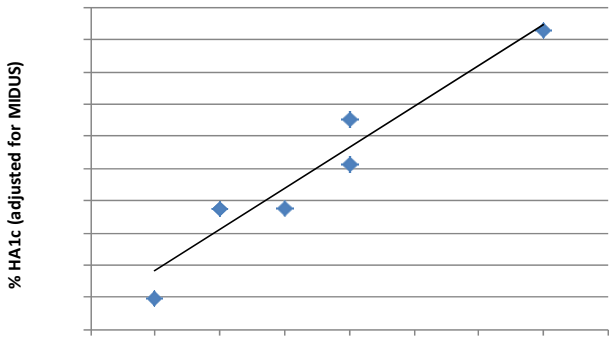
B. MIDJA saliva samples.

Assay	Assay Type	Performed at:	Assay Range	Variability	Reference Range
Cortisol (saliva)	Radioimmunoassay	ICTR, University of Wisconsin	min 0.7-70 n*M*[23]	inter-assay CV: 19-20%[24] intra-assay CV: 4.5-6.7%[35]	3-25 n*M*[34]

APPENDIX B: REGRESSION EQUATIONS

Glycosylated Hemoglobin: adjustment to pre-2006 Meriter data

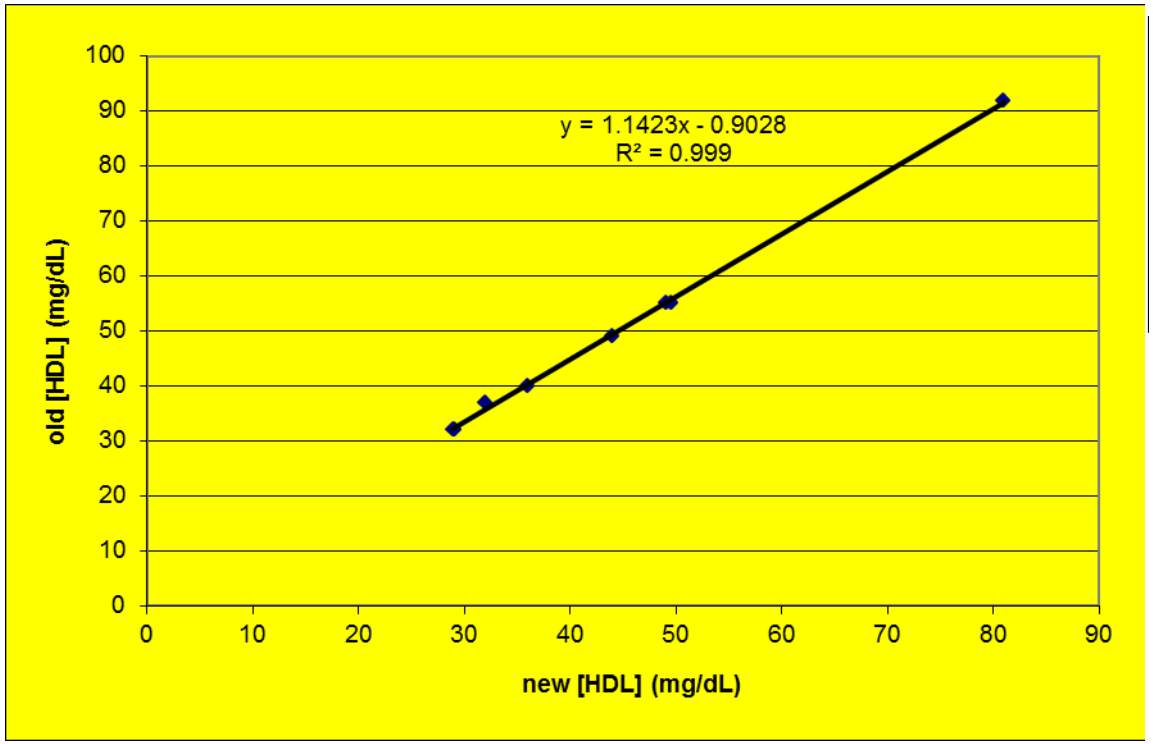




HDL Cholesterol: adjustment to pre-2007 Meriter data

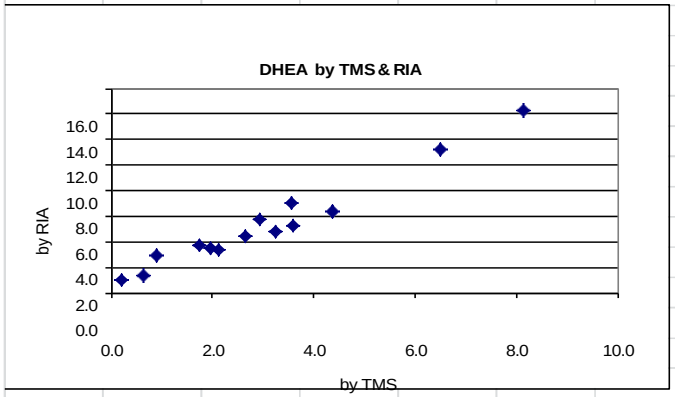
Patient#	HDL old cal	HDL new cal		n/o ratio	o/n ratio
	mean=32				
QC1	mg/dL	28.9 mg/dL		0.903	1.107
QC2	mean=55	49.6		0.902	1.109
#1	32 mg/dL	29 mg/dL		0.906	1.103
#2	37	32		0.865	1.156
#3	49	44		0.898	1.114
#4	40	36		0.900	1.111
#5	92	81		0.880	1.136
#6	55	49		0.891	1.122

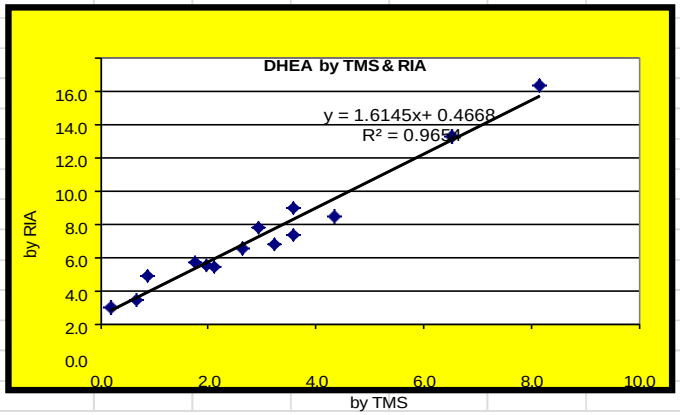
new	old
28.9	32
49.6	55
29	32
32	37
44	49
36	40
81	92
49	55



Comparison of ARUP DHEA values obtained by RIA and TMS

sample #	age	gender	RIA	TMS
10726	49	F	14.3	8.150
11604	54	F	3.4	2.110
12021	71	F	5.3	3.580
12290	69	F	3.5	1.950
12652	54	M	11.2	6.520
12658	51	F	2.9	0.881
13160	53	M	4.8	3.230
13635	53	F	1.4	0.654
13818	55	F	1.0	0.195
14753	62	M	4.5	2.640
16165	50	M	3.7	1.750
18876	50	M	5.8	2.940
18885	58	F	6.4	4.360
19109	74	F	7.0	3.570

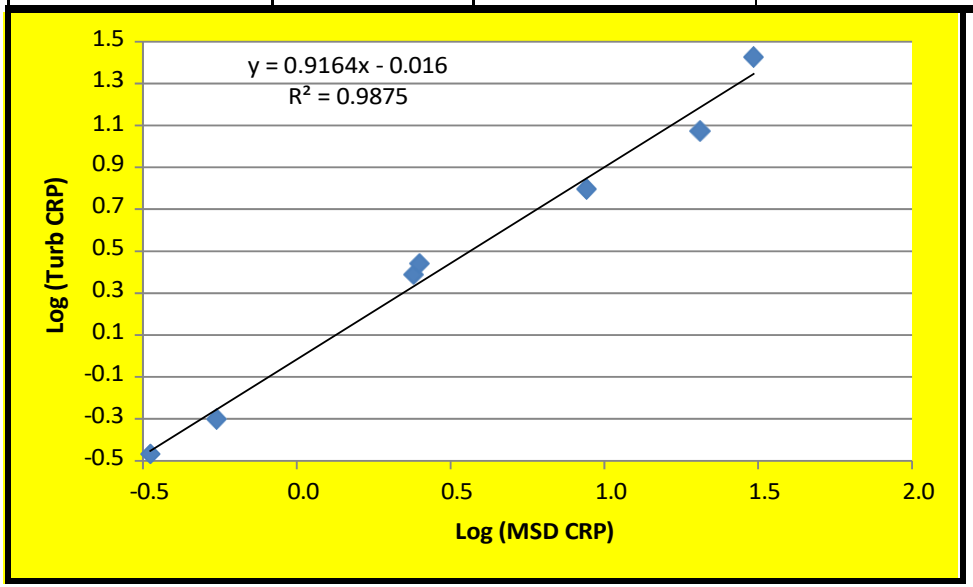




DHEA: adjustment to pre-2009 ARUP RIA data

C-reactive Protein: adjustment of MSD to turbidometric data for low-CRP samples

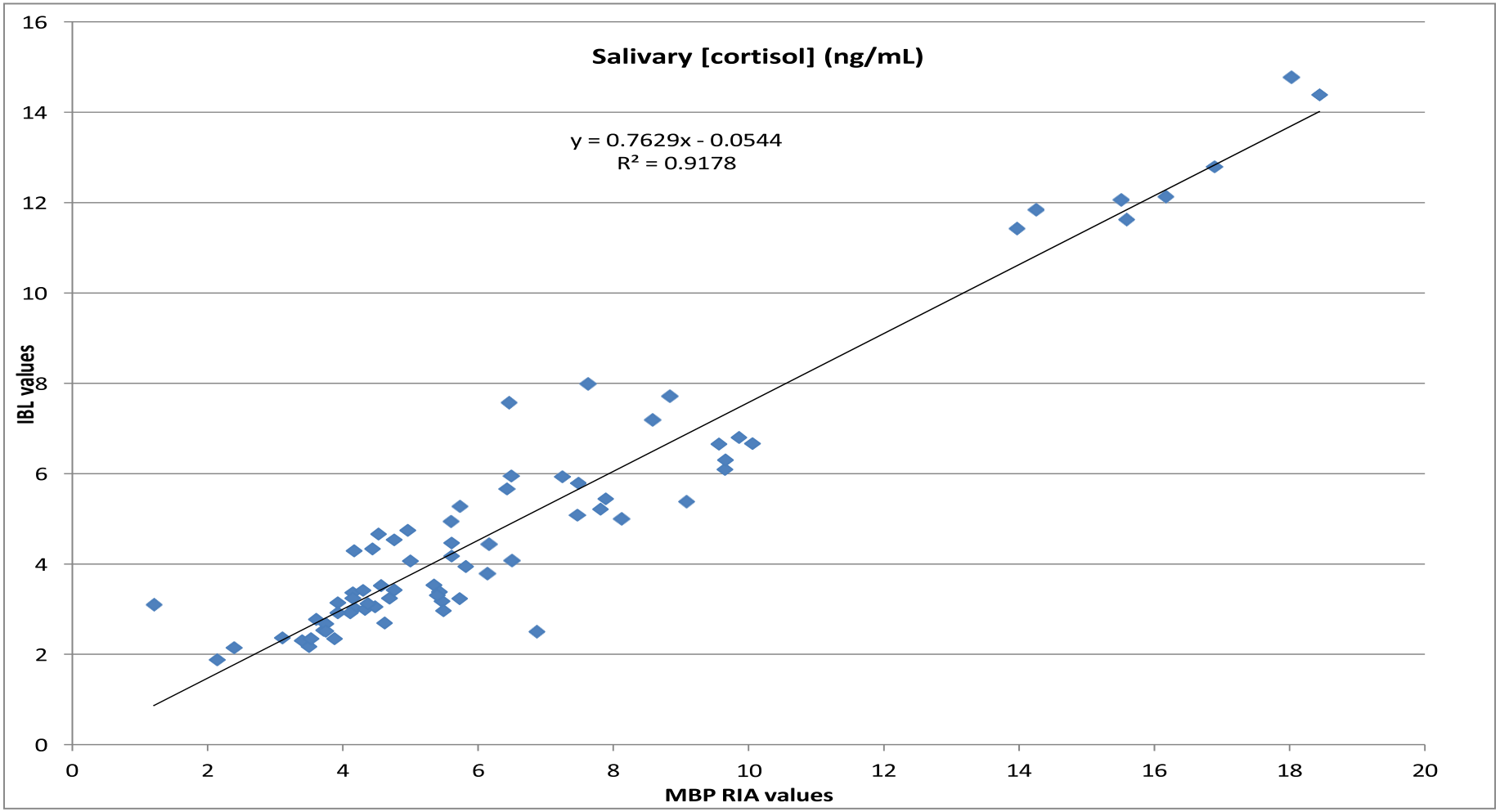
MSD CRP ug/mL	CRP ug/mL	Log (MSD CRP)	Log (Turb CRP)
0.334	0.34	-0.476	-0.468521083
0.549	0.50	-0.260	-0.301029996
2.400	2.44	0.380	0.387389826
2.500	2.75	0.398	0.439332694
8.740	6.27	0.942	0.797267541
20.500	11.80	1.312	1.071882007
30.500	26.60	1.484	1.424881637



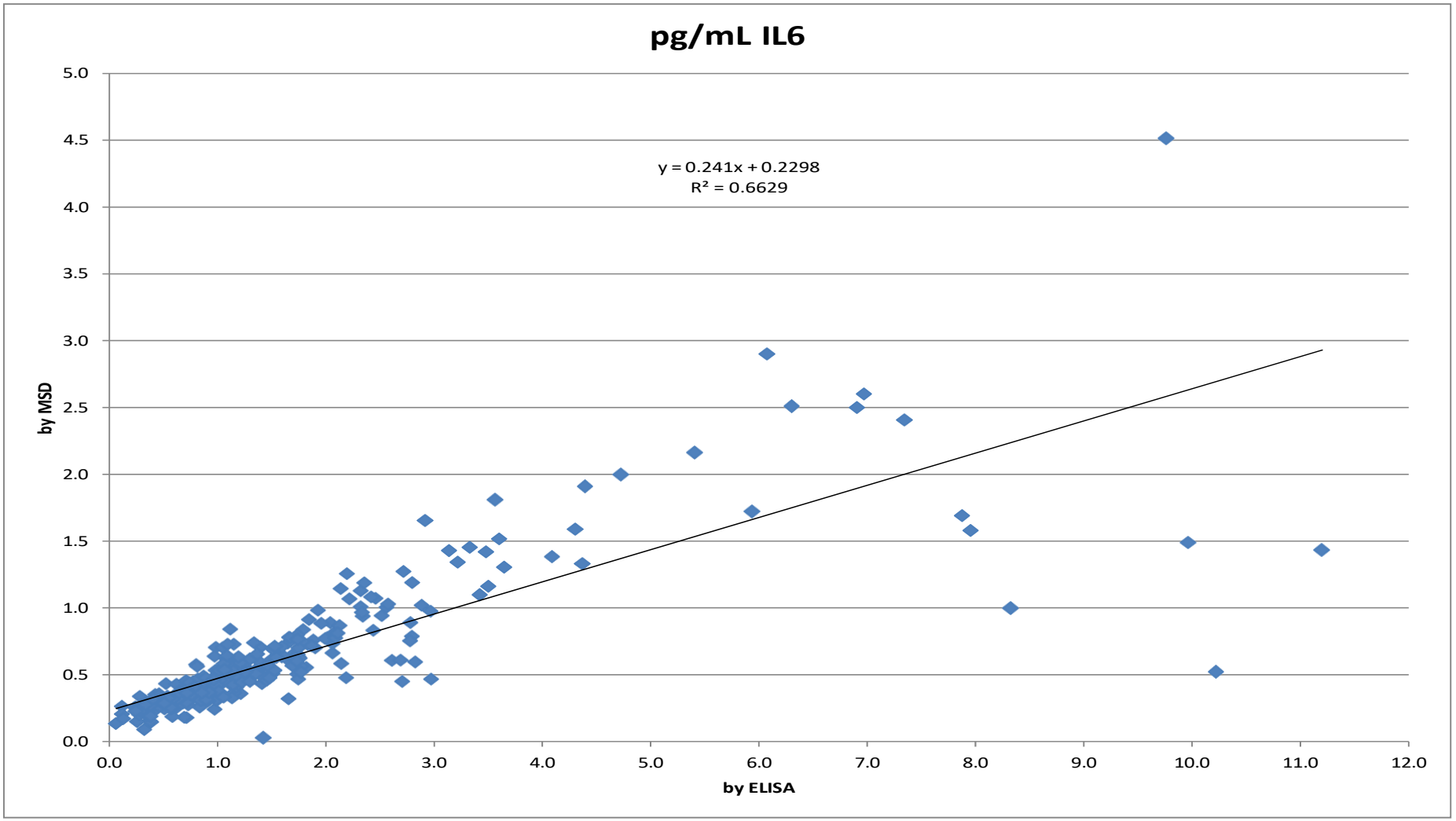
log(turb value) = 0.9164(log MSD) - 0.016

turb value = $10^{0.9164[\log(\text{MSD value})] - 0.016}$

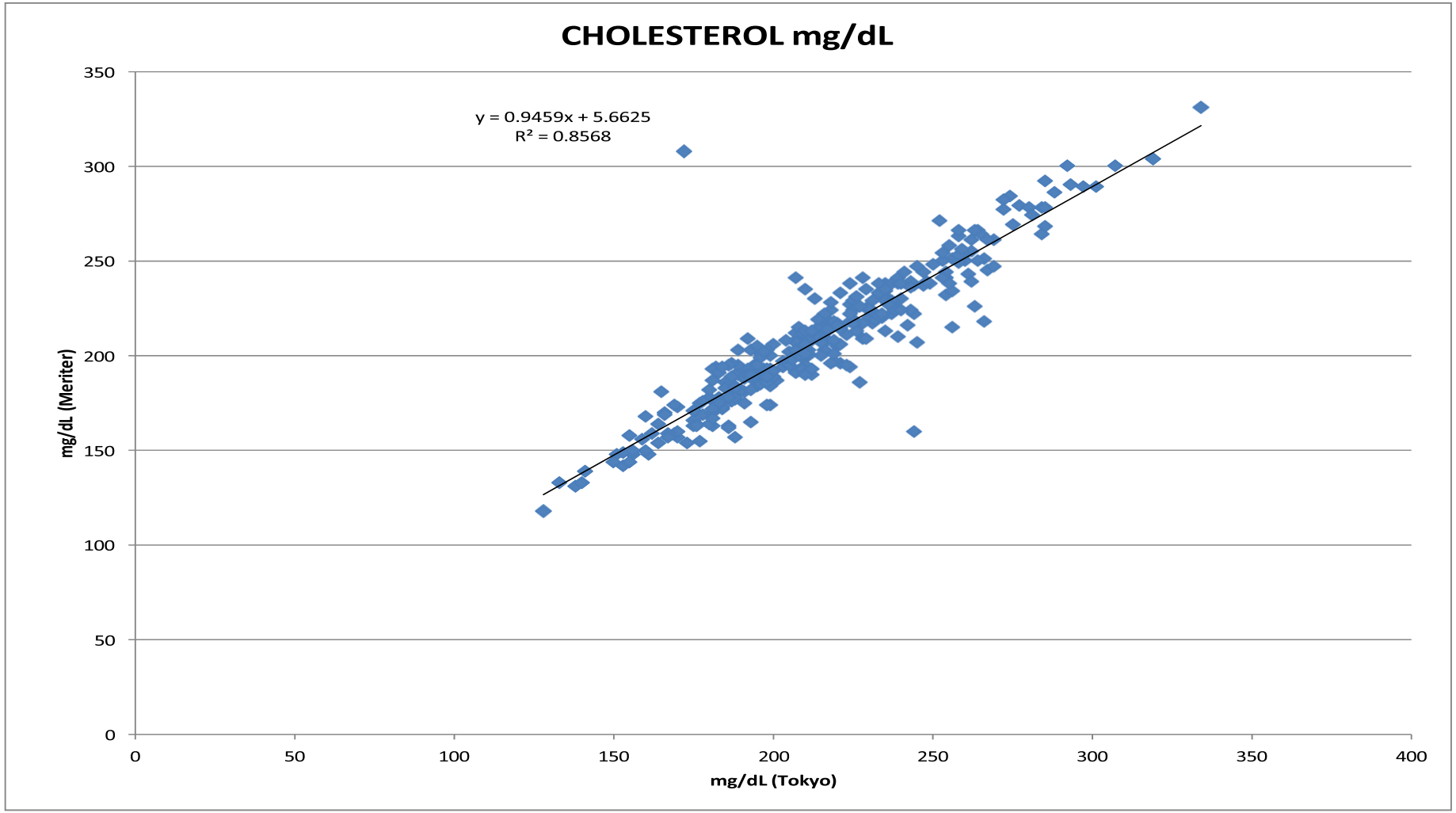
Salivary Cortisol: adjustment of ICTR values to Dresden values



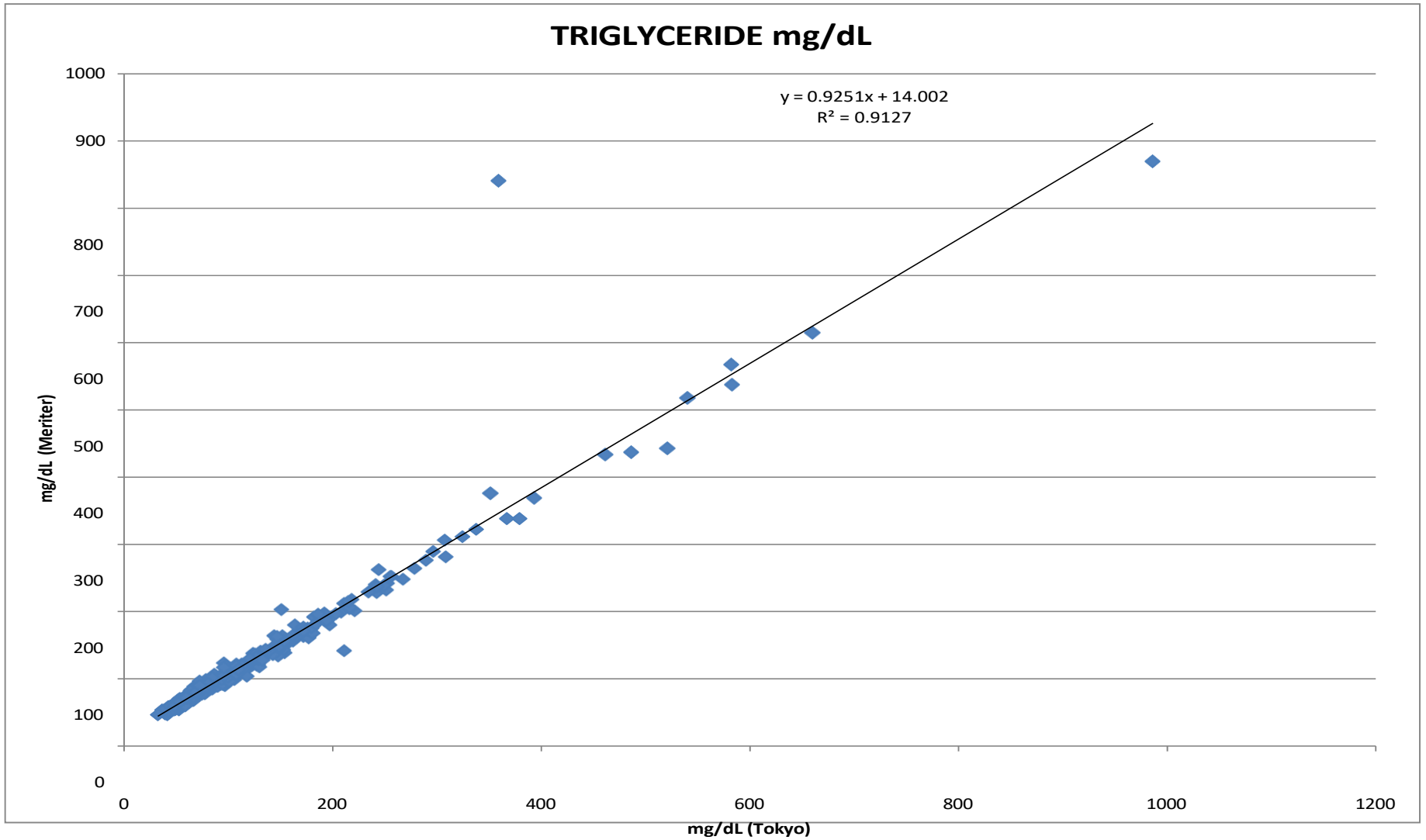
IL6: Comparison ELISA & MSD assay types



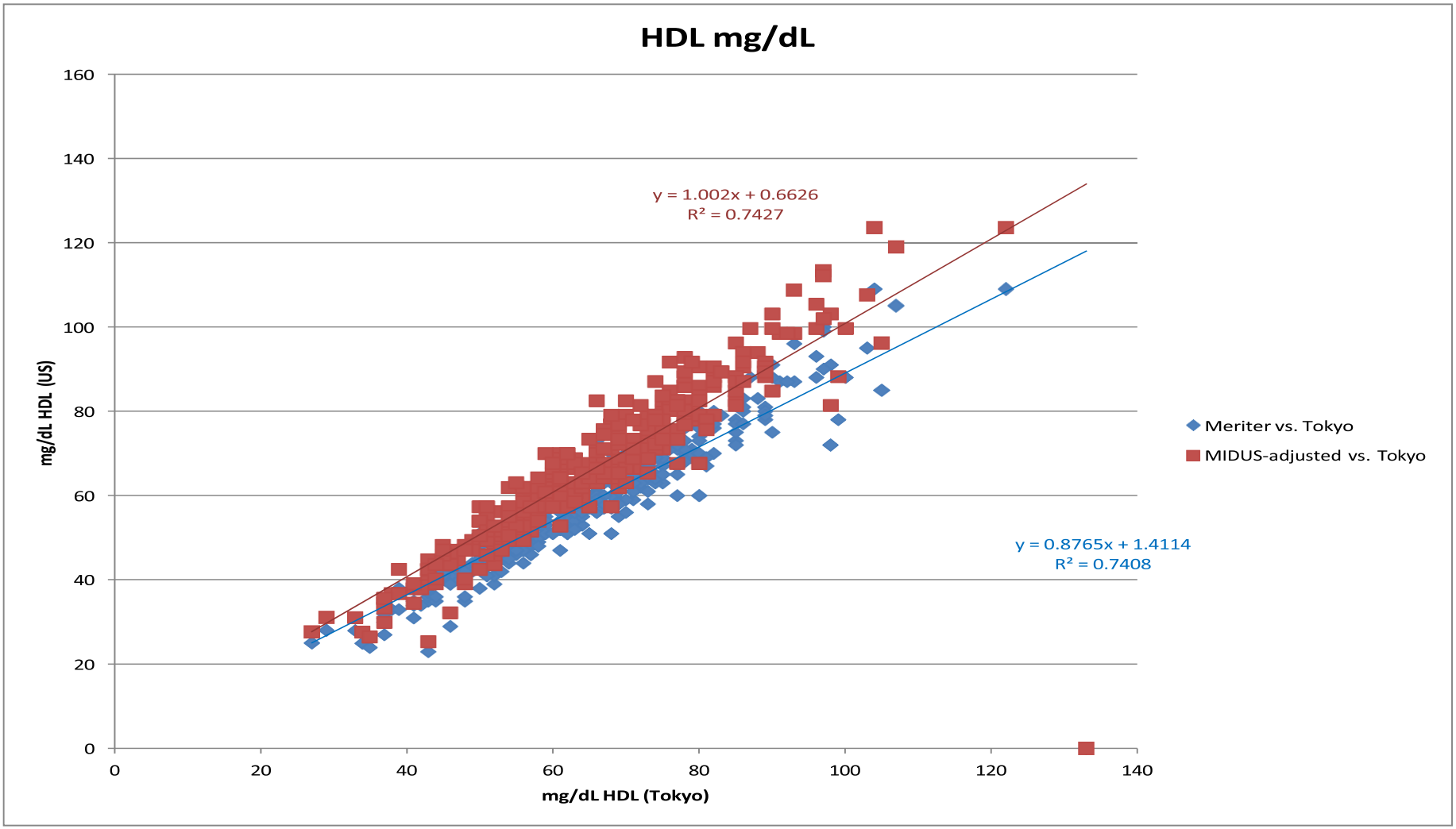
Total Cholesterol:
Comparison of measurements in Tokyo and at Meriter



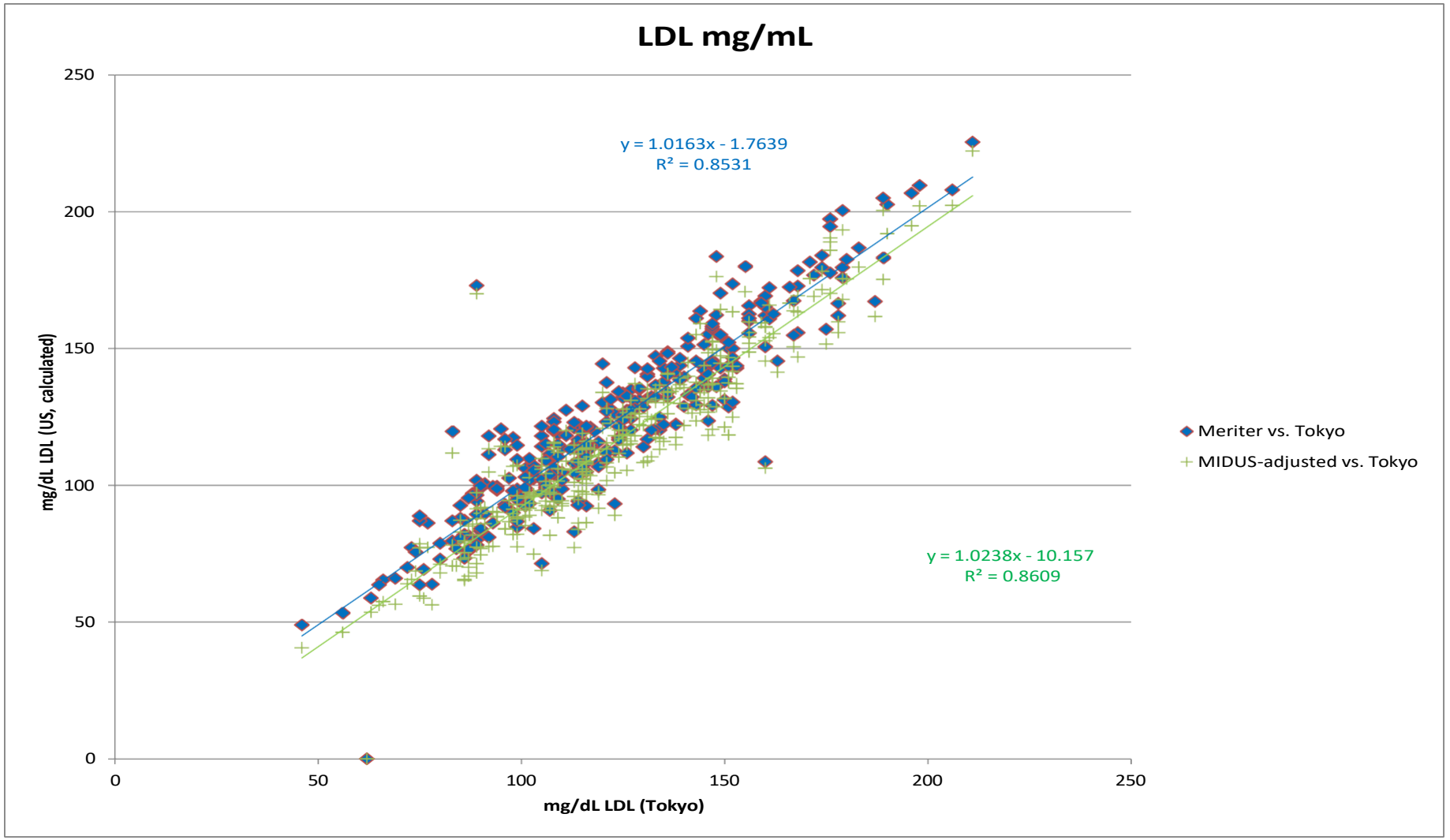
Triglycerides:
comparison of measurements in Tokyo and at Meriter



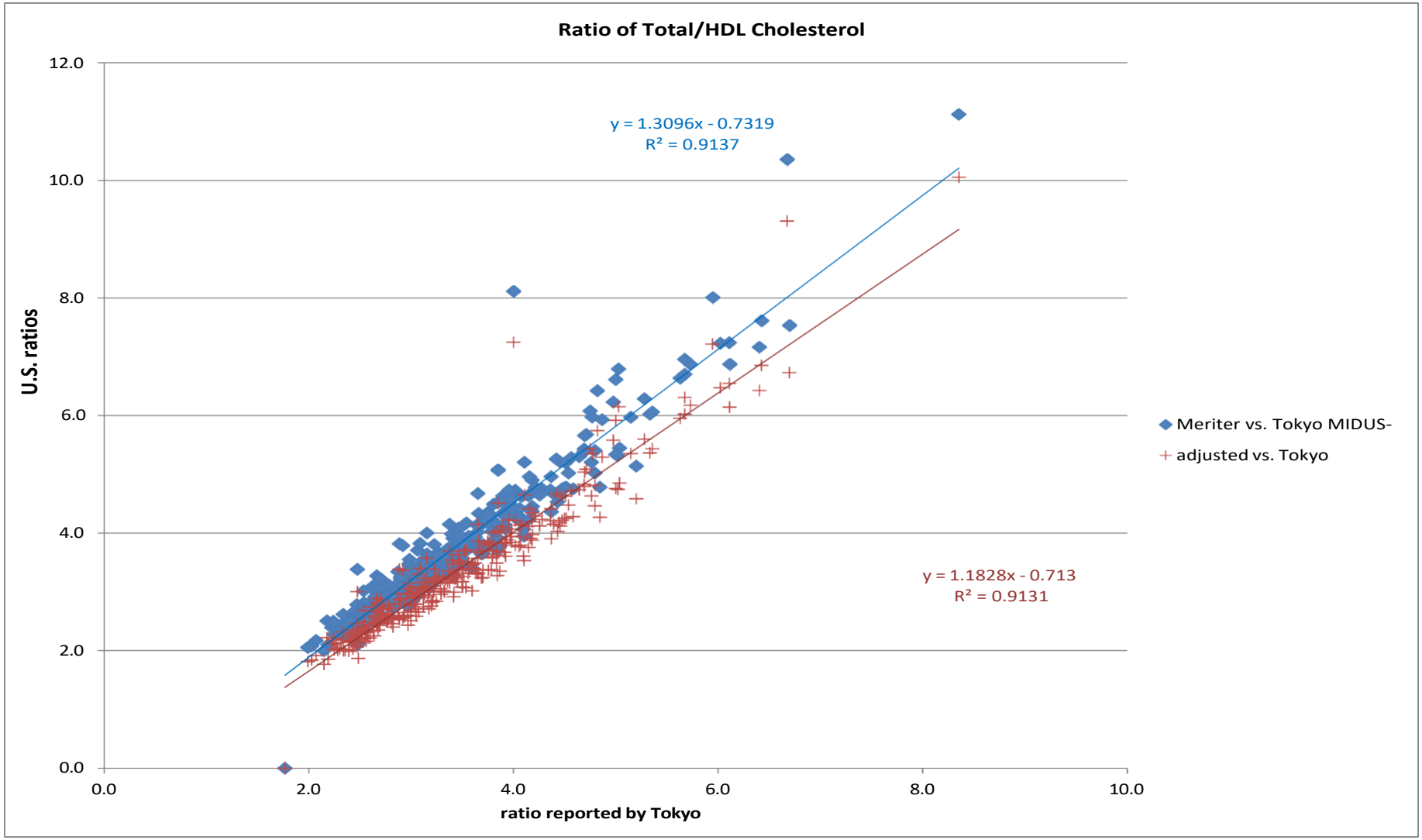
HDL Cholesterol:
Comparison of Tokyo data and Meriter data (adjusted and unadjusted)



LDL Cholesterol:
Comparison of Tokyo data and Meriter data (adjusted and unadjusted)



Ratio of Total Cholesterol to HDL Cholesterol: Comparison of Tokyo data and Meriter data (adjusted and unadjusted)



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SECTION E

MEDICATION DATA

Medication Data Collection Protocol

Data File Structure:

- **Administrative Variables**
- **Prescription and Quasi Medications**
- **Medication Allergies & Coding**
- **Therapeutic and Pharmacologic Classes**

Appendix A: Medication Chart

MEDICATION DATA COLLECTION PROTOCOL

The MIDJA Biomarker data includes details about medications study participants are taking at the time of the clinic visit. Specifically information is recorded about the following:

- Prescription Medications
- Quasi Medications (i.e. non-prescription medications, equivalent to Over-The-Counter (OTC) and Alternative (ALT) Medications in the U.S.)
- Medication Allergies

The Medication data appear at the end of the data file immediately following the Saliva data.
A copy of the medication chart with the original Japanese text and English translations is below (see Appendix A). As described in "MIDJA Biomarker Data File Notes", the naming convention organizes variables according to the data type or method used for data collection. The variable names for the medication data begin with the unique 3 character set "K2M".

The remainder of this section provides general information about data collection as well as the structure and content of the medication data. The following also includes references to additional variables related to reasons for taking medications as well as therapeutic and pharmacologic class variables added to the data file following linkage to the Lexi-Data database. These variables are created using procedures that are applied to both MIDUS and MIDJA medication data, thus details are included in a unified document, 'Documentation for MIDUS and MIDJA Medications' that can be used for both studies.

Data Collection

In Japan, to help manage medications, hospitals and pharmacies give individuals lists of the medications they are taking. Thus, participants were asked to bring all their medications to the clinic or the list of medications provided by a hospital/pharmacy. We do this to ensure that we are able to record medication names and dosages accurately. Staff recorded detailed information (medication name, dosage, etc.) on the medication chart. If a participant did not bring medications or a list, staff asked participants about medication use and recorded their responses. The Medication Chart has three pages and includes sections to record information about:

- Prescription Medications: prescribed by someone authorized/licensed in Japan, typically a physician.
- Quasi Medications: include vitamins, minerals, non-prescription pain relief, antacids, anti-diarrheals, fiber, lubricating eye or nose preparations etc. as well as other

supplements, herbs and homeopathics that the participant uses regularly and can be purchased “Over the Counter” (OTC) without a prescription.
    - The first row in this section of the chart is reserved for information about Multi-vitamin usage
    - The second row is for reporting on use of Calcium supplements.
- Medication Allergies: Any medication (prescription, OTC, alternative) that the participant reports being allergic to.

Project staff record the following information about medications on the medication chart:

- Medication name, dosage, and route of administration
- How often the medication is taken
- How long the participant has been taking a given medication
- Why they think they are taking the medication

NOTE: The order in which medications are recorded on the form is random and determined by the order in which the participant choses to report them. Thus there will be little direct correspondence between the medications or medication allergies reported in any given row of the form from wave to wave within a study or between MIDUS and MIDJA. The one exception is the first two rows in the Quasi section (OTC in MIDUS) which, as noted above, are reserved for reporting multi-vitamin and calcium supplement usage.

Data File Structure

The medication data are released in the traditional flat (wide) format as part of the larger MIDJA Biomarker aggregate data file to facilitate their use in standard between person analyses. Details about the variables in this file are included in the current document. The scope of the medication data however also lends itself to within person analysis of medication use, thus the medication data are also released in a standalone stacked (long) format. The stacked file only contains data about medications used, thus it does not include any information about study participants who do not take any medications. It also does not include any data about medication allergies. Details about the stacked file can be found in the Documentation for MIDUS and MIDJA Medications.

The aggregate (flat) file includes some administrative variables indicating whether or not the participant takes medication of the indicated type and, in some instances if yes, the number of medications. It also includes a standardized variable set characterizing each medication recorded along with information about medication allergies. The final section of the medication data is a set of variables indicating therapeutic and pharmacologic classification of the medications reported. The remainder of this section provides additional

details about these variables.

Administrative Variables

There are 3 administrative variables at the beginning of the medication section:

- K2MTM – total number of medications
- K2MPMD – Yes/No, taking any prescription medications?
- K2MQMD – Yes/No, taking any quasi medications?

The first variable at the beginning of the Prescription and Quasi medication indicates the total number of medications of a given type that the participant takes (K2MPM, K2MQM, respectively). In addition, as noted above, the first two rows of the Quasi medication section are designated for Multivitamins and Calcium Supplements, thus it also includes the following 2 administrative variables:

- K2MQMV - Yes/No, taking a multivitamin?
- K2MQCS - Yes/No, taking a calcium supplement?

Prescription and Quasi Medications: Standardized Variable Sets

These variables appear in the following order for each medication reported. The "_" in the variable name is a place holder for the character indicating the medication type (P = Prescription, Q = Quasi) while the "#' at the end of the variable names is a place holder for the row number on the chart where information about a given medication is reported.

- Created variables from the Lexi-Data database (see Documentation of MIDUS and MIDJA Medications for details):
  K2M_MID# - DrugID from the Lexi-Data database
  K2M_GN# - Generic Drug Name associated with the DrugID

- Based on information from the medication chart:
  K2M_DD#, K2M_DU# – drug dosage (dosage, units)
  K2M_F#, K2M_FU# – how often the medication is taken (frequency, frequency units)
  K2M_T#, K2M_TU# – how long has the medication been taken (number, time units)

- Mutually exclusive code variables representing the reasons why the participant thinks s/he is taking the medication:
  K2M_ICD9M -- 3 digit numeric codes representing major categories in the International Classification of Diseases, 9th Revision.
  K2M_MDC – 5 character alphanumeric variables representing a set of codes used to code reasons for taking a medication that could not be classified into an

ICD-9 category (See Documentation of MIDUS and MIDJA Medications for details about the coding process)

There are 13 sets of Prescription medication variables and 7 sets of Quasi medication variables in the MIDJA 2 Biomarker file. A few participants report more medications in a given category than the data file allows for (i.e. takes 13 Quasi medications). These additional medications are not included in the flat file as they would potentially add over a hundred variables (10 variables per medication) most of which would be designated as INAPP. These additional medications are however included in the stacked file. Cases with additional medications can be identified by looking at the frequency distribution for the number of Prescription (K2MPM) and Quasi (K2MQM) medications.

Medication Allergies

The final section of the Medication Chart is for reporting medication allergies. There are 2 administrative variables at the beginning of that section:

- K2ML – Yes/No does the respondent have Any Medication Allergies?
- K2MLM – number of allergies

There are 2 additional variables for each medication allergy reported (the # in the variable name indicates the row number in the chart):

- K2MLN# – text variable listing the medication name as reported by the participant
- K2MLRC# - Codes representing the allergic reaction to the medication (see below for details.

Note, the allergy variables are only included in the Flat file.

Coding Medication Allergy Reactions

Participant descriptions of allergic reactions to medications were recorded verbatim. The number of medication allergies reported was relatively small compared to the larger set of medication data thus they were coded using standard manual approaches. Medical definitions of common types of reactions and anaphylactic shock were used as a reference for making decisions about coding categories. Most of the categories correspond to specific individual reactions reported (e.g. rash, nausea). If the participant reported multiple individual reactions that are part of the overall anaphylactic response then the code for anaphylaxis was assigned. Sometimes participants reported reactions that could not be identified as being either part of the anaphylactic response or a known reaction to a particular medication. In those instances the reaction was coded as ‘Other’. The categories, codes, and key words are summarized below. Note, the same set of category codes is

used for both MIDJA and MIDUS thus some categories (i.e. psychosis) may not appear in all files.

Code	Category Name	Keywords
1	ANAPHYLAXIS	Anaphylaxis, difficulty breathing, faint, wheezing, swelling & hives, sweating/shaking
2	RASH	Rash, Eczema
3	HIVES	Hives, eruptions, uticaria, blisters, welts
4	VOMITING	Vomiting, throwing up,
5	SWELLING	Swelling, swollen –hands, face, feet etc.
6	STOMACH UPSET	Upset stomach, feeling sick
7	ITCHING	Itching,
8	NAUSEA	Nausea
9	PSYCHOSIS	Psychosis, hallucinations, agitation, delirium, jitters
10	OTHER	Joints ache, no energy, headache, sweats, constipation

Therapeutic and Pharmacologic Classes

The final section of the data file contains a series of variables indicating whether or not the participant takes medications in commonly occurring categories of therapeutic (TC) and pharmacologic (PC) classes identified following linkage to the Lexi-Data database. The Lexi-Data linkage and creation of these variables along with corresponding count variables are described in the Documentation of MIDUS and MIDJA Medication.

The variable names for these dummies incorporate the first 5-6 characters of the corresponding TC and PC variables as well as the numeric code for the TC or PC class. The variable label incorporates both the TC or PC class code and the category name. If a count variable was also created, then an "N" is added as the final character to the dummy variable name. For example a common parent (top tier) therapeutic class is 115=Nutritional Products. The dummy and count variables for this TC class are named/labeled as follow:

Dummy variable: K2MTC_115 = 'Multum Therapeutic Class 115 - nutritional products: YES/NO?'

Count variable: K2MTC_115_N = 'Multum Therapeutic Class 115 – nutritional products: HOW MANY?'

APPENDIX A: MIDJA MEDICATION CHART

医師の処方薬 Prescription # お薬を飲んでいらっしゃいますか?

Do you take any medicine いいえ no ・ はい yes （_____種類 Prescription: Number of prescription medications）

薬の名前、1回の分量 Medication Name/Dosage	飲み方 Route	回数 Frequency	期間? How long Taking?	どうして飲むことになりましたか? Reason for taking?
1.				
2.				
3.				
4.				
5.				
6.				
7.				

お医者の処方によらない薬(医薬部外品、サプリメントなど) Without prescription,such as quasi-drug

お薬を飲んでいらっしゃいますか? いいえ ・ はい (_____種類 Quasi-drug: Number of Quasi medications)

薬の名前、1回の分量	飲み方	回数	期間?	どうして飲むことになりましたか?
Medication Name/Dosage	Route	Frequency	How long Taking?	Reason for taking?
1 ビタミン剤 Vitamine Quasi (no prescription): Does R take multiple vitamin? Yes / No				
2 カルシウム Calcium? Yes / No				
3.				
4.				
5.				
6.				
7.				
8.				

薬物アレルギー　Medication Allergies

お薬でアレルギーがでたことがありますか? Number of allergic reactions:　　いいえ no ・ はい yes

薬の名前	アレルギー反応
1. Drug name	Reaction to drug
2.	
3.	
4.	
5.	
6.	
7.	