MIDUS 3 BIOMARKER PROJECT (P4) OVERVIEW

The Biomarker Project is one of multiple projects that comprise the MIDUS 3 (M3) "Integrative Pathways to Health and Illness" study. For the past two decades, the overarching objective of MIDUS has been to investigate linkages between sociodemographic, psychosocial, behavioral, and neurobiological variables to account for unfolding profiles of morbidity and mortality across the decades of adult life as well as the maintenance of good health and functional capacities. The study has facilitated analyses that pay attention simultaneously to age, gender, race, and socioeconomic variation in how psychosocial and neurobiological variables are linked. In addition, the M3 data permit longitudinal assessment of the impact of ongoing historical events, such as the 2008 economic recession, on the health of diverse-aged adults, which was also included in the MIDUS Refresher 1 (MR1) assessments. M3 included recruitment of additional twins to facilitate genomic analysis. Longitudinal retention rates (# of completed cases/# of eligible cases) for M3, including twins that were not part of the baseline M2 biomarker sample, are as follows:

- Overall Rate: 747/1266 =59% (Eligible = living, healthy enough to travel safely)
- Adjusted Rate: 747/1162= 64.3% (Eligible excludes: No contact/Unable to locate)

Note, due to pandemic-related interruptions in data collection, the M3 field period was extended from April 2017 to May 2022.

The M3 Biomarker Project (P4) includes assessment of multiple indicators of physiological regulation/dysregulation and health according to the basic protocol implemented in the MR1 study, which repeated and expanded the M2 biomarker protocol. The M3 protocol included bone density and body composition assessments at all sites and expansion of Actigraphy, Ankle Brachial Index (ABI) and Gait assessments to all three sites. Modifications to that basic protocol and the psychosocial assessments are noted below as appropriate. Data were collected during a 24-hour stay at one of three Clinical Research Units (CRU). The following provides basic information about the sample, recruitment, and data collection procedures. Additional information about basic assessments and the MIDUS Core Biomarker sample (i.e. MIDUS 2) can be found in:

> Love, G.D., Seeman, T.E., Weinstein, M., & Ryff, C.D.. (2010). Bioindicators in the MIDUS National Study: Protocol, Measures, Sample and Comparative Context. *Journal of Aging and Health, 22, 1059-1080*.

The Sample

MIDUS participants were eligible for M3 Biomarker data collection if they:

- Completed the M3 Survey project Phone Interview and Self-Administered Questionnaire **OR** Completed the Milwaukee 2 survey project in-person CAPI interview and Self-Administered Questionnaire
- Completed the M2 Biomarker protocol **OR** were part of the twin sample and did NOT participate in Biomarker project at M2

Eligible participants were assigned to one of the three data collection sites (UCLA/Site 1, University of Wisconsin (UW)/Site 2, or Georgetown University/Site 3) based on where they completed the M2. New twins were assigned to a data collection site based on the region (West Coast, Midwest, East Coast, respectively) in which they lived.

Recruitment

Recruitment was a two-step process. Staff at a given data collection site sent a recruitment packet (letter and brochure describing the study) to individuals assigned to their site. Within a few weeks, designated staff at that site made a follow-up call to answer any questions the individual may have had, then attempted to schedule a CRU visit and facilitated travel arrangements.

Informed Consent

Verbal consent was obtained by phone when individuals agree to participate and schedule a CRU visit. Written consent was obtained at the clinic prior to beginning study procedures.

Data Collection Procedures

All biomarker assessments, with the exception of sleep assessments, were completed during the overnight stay at the regional CRU. The visit protocol was standardized across the three sites so that assessments were completed as follows:

Day 1 (late afternoon or evening of the day the person arrived at the CRU)

- Medication Chart
- Medical History
- Self-Administered Questionnaire (SAQ), includes the Pittsburgh Sleep Questionnaire (PSQ)
- Physical Exam (Vitals)
- 12 hour Urine Collection began

Day 2 (the morning of the day the person left the CRU)

- 12 hour Urine Collection ended
- Fasting Blood Draw
- Psychophysiology Experimental Protocol
- Physical Exam (Functional Assessments)

The following assessments were completed on Day 1 or Day 2 to provide flexibility in accommodating availability of clinical staff:

- Bone Densitometry & Total Body Composition (DXA scans)
- Body Impedance
- Ankle Brachial Index
- Gait Assessment
- Muscle function (Jump/Balance). These assessments were done at UW only.

Methods for collecting psychosocial data are described below, followed by the protocols for collecting biomarker data.

Psychosocial Assessments

Psychosocial experience was assessed using the SAQ, PSQ and sections of the Medical History. Psychometric information and details about the scales included in the SAQ and PSQ can be found in the Documentation for Psychosocial Constructs and Composite Variables. Key sections and details about each instrument are highlighted below.

Self-Administered Questionnaire (SAQ)

The 25-page SAQ booklet is available as a standalone file with the other biomarker documentation and contains the following scales:

MASQ (Mood and Symptom Questionnaire
Overall Assessment of day
CES-D (Center for Epidemiologic Studies- Depression)
Perceived Stress Scale
Spielberger Anger Expression
Spielberger Trait Anger
Spielberger Trait Anxiety
Social Anxiety
Childhood Trauma Questionnaire (CTQ)
Positive Events Scale
Singelis Self-Construal Scale
Social Obligation Scale
Relational-Interdependent Self Construal (RISC)
Sympathy Scale
Adjustment Scale
Support/Strain Given to Others
Self-Control
Minimalist Wellbeing
Subjective Well-Being
UCLA Loneliness Scale

Pittsburgh Sleep Questionnaire (PSQ)

At M2 and MR1 the PSQ was administered as 2-page document that included a few questions to be answered by the participant's bed partner or roommate. Those items were dropped at M3 and the PSQ was added at the beginning of the SAQ (above). Additional details about the PSQ can be found in the Documentation for Sleep Data, along with details about collection of physiological sleep data (see below).

Medical History

Although most of this instrument assesses respondents' medical and health conditions/behaviors (see below), the final three sections were used to identify significant life events the participant

may have experienced since completing the Phone Interview component of the Survey project. These assessments include questions about:

- Changes in marital status
- Deaths of family members and/or close friends
- Other significant life events, positive or negative, that the respondent chose to tell us about.

The Medical History is administered as a computer assisted personal interview (CAPI). A Word version of the instrument, including SVNs (Short Variable Names) is available as a standalone PDF with the other biomarker documentation.

Biomarker Assessments

Medical History

In addition to the life event assessments mentioned above, the Medical History included the following. Note, to improve functionality and flow, the Medical History items were re-organized so that related items are together. Question numbers are included in parentheses with notes about change included as needed:

- Symptoms and Conditions (Q1)
- Major Health Events (broken bones (Q2), surgeries (Q13), head injuries (Q14), joint injuries (Q15), motor vehicle accidents (Q16), amputations (Q17), other (Q18))
- Immune Function: Immunizations (childhood (Q20) & adult (Q21,22)) and Allergies (Q35)
- Family Medical History (Q74-75)
- Current Health Practices:
    - Diet (Q36-49)
    - Alcohol (Q53-56)
    - Health Care and Screening (Q23-Q25)
    - Protective Practices (Q76-80)

At M2, a separate short interview was also administered to assess history of broken bones and falls, as well as other health behaviors/experiences that might affect bone health. That set of items was integrated into the MR1 Medical History interview and was carried forward into the M3 Medical History, which now also includes the following assessments.

- History of broken bones and falls for participant and immediate family (Q3-5)
- Medical conditions that might impact bone health (Q6)
- Medication/Treatment History – use of osteoporosis medication (Q7) and other medications or treatments (e.g. chemotherapy) that could affect bone health (Q8)
- History of smoking, passive smoking (these were moved later in the instrument and can be found at Q60-70), and exercise (also moved later, can be found at Q72-73)
- Women's Health - # of past pregnancies, current menarchal status (e.g. pre or post-menopausal) (these were moved earlier in the instrument and can now be at Q26-33).
- Metal in the body – provides information about pins, rods, and other metal in the body to facilitate interpretation of whole body scan data (these were moved earlier in the instrument and are now found at Q8-12)

The following new items were added at M3:

- Diabetes, included in the Symptoms and Conditions section, was expanded to include questions about Diabetes Type and Year of Diagnosis
- The Diet Section was expanded to include questions about the types of oils/fats used in cooking and a specific question about wine consumption to better assess Healthy Eating in the Mediterranean style
- A general question about Alcohol use (Q53) was expanded to better identify types/amounts of liquor participants usually drink
- Question about mode of travel and experiences related to traveling to participate in the study were added at the end of the interview (Q88-91)

Physical Exam: Short Version

The short version of the physical exam was completed at all sites following standardized procedures. It included the following assessments:

- Vital Signs: Height, weight, pulse, blood pressure, respiration, and temperature
- Waist & Hip (maximum extension & iliac crest) Measurement
- Functional Assessments: grip strength, visual acuity, peak flow, 50 foot timed walk, and, chair stands

Details about the protocol for conducting the physical exams can be found in the Documentation for Physical Exam Data. Note, the long version of the physical exam that was administered at M2 and MR1 was dropped at M3 due to changes in hospital staffing requirements.

Medication Data

Study participants were asked to bring all of their prescription, over the counter, and alternative medications to the CRU. Data about these medications were recorded on a 3-page form. One page was for prescription medications and one for over-the counter medications. The third page included a section for alternative medications and a section for recording information about medication allergies. The following information was recorded for each type of medication:

- Medication Name
- Dosage
- Route by which it is administered (e.g. by mouth, topical etc.)
- Frequency
- How long the participant has been taking it
- Why the participant thinks s/he is taking the medication

The CRU's require that information about medications to be taken during the visit be available prior to the participant's arrival. Thus, the above information was obtained or confirmed at the time of the visit.

Details about medication data collection and coding can be found in the Documentation for Medication Data. This document also includes details about therapeutic and pharmacologic class codes that are attached to the medication data via linkage to the Lexi-Data Database®. This document provides details about assigning modified ICD-10 codes (International Classification

of Diseases, version 10) and a set of MIDUS specific codes to the reasons for taking medications reported by participants.

Bone and Musculoskeletal Health Data

Bone health was assessed via questionnaire (see Medical History above), DXA (Dual X-Ray Absorptiometry) scan, muscle function assessments and assays for select blood based biomarkers. Blood samples for biomarker assay were collected at all sites for all participants and are described, separately, below.

Bone densitometry and DXA based body composition data, as well as muscle function (jump/balance) data were obtained only at Site 2 (UW) do to funding limitations. Two sites (UW, UCLA) also obtained body impedance data via different methods. Details about these protocols and data can be found in the Documentation for Musculoskeletal Health and Function Data.

Ankle Brachial Index (ABI)

Ankle Brachial Index is a check for peripheral artery disease (PAD) and is measured using the Summit Doppler Vantage ABI™ system via blood pressure cuffs placed in four locations while the participant is lying down. At the end of a 5-minute resting period, the four cuffs are activated in rotation (right arm, right ankle, left arm, left ankle) with the device automatically inflating the cuff, measuring systolic pressure and deflating the cuff. ABI is the ratio of systolic blood pressure in the arteries (typically measured at the ankle) to the brachial (upper arm) systolic blood pressure.

The ABI assessment was added to the MR1 Biomarker protocol as a pilot project conducted only at Site 1 (UCLA). It was subsequently administered at all three sites at M3. Details about this procedure can be found in the Documentation for Ankle Brachial Index.

Tissue Sample (Blood, Urine, Saliva) Assays

A 12-hour urine sample and fasting blood samples were collected from each participant. To ensure consistency, all samples were collected and processed at the CRU using standardized procedures and then both fresh and frozen samples were shipped to the MIDUS BioCore Lab for assay as follows:

- Fresh whole blood sample was sent weekly and assayed for:
  - Hemoglobin A1c
- Frozen Serum in 2 ml aliquots were shipped monthly
  - Cholesterol Panel, HDL-Function
  - Insulin, Glucose, Insulin-like Growth Factor (IGF-1)
  - DHEA and DHEA-S
  - Inflammation markers: IL-6, s-IL6-r, IL-8, IL-10, TNF-alpha, C-Reactive Protein, Fibrinogen, E-Selectin, ICAM
  - Anti-Oxidants
  - Bone Turnover: BSAP (Bone Specific Alkaline Phosphatase), NTx (n-teleopeptide type 1 collagen), P1NP (aminoterminal propeptide type 1 procollagen).

- Frozen Urine 11 ml aliquots were shipped monthly
    - Catecholamines: epinephrine, norepinephrine, dopamine
    - Cortisol
- Frozen Saliva samples were shipped monthly and assayed for Cortisol

Details about tissue sample collection and biomarker assays can be found in the Documentation for Blood, Urine and Saliva Data.

PsychoPhysiology Experimental Protocol

The protocol included two 6-minute cognitive challenges, followed by a 6-minute orthostatic (standing) challenge. Physiological reactivity during the challenge tests was monitored via measures of:

- Heart rate variability and respiration, providing an index of central-peripheral neural feedback and central nervous system-autonomic nervous system integration;
- Beat-to-beat blood pressure; and
- Collection of 5 saliva samples over time (session start, baseline, 6 minutes after the second mental challenge, 6 minutes after the end of the physical challenge, 30 minutes after the session) for cortisol assay.

Details about this protocol can be found in the Documentation for Psychophysiology Data.

Sleep Efficiency

Physiological measures of sleep were assessed only at Site 2 at M2 and MR1, but were expanded to all three sites at M3. Participants were asked to wear the Actiwatch continuously for 7 days. The data collection period was standardized to begin on the Tuesday following the day the participant arrived home after completing the CRU visit. Data collection ended a week later on the following Tuesday morning. The Actiwatch is a lightweight, watch-sized, water proof, activity monitoring device that was worn on the wrist. Participants were also asked to complete a paper and pencil daily sleep diary during the period that the Actiwatch was worn. These materials are mailed back to UW at the end of the data collection period.

The Daily Sleep Diary included assessments of daily:

- Daytime naps, exercise, caffeine and alcohol consumption, and use of sleep aids as well as other medications not taken on a daily basis.
- Subjective Sleep Quality – time to bed, time to rise, sleep disturbance, ratings of sleep quality.

Details about sleep assessments and a copy of the Daily Sleep Diary can be found in the Documentation for Sleep Data.