DOCUMENTATION

for

BLOOD, URINE, AND SALIVA DATA

in

MIDUS REFRESHER BIOMARKER PROJECT (P4)

University of Wisconsin ♦ Institute on Aging
April 2024

INTRODUCTION

This document provides an overview of the tissue samples collected in the MIDUS Refresher Biomarker Project (P4). It describes the protocols for collecting and processing tissue samples, and provides information about the construction 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 "MR1 P4 Biomarker Project Data File Notes". This document provides information about naming conventions, as well as administrative and filter variables included 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. For example, there are instances when variables were added or sections of an instrument were expanded for data entry purposes to accommodate additional information provided by the respondent.

This document will be periodically revised and updated as more information is gathered, and researchers continue to work with the MIDUS Biomarker data. If there are suggestions or comments, please submit a message through the MIDUS HelpDesk (http://midus.wisc.edu/helpdesk.php).

Table of Contents

SECTION A: OVERVIEW OF DATA AND COLLECTION PROTOCOLS

The Biomarker Project (P4) includes collection of the following tissue samples for biomarker assay:

- Fasting Blood samples
- 12 hour (overnight) Urine samples
- Saliva Samples

The biomarker data appear in the data file immediately following the Physical Exam data. As described in "MR1 P4 Biomarker Project Data File Notes", the naming convention organizes variables according to data type or the method used for data collection. The variable names for the biomarker assay data begin with the unique 4-character set "RA4B".

All sample types are obtained at all three P4 sites. The remainder of this section provides general information and indicates where additional details can be found.

Revision in The 2024 Update Release

a. Three new urine assay measures are added to this update release, all as indicators for kidney health. One new blood assay, suPAR, is added as a biomarker associated with immune and inflammatory reaction.
   - B4BALBUMIN: Urine albumin (mg/dL)
   - B4BUACR: Albumin-Creatinine Ratio (uACR)
   - B4BCYSTATINC: Urine Cystatin C (mg/L)
   - B4BSUPAR: Soluble urokinase plasminogen activator receptor (suPAR)

   Albumin and cystatin C concentrations were measured from aliquots of frozen 12-hour urine collection samples stored from MIDUS Refresher. The concentration of suPAR was measured from frozen serum samples stored from MIDUS Refresher. Detailed descriptions of these assays can be found in Section C below.

b. Fixed decimal placement error in Catecholamine values. The updates include original catecholamine variables RA4BNOREP RA4BEPIN RA4BDOPA, as well as urine creatinine adjusted variables RA4BNOCRE RA4BEPCRE RA4BDOCRE.

Biomarker Data

The biomarkers reflect functioning of the hypothalamic-pituitary-adrenal axis, the autonomic nervous system, the immune system, cardiovascular system, musculoskeletal system, antioxidants, and metabolic processes. Our tissue specimens allow for assessment of multiple indicators within these major systems as follows:

- Fasting Blood Draws
  - Cardiovascular: Cholesterol Panel (Total, HDL, and LDL) and Triglycerides
  - Hormone markers – DHEA and DHEA-S
  - Inflammation markers – IL-6, s-IL6-r, IL-8, IL-10, TNF-alpha, C-Reactive Protein, Fibrinogen, E-Selectin, and ICAM
  - Anti-Oxidant markers – *trans*-beta-carotene, 13-*cis*-beta-carotene, alpha-carotene, cryptoxanthin, lutein (*cis, trans,* total), zeaxanthin, lycopene (*13-cis, 9-cis, trans*, total), retinol, alpha-tocopherol, and gamma-tocopherol
  - Bone Turnover markers – BSAP (Bone Specific Alkaline Phosphatase), NTx (n-teleopeptide type 1 collagen), and P1NP (aminoterminal propeptide type 1 procollagen)
  - Creatinine
  - Glucose metabolism markers – glycosylated hemoglobin (HA1c), glucose, insulin, and IGF-1
- 12-Hour Urine

    - *Neuroendocrine:* Cortisol, Epinephrine, Norepinephrine, Dopamine, Creatinine, Albumin, and Cystatin C
- Saliva (Experimental Protocol)
    - *Neuroendocrine:* Cortisol

Administrative Variables

In addition to the biomarker assay results, this section of the data file also includes the following administrative, filter or computed variables:

- RA4ZBLOOD, RA4ZURINE, RA4ZSALIVA – indicate if the specified sample type was collected and whether the sample is complete or partial.
- The urine collection occurs over a 12-hour period and requires that participants notify nursing staff after each void. Thus, some voids are missed or incomplete for various reasons. The following variables can be used to identify problematic cases that data users may wish to examine more closely prior to including them in analyses.
    - RA4BUPROB – Urine collection period less than 11 hours or greater than 13 hours
    - RA4BUSTRT, RA4BUSTRT – Urine collection Start time
    - RA4BUEND, RA4BUEND – Urine collection End time
        - Note: At the Refresher, time variables were converted to a 24-hour clock with a restricted numeric format that allows leading zeros to be displayed. See 'the Refresher Biomarker (P4) Readme Data File Notes' for details about how time variables are handled in the dataset.
    - RA4BUVDYN – Any voids not collected
    - RA4BUVDN – Number voids not collected
- Saliva sufficiency flag (RA4BINSUFF). When saliva samples were prepared for assay, the testing lab found that in some instances there was insufficient saliva in the tube for the assay to be run. Thus, we created a flag variable to identify those cases along with the number of samples with insufficient saliva for the assay.

Computed Variables

The blood and urine biomarker data were also used to create several additional measures. Details about the creation of the following variables can be found in the "Documentation for Psychosocial Constructs and Composite Variables":

- RA4BHOMAIR – measure of insulin resistance
- RA4BGFR – Glomerular filtration rate
- RA4BNE12, RA4BEPI12, RA4BDOP12 – Epinephrine, Norepinephrine, and Dopamine adjusted to 12-hour values corresponding to the data collection period
- RA4BNOCRE, RA4BEPCRE, RA4BDOCRE – Epinephrine, Norepinephrine, and Dopamine adjusted for creatinine
- RA4BUACR – Albumin-Creatinine Ratio (uACR)

Tissue Sample Collection and Processing

Standardized protocols for collecting and processing tissue samples were implemented at all 3 sites. Copies of the blood and urine collection and processing protocols followed by CRU staff appear in Section B.

Saliva samples are collected during the Laboratory Challenge Study conducted during the CRU stay, thus details about sample collection are included in the documentation for that protocol (see "Psychophysiology Protocol documentation").

Biomarker Assay Descriptions

Details about the methods used to assay the tissue samples appear in Section C, which has four parts. 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. Descriptions of the assays are in

the first part of Section C; they are listed first by tissue source (blood, urine, saliva) and then by physiological system assessed. A table summarizing assay sensitivity appears next followed by the third part, which describes adjustments made to the data in response to assay changes over time. The fourth and final part of this section is a list of references cited throughout Part C of this document.

SECTION B: TISSUE COLLECTION AND PROCESSING PROTOCOLS

BLOOD AND URINE COLLECTION PROTOCOL

PREPARATION

1. Prepare urine collection supplies

- 2 urine collection containers (to hold at least 2 liters each)
- CATS COLLECTION bottle **– ACETIC ACID**
  - Add 25ml of 50% acetic acid to 1 urine collection container to acidify urine collected for catecholamine tests.
  - Place **red** CATS label from packet on side of this container!
- CORT collection bottle – **NO ACID**
  - Place **white** CORT label from packet (if MIDUS staff prepare labels) on side of collection container.
- Hat or urinal

2. Label blood tubes with MIDUS ID labels

- 3 10-ml SST vacutainers (red/black, serum separator tubes)
- 1 4-ml lavender top (EDTA tube)
- 1 4-ml lavender top (EDTA tube), wrapped with aluminum foil
- 1 4-ml or 2.7 ml blue top (sodium citrate tube)
- 1 4-mL green top (sodium heparin)
- 1 8-mL CPT (cell preparation tube with red/green top)

URINE COLLECTION

At 1900 :

- Instruct subject on 12-hour collection
- Provide urinal (men) or hat (women) and explain use
- Post reminder notice in bathroom
- Have subject void – DO NOT SAVE THIS SAMPLE
- Record date and time of void
- Instruct subject to collect all urine from then on until 0700 in specimen hat or urinal ***AND to call/notify nurse immediately after each void (even during the night).***

From 1900 until 700

- Pour **ONE HALF** of **EACH VOID** into each of the 2 urine collection containers
- Keep both collection containers in the refrigerator throughout the 12hr collection period
- **Sometime around 2100-2200***:* Remind subject (later in evening, before they go to bed) to collect urine all night
- **When subject wakes up** and /or when nurse comes in to draw blood): Remind subject to continue collecting all urine until 0700

At 0700 – Completion of urine collection

- Request final urine void from subject
- Pour **ONE HALF** into each of the 2 urine collection containers
- Let subject know that this is the end of the urine collection and there is no need to notify staff of any other voids
- Record date and time of final void
- Indicate any missed voids or urine not collected and reason urine not collected (e.g., subject refused or other reasons)

- Take urine to lab for processing and notify technician

BLOOD COLLECTION – COLLECTION OF FASTING BLOOD FROM 0630-0700

Preparing subject

- Use **non-dominant arm** if possible
- Make reasonable number of attempts to get needed samples
- Subject should avoid strenuous activity before blood draw but can take a shower

Blood draw order

- 3 10-ml **red/black SST** tubes
- First 4-ml **lavender**
  - After draw, ***gently invert*** 3-5 times
- Second 4-ml **lavender** (foil wrapped)
  - Cover the vacutainer with aluminum foil before attaching to needle.
  - Slide the foil down slightly during blood draw, leaving the top visible so that one can see up to what point in the tube the blood should be drawn. Place tube in ice bath.

After draw, ***gently invert*** 5 times

- 1 4-ml or 2.7-ml **blue** (citrated) tube
  - Fill tube completely. After draw, ***gently invert*** 3-5 times
- 1 4-mL green (heparin) tube
  - After draw, ***gently invert*** 3-5 times
- 1 8-mL red/green CPT tube

NOTE: Prevention of Backflow.

The BD Vacutainer® CPT™ Tube contains chemical additives, thus it is important to prevent possible backflow from the tube with its attendant possibility of adverse reactions to the patient. To guard against backflow, the following precautions should be taken when drawing blood into the tube:

i. Keep patient's arm in the downward position during the collection procedure.
ii. Hold the tube with the stopper uppermost.
iii. Release the tourniquet as soon as the blood starts to flow into the tube, or within 2 minutes of application.
iv. Make sure the tube contents do not touch the stopper or the end of the needle during the collection procedure.
v. After draw, gently invert 8-10 times.

- Complete specimen collection form
- Take blood tubes to lab for processing ASAP and record time on form
  - Place *lavender tube without foil* in MIDUS storage box in refrigerator **- DO NOT FREEZE**
  - Place *lavender tube with foil* **in ice bath**

BLOOD AND URINE PROCESSING PROTOCOL

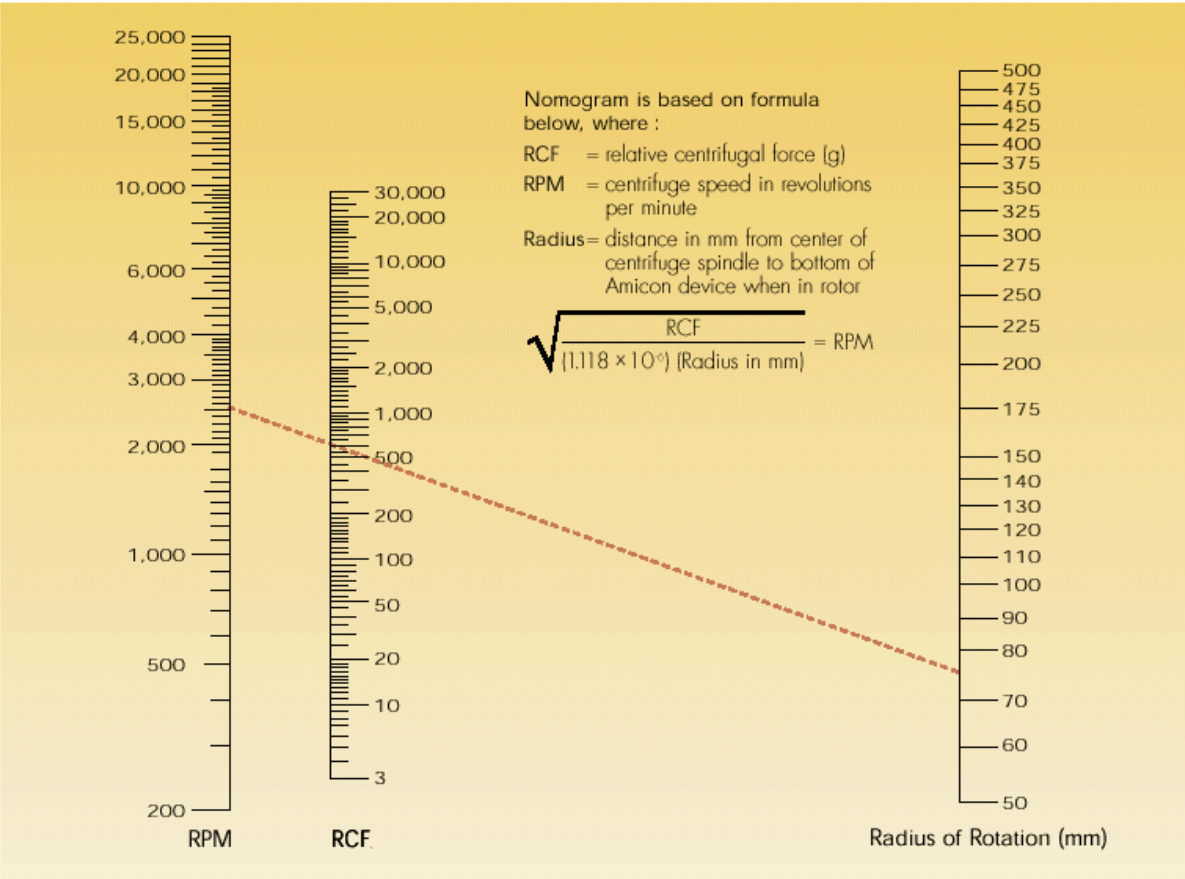
URINE PROCESSING

- Measure volume in each of the 2 urine containers and record each on the form
- Calculate and record total volume on form
- From **CORT COLLECTION** bottle
    1. Aliquot **11 ml** into each of **(2) 13-ml** plastic tubes with **white** labels.
    2. Aliquot **4 ml** into each of **(2) 5-ml** vials with **white** labels.
- From **CATS COLLECTION** bottle (the one with RED labels)
    1. Assess pH
    2. If the pH is 5 or more, then add 1 ml of acetic acid and mix thoroughly with urine
    3. Continue adding additional 1ml increments of acetic acid (and re-checking pH after each addition) until you get pH below 5. **Final pH must be below 5!** Record final pH on form.
    4. Once pH is below 5, aliquot **11 ml** into each of **(2) 13-ml** plastic tubes with **red** labels
- Store all urine samples in MIDUS box in CRU freezer (-60 to –80) for MIDUS staff pick-up.

BLOOD PROCESSING

Centrifugations described below are in Relative Centrifugal Force (RCF), otherwise referred to as “G” (times gravity). Speed (RPM) needed to achieve a particular RCF depends on the diameter of the centrifuge rotor. Most modern centrifuges have a button that allows the operator to toggle between RPM and RCF. If this is not available, lab staff are instructed to use one of the following methods to determine this speed:

1) the formula RPM = (RCF x 89445/radius in cm)$^{1/2}$; 2) the online calculator at www.djblabcare.co.uk, or 3) the following nomogram



1. **First** lavender tube without foil (whole blood for HbA1c test and DNA extraction)

- Check that tube was placed in MIDUS storage box in refrigerator at 4 degrees Centigrade **(DO NOT FREEZE).** Record time stored in refrigerator.

2. **Blue citrated tube**
   a. Centrifuge **as soon as possible** to separate plasma (within 15 minutes of draw).
      1. Keep on ice after inversion
      2. Use refrigerated centrifuge (4°C).
      3. Centrifuge samples for **15** minutes at **1000 g (RCF)**.
      4. On the form, record time centrifuge begun.
   b. From **blue**, aliquot 1 ml plasma into each of 2 **blue** labeled 2-ml vials.
   c. On form, indicate number of aliquots filled of each type. If none filled or fewer than number specified, indicate why.
   d. Store in MIDUS storage box in GCRC freezer (-60 to -80 degrees) for MIDUS staff pick up.
   e. On form, record time specimens stored in freezer.

3. **1 8-ml CPT tube**
   a. Centrifuge tube as soon as possible for 20 minutes at **1800 g (RCF)** without brake, at room temperature. (Invert tube 8-10 times immediately prior to centrifugation.)
   b. Using a transfer pipette, discard the clear plasma from the uppermost layer. Avoid disturbing the whitish/cloudy cell layer below the plasma that contains the PBMC.
   c. Pipette the cell layer into a 3.6 mL cryo-vial (i.e. Nunc-style tube). **Re-centrifuge** for 5 minutes at **1200 g (RCF)**.
   d. Without disturbing the white cell pellet at the bottom of the tube, remove most of the remaining fluid and discard. Only the cell pellet and a small amount of plasma should remain.
   e. On the form, indicate the number of aliquots filled. If none, indicate why.
   f. Label the tube with white label and 'PBMC'.
   g. Store in MIDUS storage box in GCRC freezer (-60 to -80 degrees) for MIDUS staff pick up.
   h. On form, record time specimen was stored in freezer.

4. **1 4-ml green top sodium heparin tube**
   a. Centrifuge within 30 minutes from time of draw at **1500 g (RCF)** for 10 minutes at 4 degrees C.
      (If refrigerated centrifuge is not available, keep the blood cold; refrigerated is fine.)
   b. Aliquot 1 ml plasma into each of two 2-ml Nalgene cryovials (clear caps) labeled HDLF.
   c. Store in MIDUS storage box in GCRC freezer (-60 to -80 degrees) for MIDUS staff to pick up.
   h. On form, record time specimen was stored in freezer.

5. **Foil-wrapped** lavender tube
   a. Get tube from ice-bath within 30-45 minutes.
   b. **Under dim lights**, separate plasma.
      1. Use refrigerated centrifuge (4°C).
      2. Centrifuge specimen for **15** minutes.
      3. Set speed at **1000 g (RCF)**, as described above.
      4. On the form, record time centrifuge begun.
   c. Aliquot **0.5 ml** plasma into **2 yellow**-labeled 2-ml vials.
   d. On form, indicate number of aliquots filled. If less than 2 filled, indicate why.
   e. Store in MIDUS storage box in GCRC freezer (-60 to -80 degrees) for MIDUS staff pick up.

6. **3 10-ml SST tubes**

a. Let stand for 15-30 minutes after draw (maximum of 2 hours allowed between draw and centrifuging, must be kept at room temp for a minimum of 40 minutes BEFORE centrifuging).

b. Centrifuge vacutainers to separate sera.
   1. Use refrigerated centrifuge (4°C).
   2. Centrifuge samples for **20** minutes at **1000 g (RCF)** as described for the blue-topped citrated tube (above).
   3. On form, record time centrifuge begun.

c. From SSTs, aliquot 1ml serum into each labeled 2-ml vial as follows:
   Red – 2 vials
   White – 2 vials
   Green – 3 vials
   Orange – 5 vials

d. On form, indicate number of aliquots filled of each type. If none filled or fewer than number specified, indicate why.

e. Store in MIDUS storage box in GCRC freezer (-60 to -80 degrees) for MIDUS staff pick up.

f. On form, record time specimens stored in freezer.

SECTION C: BIOMARKER ASSAY DOCUMENTATION

BIOMARKER ASSAY DESCRIPTIONS

Overview

The following provides information about laboratory assays of tissue samples collected as part of the MIDUS Biomarker Project (Project 4) including details about assay method and sensitivity for inclusion in manuscripts. Unless otherwise noted assays were performed on samples collected at both MIDUS 2 and the MIDUS Refresher. If the assay method changed during or since MIDUS 2, information about the change and any corrections to the data are also provided. The latter are reported in the Data Adjustments section. Assays are listed first by tissue source (blood, urine, 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 the MIDUS BioCore. A summary table listing all the assays along with the specimen type, assay type and sensitivity follows these descriptions. Data adjustments are described next. This section ends with a list of the references cited throughout this section.

I. Blood Assays

A. Sample Collection:

Fasting blood samples are collected from each participant before breakfast on Day 2 of their hospital stay. To ensure consistency, all samples are collected and processed at the CRU using standardized procedures described in Section B. Frozen samples are stored in a -60° C to -80° C freezer until shipped on dry ice to the MIDUS BioCore Lab. Samples are subsequently stored in a -65° C freezer until assayed.

- *Fresh whole blood* samples are refrigerated and shipped to the MIDUS BioCore Lab weekly and assayed for Hemoglobin A1c.

- *Frozen serum and plasma* in 1 mL aliquots are shipped to the MIDUS BioCore Lab monthly for the following biomarker assays:
  - Cardiovascular markers – Cholesterol Panel and triglycerides
  - Hormone markers – DHEA and DHEA-S
  - Inflammation markers – IL-6, s-IL6-r, IL-8, IL-10, TNF-alpha, C-Reactive Protein, Fibrinogen, E-Selectin, ICAM, and suPAR
  - Anti-Oxidant markers – *trans*-beta-carotene, 13-*cis*-beta-carotene, alpha-carotene, cryptoxanthin, lutein (*cis, trans,* total), zeaxanthin, lycopene (*13-cis, 9-cis, trans*, total), retinol, alpha-tocopherol, and gamma-tocopherol
  - Bone Turnover markers – BSAP (Bone Specific Alkaline Phosphatase), NTx (n-teleopeptide type 1 collagen), and P1NP (aminoterminal propeptide type 1 procollagen)
  - Creatinine
  - Glucose metabolism markers – glucose, insulin, and IGF-1

B. Assay Details

B1. Assay Details - Cardiovascular Markers

The Hemoglobin A1c and Cholesterol panel (lipid) assays are performed at Meriter Labs (Madison, WI) using a Roche Cobas analyzer (Roche Diagnostics, Indianapolis, IN).[1]

***Glycosylated Hemoglobin**:*

The instrument measures the total hemoglobin concentration of the EDTA-anticoagulated blood colorimetrically, and the concentration of glycosylated hemoglobin (A1c) using a turbidometric inhibitor

procedure; it then divides the A1c concentration by the total hemoglobin concentration and reports this as a percentage.[2]

The A1c assay changed over time, see the Data Adjustment section for more information.

Cholesterol Panel (Lipid assays):

Cholesterol: The instrument uses enzymes to convert the cholesterol to a colored product and measures absorbance of the product.[3]

HDL-cholesterol: The instrument removes beta-lipoproteins from the serum by precipitation using polyethylene glycol, and then measures HDL (the alpha-fraction) as described above for total cholesterol.[4] The HDL assay changed over time, see the Data Adjustment section for more information.

Triglycerides: The instrument adds enzymes to the serum to obtain a colored product, and measures oabsorbance of the product.[5]

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:

LDL = TC - HDL - TG/5

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.[6]

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:

This was a radioimmunoassay performed using kit #DSL8900 from Diagnostic Systems Laboratories (Webster, TX). The procedure follows the basic principle of radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of [I-125]-labeled DHEA bound to the antibody is inversely proportional to the concentration of the unlabeled DHEA present. The separation of free and bound antigen is easily and rapidly achieved using a double antibody system.[7]

On May 18, 2009, ARUP began using LC-MS/MS (Liquid Chromatography tandem Mass Spectrometry) to assay DHEA. Data collection ended on May 31, 2009; thus the final 75 samples for MIDUS 2 (as well as all the samples for MIDUS R) were assayed under the new method. This method uses the Agilent 1200 HPLC (Agilent Technologies, Santa Clara, C A) to fractionate the serum and the API 5500 Mass Spectrometer (Applied Biosystems, Waltham, MA) for detection.

See the Data Adjustment section for additional information.

DHEA-Sulfate:

This assay is an electrochemiluminescent immunoassay and is run on a Roche Cobas e602 analyzer (Roche Diagnostics).

B3. Assay Details – Inflammation Markers

Cytokines and IL-6sr are assayed in the MIDUS BioCore Laboratory (University of Wisconsin, Madison, WI). The ICAM, E-Selectin, 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.[8]

IL6 by ELISA:

IL6 is 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).[9]

Soluble Receptors for IL6 (IL6-sr):

Concentration of IL6-sr is 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 is positively correlated to the concentration of IL6-sr in the samples.[10]

sICAM-I (Human Soluble Intercellular Adhesion Molecule-1):

The sICAM-1 assay is a sandwish ELISA using Quantikine® kit #SCD540 (R&D Systems, Minneapolis, MN). sICAM-1 in the serum is sandwiched by an immobilized monoclonal antibody and the enzyme-linked monoclonal antibody. The amount of ICAM-1 present is determined by colorimetric reaction.[11]

There were lot-to-lot changes in the sICAM-1 assay over the course of the data collection period. Correction factors were applied to the data at the Tracy lab.

Soluble E-Selectin (sE-Selectin):

sE-selectin, also known as endothelial leukocyte adhesion molecule-1 (ELAM-1) and CD62E, is measured in serum by sandwich ELISA using Quantikine® kit #SSLE00 (R&D Systems, Minneapolis, MN). The sE-Selectin assay utilizes two antibodies directed against different epitopes on the sE-Selectin molecule. The amount of sE-selectin bound is determined colorimetrically.[12]

There were lot-to-lot changes in the sE-Selectin assay over the course of the data collection period. Correction factors were applied to the data at the Tracy lab.

Fibrinogen:

Fibrinogen antigen is measured in plasma using the BNII nephelometer (N Antiserum to Human Fibrinogen; Siemens, Malvern, PA). 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.

C-reactive protein (CRP):

CRP was initially measured in plasma using the BNII nephelometer from Dade Behring utilizing 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.

Samples (from both MIDUS 2 and MIDUS R) falling below the assay range for CRP by this method were re-assayed by immunoelectrochemiluminescence using a high-sensitivity assay kit (Meso Scale Diagnostics #K151STG).[19]

Beginning in 2016, all the CRP assays were performed on the MSD platform. Beginning in 2016, because of technical difficulties associated with the use of plasma in MSD kits, CRP was assayed in serum. Corrections were applied at the MIDUS BioCore, see Data Adjustments for more information.

Soluble urokinase plasminogen activator receptor (suPAR): Note, suPAR assays were completed using stored MIDUS refresher samples.

suPAR (soluble urokinase plasminogen activator receptor) is a biomarker associated with immune and inflammatory reactions. suPAR levels typically increase with age. Healthy individuals have suPAR levels falling in the range of 2 to 4 ng/mL.[26] MSD's R-Plex Human U-PAR Assay kit[25] was utilized for the quantification of this biomarker. Blank MSD GOLD 96-well Small Spot Streptavidin plates were coated with R-Plex human U-PAR capture antibodies and left to incubate. After three washes to remove any unbound capture antibodies not fixated to the plate, thawed and diluted serum samples were added and incubated. After an additional three washes to remove any unbound sample antigens from the well, detection antibody was added which was conjugated with "SULFO-TAG" electrochemiluminescent labels and incubated. This detection antibody attaches to antibody-antigen complexes and allows for quantification of the desired analyte. The last incubation step was followed by another wash step to remove unbound detection antibodies. Read buffer was then added to the plate and analyzed with the MESO QuickPlex SQ 120 machine.

B4. Assay Details – Anti-oxidant Markers

Trans-beta-carotene, 13-*cis*-beta-carotene, alpha-carotene, cryptoxanthin, *cis*-lutein, *trans*-lutein, total lutein, zeaxanthin, 13-*cis*-lycopene, 9-*cis*-lycopene, *trans*-lycopene, total lycopene, retinol, alpha-tocopherol, and gamma-tocopherol assays are performed at the Antioxidants Research Laboratory (Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA).

Plasma samples (1.0 mL) are extracted with chloroform:methanol, and internal standards for carotenoids, retinoids, and tocopherols are added, along with saline. A reverse-phase gradient HPLC method with UV/VIS

and fluorescence detection is used for the simultaneous determination of these markers, according to a modification of the methods described by Ribaya-Mercado et al. (1992) and.Yeum et al. (1998).[13, 14,15]

B5. Assay Details – Bone Turnover Markers

The Bone Turnover assays (Bone Specific Alkaline Phosphatase, n-teleopeptide type 1 collagen, and aminoterminal propeptide type 1 procollagen), are performed in Dr. Neil Binkley's laboratory at the University of Wisconsin (Madison, WI).

n-teleopeptide type 1 collagen (NTx):

Osteomark® kits, Cat. #9021 (Alere Scarborough, Inc., Scarborough, ME) were used for all NTx analyses. This assay is a competitive-inhibition sandwich ELISA. Assay values are reported in nanomoles bone collagen equivalents (nm BCE) per liter (nM BCE/L).[16]

Aminoterminal propeptide type 1 procollagen (P1NP):

UniQ P1NP RIA (catalog # 67034) (Orion Diagnostica, Espoo, Finland) was used for all P1NP analyses. This is a competitive radioimmunoassay.[17]

Bone Specific Alkaline Phospatase (BSAP):

MicroVue BAP kits (catalog number 8012, Quidel Corporation, San Diego, Ca) were used for all BSAP analyses.This procedure is a sandwich ELISA.[18]

B6. Assay Details – Serum Creatinine

Serum Creatinine:

This assay is run on a Roche Cobas Analyzer (Roche Diagnostics) and is an enzymatic colorimetric assay, in which the instrument measures absorbance on the colored end product of an enzymatic reaction.[20]

B7. Assay Details - Glucose Metabolism

These assays are performed at ARUP laboratories in Salt Lake City, UT.

Glucose:

This assay is run on the Cobas c502 analyzer (Roche Diagnostics) and is an enzymatic colorimetric assay.[21]

Insulin:

This assay is performed on the ADVIA Centaur (Siemens) and is a two-site sandwich immunoassay using direct chemiluminescent technology. [22]

Insulin-like Growth Factor-1 (IGF-1):

This assay is run on the DiaSorin Liaison® XL and is two-site sandwich immunoassay using a chemiluminescent detection system.[40]

II. Urine Assays

A. Sample Collection:

The 12-hour overnight (7:00 pm – 7:00 am) urine samples are collected from each participant during the CRU visit. Prior to collecting the first sample CRU staff prepare two containers. The first, for catecholamine assay, has 25 mL of 50% acetic acid added to it and is labeled "CATS –ACID". The second, for cortisol assay, is labeled "No ACID". Each void is split between two urine collection containers. Details can be found in Section B.

13 mL aliquots of both acidified and untreated urine are stored in a -60º C to -80º C freezer and then shipped to the MIDUS BioCore lab on a monthly basis for neuroendocrine markers: catecholamines (epinephrine, norepinephrine, dopamine) and corticosteroids (cortisol, cortisone), as well as creatinine.

B. Assay Details

B.1 Assay details – Catecholamines
Catecholamine assays are performed at the Institute for Clinical and Translational Research (ICTR) at the University of Wisconsin at Madison for the MIDUS Refresher phase. At MIDUS 2 the assays were done at Mayo Labs. See the Data Adjustment section for more information

Epinephrine, Norepinephrine, Dopamine:

High-Pressure Liquid Chromatography (HPLC) is used for free catecholamine fractionation. They are quantified using electrochemical detection (ECD).

B2. Assay Details – Cortisol
Urine cortisol & cortisone assays are performed at the ICTR for the MIDUS Refresher phase. At MIDUS 2, the assays were done at Mayo Labs. See the Data Adjustment section for more information

Cortisol & Cortisone:

These are fractionated using HPLC and quantified using mass spectrometry (MS).

B3. Assay Details – Creatinine
Urine creatinine assays are performed at the ICTR for the MIDUS Refresher phase. At MIDUS 2 the assays were done at Mayo Labs. See the Data Adjustment section for more information

Creatinine:

Creatinine is measured by a colorimetric assay known as the Jaffe reaction. The urine is mixed with an alkaline picrate solution, which reacts with creatinine to produce an orange-yellow end product.

B4. Assay Details – Albumin and Cystatin C
Urine albumin and cystatin C are assayed in the MIDUS BioCore Laboratory (University of Wisconsin – Madison) using stored MIDUS refresher samples.

Albumin and Cystatin C:

Albumin and cystatin C are both biomarkers of kidney health and can be found in the blood and detected in urine. High levels of urinary albumin (albuminuria) can indicate poor kidney filtration since this organ normally prevents the flow of albumin into the urine. High levels of urinary cystatin C can indicate that the kidney tubules are not reabsorbing and catabolizing cystatin C. Albumin and cystatin C concentrations were measured from aliquots of frozen 12-hour urine collection samples derived from the M2 and MR1 phases of MIDUS. The BioCore designed a multiplex sandwich immunoassay that simultaneously tested these two analytes through electrochemiluminescent technology. MSD's R-Plex capture antibodies for both cystatin C[29] and albumin[27] were coated to the U-Plex Development SECTOR Plates (Meso Scale Discovery; Rockville, MD). Capture antibodies were attached to opposite ends of each individual well to help reduce signal overlap. Following three rinses with MSD wash buffer, thawed urine samples were diluted and added to the coated plate. After sample incubation and additional wash step, detection antibodies were added to each well that were conjugated with "SULFO-TAG" electrochemiluminescent labels. After a 1-hour incubation with the detection antibody, the plate was rinse 3 times with MSD wash buffer. Read buffer was then added to the plate and analyzed with the MESO QuickPlex SQ 120 instrument.

III. Saliva Assays

A. Sample Collection:
Saliva samples are collected during the Psychophysiology Challenge Protocol to complement assessments of stress reactivity based on Heart Rate Variability. Five samples are collected (pre-protocol, baseline, post-cognitive challenge, immediately post-orthostatic challenge, and 30 minutes post-protocol). Samples are then

frozen and shipped to the MIDUS BioCore lab for storage and subsequent Cortisol assay. See the Psychophysiology Protocol documentation for details about saliva sample collection.

B. Assay details

B1. Assay Details – Saliva Cortisol

Salivary cortisol is assayed at the ICTR for the MIDUS Refresher phase. At MIDUS 2 the assays were done at Dresden Lab Service. See the Data Adjustment section for more information.

The samples for this assay are collected on cotton swabs in salivettes (Sarstedt Cat. #51.1534) and frozen. At the time of assay, they are thawed and centrifuged at 3000 rpm for 5 min, resulting in a particulate-free, clear fluid of low viscosity.

Salivary Cortisol:

Concentrations of free cortisol (the only type found in saliva) were determined using radioimmunoassay (catalog #07-221106 from MP Biomedicals, Solon, OH).

ASSAY SENSITIVITY SUMMARY TABLE

A. P4 Bioassays from blood samples (specimen type)

Assay	Assay Type	Performed at:	Assay Range[24]	Variability	Reference Range
HA1c (blood)	Immunoturbidometric	Meriter	4.0-20.1 mg/dL min 4%	inter-assay CV: 0 % intra-assay CV: 2.2-2.3%	4.0-5.9%[28]
Total Cholesterol (serum)	Enzymatic colorimetric	Meriter	3.86-800 mg/dL min 3.86 mg/dL	inter-assay CV: 4.13 % intra-assay CV: 0.51-0.81%	150-199mg/dL[31]
Triglycerides (serum)	Enzymatic colorimetric	Meriter	8.85-885 mg/dL min 8.85 mg/dL	inter-assay CV: 2.52 % intra-assay CV: 1.6 %	Male: 40-160mg/dL[28] Female: 35-135mg/dL[28]
HDL-cholesterol (serum)	Enzymatic colorimetric	Meriter	3-120 mg/dL min 3 mg/dL	inter-assay CV: 3.56 % intra-assay CV: 1.1-1.4%	Male: >45mg/dL[28] Female: >55mg/dL[28]
LDL-cholesterol (serum)	Calculated	Univ. of Wisconsin (MIDUS BioCore Lab)	N/A	inter-assay CV: 4.71 %	≤130 mg/dL[28]
DHEA-sulfate (serum)	Immunoelectro-chemiluminescent	Associated Regional & University Pathologists (ARUP)	1-1000 ug/dL[41] min 1 µg/dL	inter-assay CV: 3 % intra-assay CV: 2.4 %	Males: 1.3-5.5mg/mL[31] Females: 0.6-3.3mg/mL[31]
DHEA (serum)	HPLC-tandem MS	ARUP	0.05-40 ng/mL min 0.01 ng/mL	inter-assay CV: 7.4 % intra-assay CV: 8.5 %	Ages ≥40: 0.63-4.70 ng/mL [28, 39] Postmenopausal: 0.60-5.73 ng/mL[28, 39]
Creatinine (serum)	Enzymatic colorimetric	Meriter	0.06-30.5mg/dL min 0.06 mg/dL	inter-assay CV: 2.08 %	Male: 0.6-1.2mg/dL[28] Female: 0.5-1.1mg/dL[28]
IL-6 (serum)	ELISA	Univ. of Wisconsin (MIDUS BioCore Lab)	0.156-10 pg/mL min 0.11 pg/mL	inter-assay CV: 15.66 % intra-assay CV: 3.73 %	0.45-9.96 pg/mL[9, 32]
IL6-sr (serum)	ELISA	Univ. of Wisconsin (MIDUS BioCore Lab)	31.2-2000 pg/mL min 15.1 pg/mL	inter-assay CV: 5.33 % intra-assay CV: 1.31 %	13,547-44,942 pg/mL[10]
IL-6 (serum)	Immunoelectro-chemiluminescent	Univ. of Wisconsin (MIDUS BioCore Lab)	1.58-488 pg/mL min 0.06 pg/mL	inter-assay CV: 5-15 % intra-assay CV: 4.73 %	0.16-27.2 pg/mL[8, 32]
IL-8 (serum)	Immunoelectro-chemiluminescent	Univ. of Wisconsin (MIDUS BioCore Lab)	1.13-375 pg/mL min 0.04 pg/mL	inter-assay CV: 6-7 % intra-assay CV: 2.88 %	1.48-1720 pg/mL[8]
IL-10 (serum)	Immunoelectro-chemiluminescent	Univ. of Wisconsin (MIDUS BioCore Lab)	0.68-233 pg/mL min 0.03 pg/mL	inter-assay CV: 11-14 % intra-assay CV: 5.78 %	0.06-3.08 pg/mL[8]
TNF-α (serum)	Immunoelectro-chemiluminescent	Univ. of Wisconsin (MIDUS BioCore Lab)	0.69-248 pg/mL min 0.04 pg/mL	inter-assay CV: 7 % intra-assay CV: 3.19 %	0.1-1.75 pg/mL[8]
Fibrinogen (citrated plasma)	Immunoturbidometric	Univ. of Vermont (Tracy Lab)	2.8-4560 mg/dL	inter-assay CV: 4.13-6.64 % intra-assay CV: 2.7 %	200-400 mg/dL[28, 33]
CRP (citrated plasma)	Immunoturbidometric	Univ. of Vermont (Tracy Lab)	0.164-800 ug/mL	inter-assay CV: 1.08-4.3 % intra-assay CV: 2.3 – 4.4 %	<1.0 mg/dL[28]
CRP (serum)	Immunoelectro-chemiluminescent	Univ. of Vermont (Tracy Lab)	0.014-216 ug/mL min 10^{-6} ug/mL	inter-assay CV: 4.72-5.16 % intra-assay CV: 2.2-4.1	<1.0 mg/dL[28]

Assay	Assay Type	Performed at:	Assay Range[24]	Variability	Reference Range
suPAR	Electrochemiluminescent	Univ. of Wisconsin (MIDUS BioCore Lab)	0.01-50 ng/mL	inter-assay CV: 4.96% intra-assay CV: 2.56%	2-4 ng/mL[26]
sE-Selectin (serum)	ELISA	Univ. of Vermont (Tracy Lab)	1.25-80 ng/mL min 0.027 ng/mL	inter-assay CV: 7.1-11.15 % intra-assay CV: 5.2-6.6 %	17.9-79.2 ng/mL[12]
sICAM-1 (serum)	ELISA	Univ. of Vermont (Tracy Lab)	31-1000 ng/mL min 0.254 ng/mL	inter-assay CV: 7.49-8.16 % intra-assay CV: 3.7-5.2 %	98.8-320 ng/mL[11]
P1NP (serum)	RIA	Univ. of Wisconsin (Binkley Lab)	5-250 ug/L min 2 ug/L	inter-assay CV: 6-9.8 % intra-assay CV: 6.5-10.2%	Premenopausal female: 19-101 µg/L[28] Postmenopausal female: 16-96 µg/L[28] Male: 22-105 µg/L[28]
sBAP (serum)	ELISA	Univ. of Wisconsin (Binkley Lab)	0-140 U/L min 0.7 U/L	inter-assay CV: 5-7.6 % intra-assay CV: 3.9-5.8%	Premenopausal female: 11.6-29.6 U/L[18] Postmenopausal female: 14.2-42.7 U/L[18] Male: 15.0-41.3 U/L[18]
sNTx (serum)	ELISA	Univ. of Wisconsin (Binkley Lab)	3.2-40 n*M* BCE (bone collagen equivalents)	inter-assay CV: 6.9 % intra-assay CV: 4.6 %	Female: 6.2-19.0 nM BCE[28] Male: 5.4-24.2 nM BCE[28]
***trans*-β-Carotene (EDTA-plasma)**	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 0.2 picomole	inter-assay CV: 5.6 % intra-assay CV: <5 %	None established
13-*cis*-β-Carotene (EDTA-plasma)	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 0.2 picomole	inter-assay CV: 9.77 % intra-assay CV: <5 %	None established
α-Carotene (EDTA-plasma)	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 0.2 picomole	inter-assay CV: 4.39 % intra-assay CV: <5 %	None established
β-Cryptoxanthine (EDTA-plasma)	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 0.2 picomole	inter-assay CV: 5.11 % intra-assay CV: <5 %	None established
Lutein (EDTA-plasma)	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 0.2 picomole	inter-assay CV: 5.97-8.82% intra-assay CV: <5 %	None established
Zeaxanthine (EDTA-plasma)	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 0.2 picomole	inter-assay CV: 7.08 % intra-assay CV: <5 %	None established
Lycopene (EDTA-plasma)	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 0.2 picomole	inter-assay CV: 6.96-13.42% intra-assay CV: <5 %	None established
Retinol (EDTA-plasma)	HPLC/colorimetric	Tufts Univ. (Blumberg Lab)	min 2 picomoles	inter-assay CV: 5.95 % intra-assay CV: <5 %	32.5-78µg/dL[34, 35]
α-Tocopherol (EDTA-plasma)	HPLC/fluorometric	Tufts Univ. (Blumberg Lab)	min 2.7 picomoles	inter-assay CV: 5.82 % intra-assay CV: <5 %	5.5-17mg/L[35]
γ-Tocopherol (EDTA-plasma)	HPLC/fluorometric	Tufts Univ. (Blumberg Lab)	min 2.7 picomoles	inter-assay CV: 5.85 % intra-assay CV: <5 %	None established

Assay	Assay Type	Performed at:	Assay Range[24]	Variability	Reference Range
Fasting glucose (serum)	Enzymatic colorimetric	ARUP	2-750 mg/dL min 2 mg/dL	inter-assay CV: 1 % intra-assay CV: 1.1 %	18-59 yr: 74-106mg/dL[28] 60-90 yr: 82-115mg/dL[28] >90 yr: 75-121mg/dL[28]
Insulin (serum)	Immunochemiluminescent	ARUP	1-300 uIU/mL min 1 uIU/mL	inter-assay CV: 4.6 % intra-assay CV: 4.0 %	6-26 µU/mL[28]
IGF-1 (serum)	Immunochemiluminescent	ARUP	10-1200 ng/mL min 10 ng/mL	inter-assay CV: 6.5 % intra-assay CV: 4.2 %	42-110ng/mL[28]

B. P4 Bioassays from urine and saliva samples (specimen type)

Assay	Assay Type	Performed at:	Assay Range[24]	Variability	Reference Range
Creatinine (urine)	Colorimetric	ICTR	0-400 mg/dL	inter-assay CV: 2.59-4.13% intra-assay CV: 1.3-1.75%	Male: 800-2000 mg/24h[36] Female: 600-1800 mg/24h[36]
Cortisol(urine)	HPLC/mass spectrometry	ICTR	min 0.03125 ug/dL	inter-assay CV: 8.08-15.49%	<100 µg/24hr[28]
Cortisol/creatinine ratio(urine)	Calculated	Univ. of Wisconsin (MIDUS BioCore Lab)	N/A	N/A	Male: <32 µg/g[37] Female: <24 µg/g[37] Pregnant: <59 µg/g[37]
Cortisone (urine)	HPLC/mass spectrometry	ICTR	min 0.03125 ug/dL	inter-assay CV: 7.92-14.29%	Male: 17-141 µg/24h [38] Female: 15-122 µg/24h[38]
Cortisone/creatinine ratio (urine)	Calculated	Univ. of Wisconsin (MIDUS BioCore Lab)	N/A	N/A	N/A
Albumin (urine)	Electrochemiluminescent	Univ. of Wisconsin (MIDUS BioCore Lab)	0.000084-2 mg/dL	inter-assay CV: 6.26-7.34% intra-assay CV: 7.71%	<2 mg/dL[28]
Cystatin C (urine)	Electrochemiluminescent	Univ. of Wisconsin (MIDUS BioCore Lab)	0.00036-0.46 mg/L	inter-assay CV: 4.35-5.56% intra-assay CV: 8.1%	0.03-0.18 mg/L[30]
Norepinephrine (acidified urine)	HPLC, electrochemical	ICTR	min 0.488 ng/mL	inter-assay CV: 10.43-16.77%	<100µg/24hr[28]
Epinephrine (acidified urine)	HPLC, electrochemical	ICTR	min 0.488 ng/mL	inter-assay CV: 12.59-18.61%	<20 µg/24hr[28]
Dopamine (acidified urine)	HPLC, electrochemical	ICTR	min 0.488 ng/mL	inter-assay CV: 10.15-16.68%	65-400 µg/24hr[28]
Cortisol (saliva)	RIA	ICTR	0.69-68.98 n*M*[23]	inter-assay CV: 29-44% intra-assay CV: 7.16-8.66%	7am-9am: 100-750ng/dL[28] 3pm-5pm: <401ng/dL[28] 11pm-midnight: <100ng/dL[28]

REFRESHER DATA ADJUSTMENTS TO REFLECT ASSAY CHANGES

I. Hemoglobin A1c

Due to a change in the calibration protocol at Meriter Labs on 5/21/06, a change in a reagent on 2/19/07, and a platform change on 4/17/10, an adjustment had to be made to the reported data to get them in line with the data reported at the beginning of MIDUS 2. MIDUS R HA1c values are adjusted using the following equation, which was derived by combining all three adjustments:

RA4BHA1C = 1.3849 * (reported value) – 2.1438

In addition to this adjustment, ten HA1c values underwent another adjustment, as a result of a new lot of reagent cartridges in June, 2015. These caused an upward bump in the control values. Their assay was recalibrated to compensate for this shift, but ten values were reported to us before the recalibration. Using samples which Meriter ran with and without recalibration, the following regression curve was obtained:

Corrected value = 0.8856 * (reported value) + 0.0015

This adjustment was made to the ten affected values before entering them in the data spreadsheet.

II. HDL-cholesterol

The vendor restandardized the assay performed at Meriter on August 6, 2007. Thereafter the reported data is adjusted using the following equation:

RA4BHDL = 1.1423 * (reported value) – 0.9028

III. DHEA

On May 18, 2009, ARUP Laboratories changed this assay from RIA to HPLC-tandem MS. The reported data is adjusted using the following equation:

RA4BDHEA = 1.6145 * (reported value) + 0.4668

IV. Soluble E-selectin and ICAM-1

The Tracy Lab at the University of Vermont reports lot-to-lot variation in data for these markers, as well as correction factors to apply to correct for this. Both values are entered in the data file, with the corrected values entered as RA4BSESEL and RA4BSICAM, respectively. These assays were run in three batches with the following corrections applied as follows:

Batch 1 - RA4BSESEL = 1.0905 * (reported value)

Batch 2 - RA4BSESEL = 1.3716 * (reported value)

Batch 3 - RA4BSESEL = 1.0000* (reported value)

Batch 1 & 2 - RA4BSICAM = 1.2844* (reported value)

Batch 3 - RA4BSICAM = 1.0000* (reported value)

V. C-reactive protein

The Tracy Lab initially measured CRP using a nephelometric assay; however, this was not sensitive enough to detect very low levels of this biomarker. Samples with undetectable CRP were re-assayed using the MSD immunoelectrochemiluminescent platform. Comparison data showed the following relationship between CRP levels reported using the two assays:

neph value = 0.7828 * (MSD value) – 0.0841

Because of the negative y-intercept in the above equation, it could not be applied to many of the low CRP values obtained using the MSD assay. So the following log-transformed equation was used:

$$RA4BCRP^{49} = 10^{0.9164[\log(\text{MSD value})] - 0.016}$$

In 2016 the Tracy lab started running all the CRP assays using the MSD technology, and on serum rather than citrated plasma because of technical difficulties associated with using plasma.
Comparison data relating MSD values using serum and plasma produced the following equation:

$$\text{Plasma MSD CRP value} = 0.6268 * (\text{serum MSD CRP value}) + 0.3301$$

Combining this equation with the linear equation [neph value = 0.7828 * (MSD value) – 0.0841] gives:

$$RA4BCRP = 0.4906 * (\text{serum MSD value}) + 0.1743$$

VI. Urinary norepinephrine

During the MIDUS 2 phase, catecholamines were assayed at Mayo Labs. For MIDUS R, this was done at the ICTR at the UW-Madison. Comparison data yielded the following equation to adjust the ICTR data:

$$RA4BNOREP = 0.9856 * (\text{reported value}) – 0.0685$$

In 2016, ICTR started using different columns for HPLC, necessitating another adjustment. Thereafter a new equation was used combining the results of both sets of comparison data:

$$RA4BNOREP = 0.617 * (\text{reported value}) + 2.625$$

VII. Urinary epinephrine

For epinephrine, no good regression curve comparing Mayo and ICTR values could be obtained in 2013. Since both the mean and the median of the first set of ICTR data (reported in November 2013) were similar to the mean and the median, respectively, for MIDUS 2 data, the ICTR epinephrine values were not adjusted from 2013 to 2015.

Comparison data from the 2016 assay change yielded the following equation:

$$RA4BEPIN = 0.851 * (\text{reported value}) – 0.8554$$

VIII. Urinary dopamine

Comparison data for dopamine assayed at both Mayo and ICTR in 2013 yielded the following regression equation:

$$RA4BDOPA = 0.9371 * (\text{reported value}) – 12.942$$

After the 2016 change, another equation was obtained which, when combined with the earlier one, produced this equation:

$$RA4BDOPA = 0.500 * (\text{reported value}) + 1.440$$

IX. Urinary cortisol

This was assayed at Mayo Labs for MIDUS 2, and at ICTR for MIDUS R. Comparison data generated the following equation for adjusting the MIDUS R reported values:

$$RA4BCORTL = 0.6823 * (\text{reported value}) + 0.9759$$

X. Urinary cortisone

ICTR values reported for MIDUS R were adjusted as follows, based on comparison data for samples previously assayed at Mayo for MIDUS 2:

RA4BCORTO = 0.8518 * (reported value) – 0.1979

XI. Urinary creatinine

Mayo Labs used an enzymatic colorimetric assay during MIDUS 2. For MIDUS R, ICTR used a non-enzymatic colorimetric method (the Jaffe reaction) for assaying creatinine. Comparison data produced the following adjustment:

RA4BUCREA = 1.0019 * (reported value) – 5.4888

XII. Salivary cortisol

During MIDUS 2, cortisol was measured at Dresden Lab Service by ELISA using kits manufactured by IBL International. For MIDUS R, saliva samples were sent to ICTR, where they were initially assayed using ELISA kits from Salimetrics LLC (Carlsbad, CA). The data obtained with these assays was deemed unreliable, so the lab re-assayed all the samples by RIA using kits from MP Biomedicals. From their comparison data the following equation was generated to adjust their RIA data to get it in line with the earlier IBL-ELISA data:

[cortisol] = 0.7629 (reported value) – 0.0544

By the time ICTR decided to try RIA, some of these early saliva samples were depleted. For these samples the Salimetrics data were adjusted to align them with IBL data using the following equation:

[cortisol] + 1.7973 * (Salimetrics value) + 1.0035

REFERENCES FOR BIOASSAY DOCUMENTATION AND SENSITIVITY TABLE

1. https://www.testmenu.com/meriter/TestDirectory/SiteFile?fileName=sidebar%5CTA%20New%20Chemistry%20Analyzers%20040524.pdf
2. Roche Diagnostics product insert: "Cobas Integra 400/700/800, Hemoglobin A1c Whole Blood Application - Standardized according to IFCC", November 2003.
3. Roche Diagnostics product insert: "Cobas Integra 400/700/800, Cholesterol Gen.2", May 2003.
4. Roche Diagnostics product insert: "Cobas Integra 400/700/800, HDL-Cholesterol Direct", November 2001.
5. Roche Diagnostics product insert: "Cobas Integra 400/700/800, Triglycerides", October 2000.
6. Wang TY, Haddad M, Wang TS. Low triglyceride levels affect calculation of low-density lipoprotein cholesterol values. Arch Pathol Lab Med. 2001 Mar;125(3):404-5. doi: 10.5858/2001-125-0404-LTLACO. PMID: 11231492.
7. Diagnostic Systems Laboratories, Inc. product insert, "Dehydroepiandrosterone (DHEA) RIA DSL-8900", April 4, 2005.
8. MSD product insert, “Proinflammatory Panel 1 (human) Kits: V-Plex”, 2016.
9. "Human IL-6 Quantikine HS ELISA Kit”, downloaded from R & D website, April 2017.
10. “Human IL-6 R alpha Quantikine ELISA Kit”, downloaded from R & D website, April 2017.
11. “Human ICAM-1/CD54 Allele-specific Quantikine ELISA Kit”, downloaded from R & D website, April 2017.
12. “Human sE-Selectin/CD62E Quantikine ELISA Kit”, downloaded from R & D website, April 2017.
13. Antioxidants Research Laboratory (TUFTS), "Antioxidant Assay Protocol: Collection and Analyses of Plasma Carotenoids, Retinol, and Tocopherols, 2009.
14. Ribaya-Mercado JD, Fox JG, Rosenblad WD, Blanco MC, Russell RM. ß-Carotene, retinol and retinyl ester concentrations in serum and selected tissues of ferrets fed ß-carotene. Journal of Nutrition 1992;122:1898-1903.
15. Yeum KJ, Ahn SH, de Paiva SAR, Lee-Kim YC, Krinsky NI, Russell RM. Correlation between carotenoid concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer. Journal of Nutrition 1998;128:1920-6.
16. Osteomark product insert for serum NTx assay kit, August 2012.
17. Orion Diagnostica product insert, “UniQ P1NP RIA”, January 2008.
18. MicroVue Bone BAP EIA An Enzyme Immunoassay for the quantitation of bone-specific alkaline phosphatase (BAP) in human serum [package insert]. Quidel Corporation, 2019. Retrieved February 15, 2023, from https://www.quidel.com/sites/default/files/product/documents/PI8012003EN00_%2811_19%29_MicroVue_BAP_EIA_Pkg_Insert.pdf
19. MSD product insert, “Vascular Injury Panel 2 (human) Kits: V-Plex, August 2014.
20. Meriter Laboratories, "Creatinine level in serum assay", September 21, 2004.
21. ARUP, "Glucose. Serum and Body Fluid by Roche Molecular Analytics P", August 2009.
22. ARUP, "Insulin By Siemens ADVIA: Centaur", April 2010.
23. Stone A, Schwartz J, Smyth J, Kirschbaum C, Cohen S, Hellhammer D, Grossman S. Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. *Psychoneuroendocrinology* 26:295-306 (2001).
24. Assay ranges reflect the standard curves of the assays. The minimum can be below the lower level of the standard curve (by extrapolation), or above the lower level of the standard curve (when the points at the lower end of the standard curve do not fit the curve very well). There is no maximum detectable value, since samples with high levels of analyte can be diluted prior to assay.
25. Meso Scale Diagnostics Human U-PAR R-PLEX Antibody Sets and Assays, August 2022.
26. Rasmussen LJH, Caspi A, Ambler A, Danese A, Elliott M, Eugen-Olsen J, Hariri AR, Harrington H, Houts R, Poulton R, Ramrakha S, Sugden K, Williams B, Moffitt TE. Association Between Elevated suPAR, a New Biomarker of Inflammation, and Accelerated Aging. J Gerontol A Biol Sci Med Sci. 2021 Jan 18;76(2):318-327. doi: 10.1093/gerona/glaa178. PMID: 32766674; PMCID: PMC7812430.
27. Meso Scale Diagnostics Human Albumin R-PLEX Antibody Sets and Assays, August 2022.

28. Pagana KD, Pagana TJ, Pagana TN. Mosby's Diagnostic & Laboratory Test Reference. 14th ed. St. Louis, MO: Elsevier, 2019.
29. Meso Scale Diagnostics Human Cystatin C U-PLEX Antibody Sets and Assays, November 2023.
30. Conti M, Moutereau S, Zater M, Lallali K, Durrbach A, Manivet P, Eschwège P, Loric S. Urinary cystatin C as a specific marker of tubular dysfunction. Clin Chem Lab Med. 2006;44(3):288-91. doi: 10.1515/CCLM.2006.050. PMID: 16519600.
31. *The Merck manual*. Merck Sharp & Dohme Research Laboratories. https://www.merckmanuals.com/professional.
32. https://www.mayocliniclabs.com/test-catalog/overview/618776#Clinical-and-Interpretive
33. https://www.uptodate.com/contents/cryoprecipitate-and-fibrinogen-concentrate
34. *Vita - Overview: Vitamin A, serum*. VITA - Overview: Vitamin A, Serum. (n.d.). https://www.mayocliniclabs.com/test-catalog/overview/42357#Clinical-and-Interpretive
35. ABIM laboratory test reference ranges–January 2024. (n.d.). https://www.abim.org/Media/bfijryql/laboratory-reference-ranges.pdf
36. Chernecky, C., & Berger, B. (2013). Creatinine - Urine. In *Laboratory Tests and Diagnostic Procedures* (pp. 398–402). Elsevier.
37. *Cortisol Urine Free by LC-MS/MS*. Meriter Laboratories Test Directory. Retrieved February 15, 2023, from https://www.testmenu.com/meriter/Tests/1036555
38. Taylor, R. L., Machacek, D., & Singh, R. J. (2002). Validation of a high-throughput liquid chromatography–tandem mass spectrometry method for urinary cortisol and Cortisone. *Clinical Chemistry*, 48(9), 1511–1519. https://doi.org/10.1093/clinchem/48.9.1511
39. *Dehydroepiandrosterone, serum or plasma: Arup Laboratories Test Directory*. Dehydroepiandrosterone, Serum or Plasma | ARUP Laboratories Test Directory. (n.d.). https://ltd.aruplab.com/Tests/Pub/2001640
40. ARUP, "Insulin-Like Growth Factor I (IGF-1) By Siemens IMMULITE® 2000", November 2009.
41. ARUP, "Dehydropiandrosterone [sic] Sulfate by Roche Modular Analytics E170: New Instrument/Assay Validation Worksheet", April 2003.