Multi-Ethnic Study of Atherosclerosis (MESA)

Exam 7 Protocol

Version 98 December~~November~~ 8, 2023

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1. Summary of the Multi-Ethnic Study of Atherosclerosis

The Multi-Ethnic Study of Atherosclerosis (MESA) was initiated in July 2000 to investigate the prevalence, correlates, and progression of subclinical CVD (disease detected non-invasively before it has produced clinical signs and symptoms) in a population-based sample. MESA recruited 6814 white (28%), Asian (12%, predominantly of Chinese descent), African-American (28%), and Hispanic (22%) men and women aged 45-84 who were free of clinical CVD from six communities. These participants were extensively characterized during the baseline and five subsequent examinations with respect to established and putative risk markers and subclinical atherosclerosis measures.

The cohort was recruited from six field centers and characterized for coronary calcification, ventricular mass and function, flow-mediated endothelial vasodilation, carotid intimal-medial wall thickness and presence of echogenic lucencies in the carotid artery, lower extremity vascular insufficiency, arterial wave forms, electrocardiographic measures, standard coronary risk factors, sociodemographic factors, lifestyle factors, and psychosocial factors. Selected repetition of subclinical disease measures and risk factors allowed the study of the progression of disease. Blood samples were assayed for putative biochemical risk factors and stored for case-control studies. DNA was extracted and lymphocytes immortalized to study candidate genes and genome-wide scanning. Participants have been followed for identification and characterization of cardiovascular disease events, including acute myocardial infarction and other forms of coronary heart disease (CHD), stroke, and congestive heart failure; mortality; and for cardiovascular disease interventions.

In addition to the six Field Centers, the study involves a Coordinating Center, a Central Laboratory. Previously, MESA also included reading centers for Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Ultrasound, Retinal Photography, and Electrocardiography. Protocol development took place in the first 18 months, and staff training and pilot testing occurred prior to each exam. The first examination took place over 25 months, followed by five follow-up exams. Participants have been contacted every 9-12 months throughout the study to assess clinical morbidity and mortality. MESA is currently in its fourth contract period.

The original MESA objectives, research questions, background, rationale, and study design can be reviewed in the Exam 1 MESA Protocol at https://www.mesa-nhlbi.org/MesaInternal/documents/2004/mesaprot000225-updated.doc.

The Exam 1 MESA protocol also includes the following information:

- Original sample size and power calculations
- Description of the field center communities and source populations
- Study population and sampling
- Eligibility and exclusion criteria
- Recruitment method

Published manuscripts information is provided on the MESA website at https://www.mesa-nhlbi.org/PublishedPapers/PublishedPapers_Chronological.docx.

2. Introduction and Specific Aims

Cardiovascular diseases (CVD) remain the leading cause of death in the United States. Incidence rates of MI, stroke, or other symptomatic CVD remain high, and the burden of heart failure is increasing. The decrease in CVD mortality observed may relate to improvement in the control of some CVD risk factors (smoking, hypertension, dyslipidemia); however, there are adverse trends in obesity and diabetes, and the population is aging. The degree to which biological and behavioral risk factors, genetics, or socio-economic factors contribute to the expression of subclinical CVD and the subsequent incidence of clinical events remains an active area of investigation. The original goals of MESA were to assess the importance of subclinical vascular disease markers on CVD outcomes and to better understand the predictors of subclinical disease progression in a diverse cohort. These goals remain incomplete, especially within race/ethnicity, age, and sex strata.

The aims of the 7th clinical examination include:

Aim 1: Enhance statistical power to perform analyses of predictors of clinical outcomes, particularly in informative subgroups
Aim 2: Study the progression of subclinical to clinical CVD
Aim 3: Identify new risk factors or interactions among factors that inform disease pathophysiology.

This exam will build on the successful components of MESA to provide further insights into strategies to reduce the burden of CVD and eliminate/reduce racial/ethnic CVD disparities.

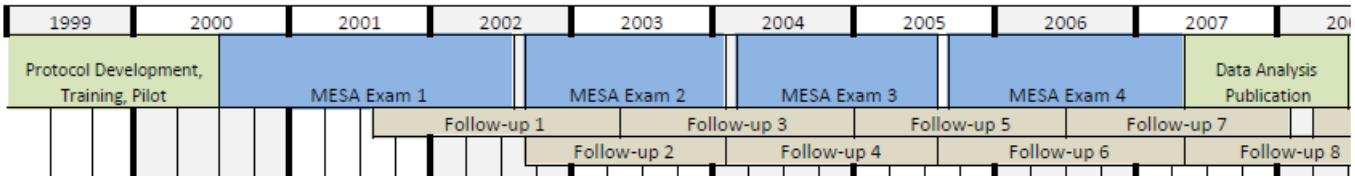
3. Exam 7 Study Design

3.1. Structure for the 7th Examination

The current MESA Contract for Exam 7 is structured similarly to that of Exam 6. Unlike Exams 1-5, the core exam is small with only a few key components, including demographics, anthropometry, blood pressure, smoking history, medical history (including medications), and phlebotomy. Grant proposals were solicited by NHLBI for MESA Exam 7 ancillary studies under the assumption that Exam 7 would become economically feasible when a sufficient quantity of ancillary studies received grant funding. The intent was for these ancillary studies to provide the necessary financial support for the Coordinating Center, the Field Centers, and the Lab to integrate the activity into the Core Exam. The advantage of this approach is that it allows the science of Exam 7 to be determined based on a full peer-review within the standard NIH grant system. The challenge has been creating a unified, seamless exam from multiple ancillary studies that were not proposed in conjunction with one another.

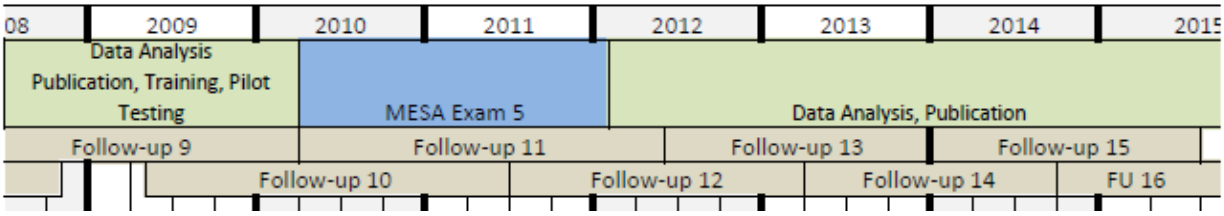
3.2. Contract Periods

First contract period (January 15, 1999 – August 14, 2008):



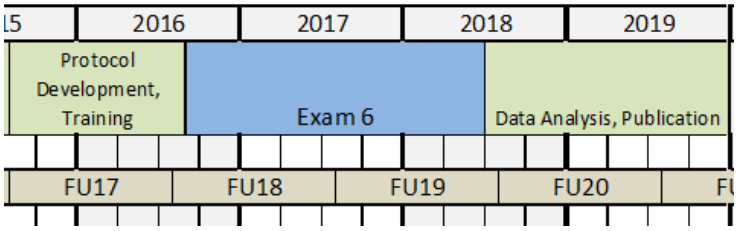
Protocol development, training, pilot testing	January 15, 1999 - July 14, 2000
Examinations 1-4, surveillance (data analysis/publication from 2002)	July 15, 2000 - July 14, 2007
Events surveillance, data analysis/publication	July 15, 2007 - January 14, 2008

Second contract period (August 15, 2008 – August 14, 2015):



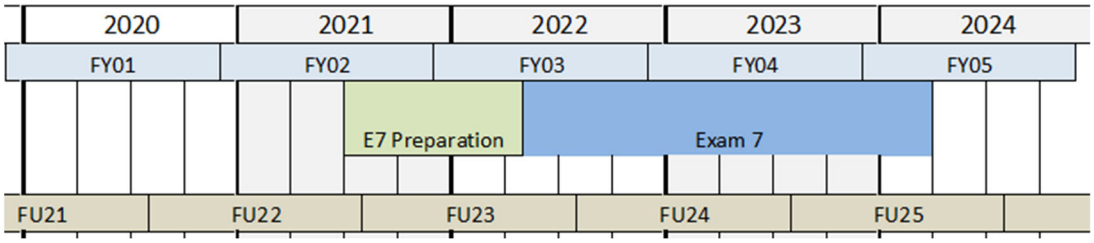
Events surveillance, protocol development, training, pilot testing, data analysis/publication	August 15, 2008 – March 31, 2010
Examination 5, events surveillance, data analysis/publication	April 1, 2010 – September 30, 2011
Events surveillance, data analysis/publication	October 1, 2011 - August 14, 2015

Third contract period (August 15, 2015 – December 18, 2019):



Events surveillance, protocol development, training, pilot testing, data analysis/publication	August 15, 2015 – August 31, 2016
Examination 6, events surveillance	September 1, 2016 – March 31, 2018
Events surveillance, data analysis/publication	March 31, 2018 - August 14, 2019
Data analysis/publication	August 15, 2019 – December 18, 2019

Fourth contract period (December 19, 2019 – December 18, 2024):



Events surveillance, protocol development, training, pilot testing	December 19, 2019 – March 4, 2022
Examination 7, Events surveillance	May 5, 2022 – March 29, 2024
Events surveillance, data cleaning, data analysis/publication	April 1, 2024 – December 18, 2024

3.3. Previous Exam Components

Table 1 below shows components performed in Exams 1-6 and those planned for Exam 7

- **X** = MESA Classic Cohort (all available assumed unless number provided)
- A = Ancillary Study procedure
- Counts provided below are reflective of data available in datasets. Some variation in the number available for a specific analysis is expected.
- Data collected outside main exams are added to the closed exam column. For example, MESA Kidney GFR measures from 2012-2014 are included in the Exam 5 column.

Table 1: Components included in Exams 1-7

	Exam 1 Jul00- Aug02 24 Months	Exam 2 Sep02- Feb04 18 Months	Exam 3 Mar04- Sep05 18 Months	Exam 4 Sep05- May07 21 Months	Exam 5 Apr10- Dec11 21 Months	Exam 6 Sept16- June18 22 Months	Exam 7 May22- Nov23 18 Months
	N=6814	N=6232	N=5939	N=5704	N=4655	N=3303	N=3000*
Questionnaires							
Air Pollution Questionnaire			X		X		A
Brain Injury Questionnaire						A_{1030}	
COPD Assessment						A_{220}	A
Family Hx		X					
Food Frequency (Diet)	X				X		
Heart Failure Symptoms/Risk Factors						A	
Medical Hx	X	X	X	X	X	X	X
Medications	X	X	X	X	X	X	X
Occupation/employment	X	X	X			X	X
Personal Hx	X	X	X	X	X	X	X
Physical Activity	X	X	X		X	A	
Physical Function						A	
Psycho-Social	X	X	X	X	X		X
Residential/neighborhood		X			A		A
Sleep Hx		X		X	A_{2222}	A_{219}	A
Stress Questionnaire			A_{1000}		A_{1080}		A
Urinary Incontinence						A	
Procedures/Assessments							
Air Pollution Monitoring				A	A		

	Exam 1 Jul00- Aug02 24 Months	Exam 2 Sep02- Feb04 18 Months	Exam 3 Mar04- Sep05 18 Months	Exam 4 Sep05- May07 21 Months	Exam 5 Apr10- Dec11 21 Months	Exam 6 Sept16- June18 22 Months	Exam 7 May22- Nov23 18 Months
Ambulatory Blood Pressure Monitor							A
Ankle Brachial Index (ABI)	X		X		X		
Anthropometry	X	X	X	X	X	X	X
Arterial Wave Form/Arterial Stiffness	X_{6335}				A_{4130}	A	A
Blood Pressure	X	X	X	X	X	X	X
Blood Sample collection	X	X	X	X	X	X	X
Brain Amyloid PET Imaging						$A_{\sim 216}$	A
Cardiopulmonary Exercise Testing					A_{102}	A_{300}	
Clinician's Neuro-Physical Exam						A	A
Cognitive Function (CASI, Digit symbol, Digit spam)					X	A_{2051}	A
Cognitive Function (UDS)						$A_{\sim 700}$	A
CT Aortic (abdomen)		X_{1000}	X_{1000}				
CT Coronary (Chest)	X_{6814}	X_{2955}	X_{2805}	A_{1405}	A_{3302}		
CT Lung					A_{3122}	A_{3260}	A
Echocardiogram						A	
Electrocardiogram (ECG)	X_{6765}				X_{4625}	A	
Epigenetics DNA Methylation					A_{1264}	A_{1726}	
Genotyping		A	A	A	A	A	
GFR measurement					A_{294}		
Glucose Monitor (14d)							A
MRI Brain						$A\sim_{1896}$	A
MRI Cardiac	X_{5000}	X_{650}		X_{1350}	X_{3027}		
MRI Carotid		X_{1000}					
MRI Coronary Wall				A_{300}			
MRI Cardiac, Tagging		A_{1500}			A_{1200}		
MRI Lower Extremity Tissue Sodium						A_{1157}	
Nasal Brushing/Hair Collection							A
PET MRI Carotid						$A_{\sim 350}$	
PET Brain Amyloid						A	A

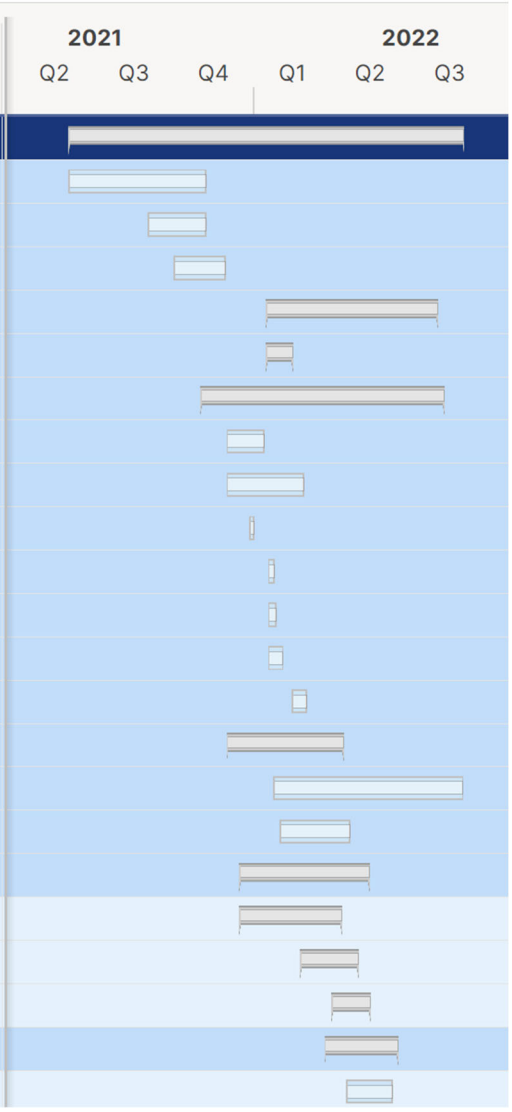
	Exam 1 Jul00- Aug02 24 Months	Exam 2 Sep02- Feb04 18 Months	Exam 3 Mar04- Sep05 18 Months	Exam 4 Sep05- May07 21 Months	Exam 5 Apr10- Dec11 21 Months	Exam 6 Sept16- June18 22 Months	Exam 7 May22- Nov23 18 Months
Polysomnography and actigraphy					A_{2060}		A
Retinal Photography		X_{6080}			X_{4350}		
Salivary cortisol collection			A_{1000}		A_{1080}		
Short Physical Performance Battery						A	A
Six Minute Walk Test					A_{302}	A	
Spirometry				X_{3600}	A_{3220}	A_{2564}	A
Urine collection	X	X	X		X	X	A
US Carotid Distensibility	X_{6530}						
US Carotid IMT	X_{6725}	X_{2955}	X_{2805}	A_{1360}	A_{3429}	A_{1188}	
US Plaque	X			A_{836}	A_{3447}	A_{1188}	
US Endothelial Function (FMD)	X_{3500}						
Vision/Refraction		X_{6080}			X_{4350}		
Vitamin D Clinical Trial						$A_{\sim 680}$	
ECG Monitor Patch						A_{1557}	A

*expected for Exam 7

3.4. Exam 7 Timeline

Table 2: Timeline for MESA Exam 7 Preparation

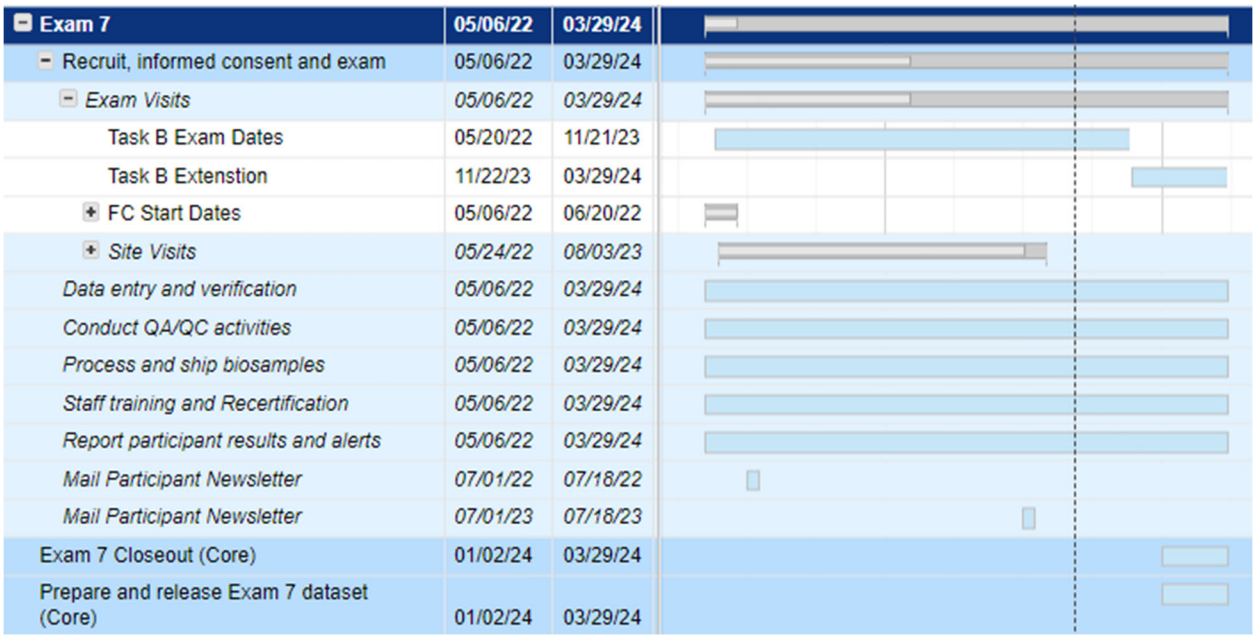
Task Name	Start Date	End Date
Exam 7 Preparation	**06/01/21**	**09/01/22**
Exam 7 Protocol Development	06/01/21	11/08/21
Draft Consent Form	09/01/21	11/08/21
Finalize Questionnaires	10/01/21	11/30/21
Participant Results Letters	01/15/22	08/03/22
Manuals of Operations	01/15/22	02/15/22
Program data collection software	11/01/21	08/09/22
Central Training Preparation	12/01/21	01/14/22
Hire Technicians	12/01/21	03/01/22
Order retention gifts	12/27/21	12/31/21
Laptops to sites	01/18/22	01/26/22
Order biospecimen labels	01/18/22	01/28/22
Central Training	01/18/22	02/04/22
Mail Participant Newsletter	02/14/22	03/04/22
Training/Certification	12/01/21	04/15/22
Translations	01/24/22	09/01/22
Establish Remote Consent Protocol	01/31/22	04/22/22
sIRB Submission	12/15/21	05/16/22
Local IRB/Radiation review/consent	*12/15/21*	*04/14/22*
UW sIRB ICF/Site Activation (Pilot)	*02/24/22*	*05/03/22*
UW sIRB ICF/Site Activation (Exam 7)	*04/01/22*	*05/16/22*
Exam 7 Pilot	03/24/22	06/17/22
Implement changes based on pilot	*04/18/22*	*06/10/22*



A general timeline for the Exam 7 time period is provided in Table 3.

Table 3: Timeline for MESA Exam 7

Exam 7	05/06/22	03/29/24
Recruit, informed consent and exam	05/06/22	03/29/24
Exam Visits	*05/06/22*	*03/29/24*
Task B Exam Dates	05/20/22	11/21/23
Task B Extenstion	11/22/23	03/29/24
FC Start Dates	05/06/22	06/20/22
Site Visits	*05/24/22*	*08/03/23*
Data entry and verification	*05/06/22*	*03/29/24*
Conduct QA/QC activities	*05/06/22*	*03/29/24*
Process and ship biosamples	*05/06/22*	*03/29/24*
Staff training and Recertification	*05/06/22*	*03/29/24*
Report participant results and alerts	*05/06/22*	*03/29/24*
Mail Participant Newsletter	*07/01/22*	*07/18/22*
Mail Participant Newsletter	*07/01/23*	*07/18/23*
Exam 7 Closeout (Core)	01/02/24	03/29/24
Prepare and release Exam 7 dataset (Core)	01/02/24	03/29/24



3.5. MESA Population

The MESA cohort was aged 45-84 in 2000-2002 and recruited from the following source populations:

- Wake Forest: The resident population of Forsyth County drawn from North Carolina Division of Motor Vehicles list, supplemented with voter registration and consumer lists.
- Columbia: Local 1199 National Benefit Fund (NBF) members, retirees, and their spouses residing in 18 contiguous zip codes of Northern Manhattan and the Bronx, drawn from a listing of all age-eligible, members, retirees, and their spouses; and other residents recruited by random dialing into the same set of zip codes.
- Johns Hopkins: Residents of a series of census tracts that run along the rapid transit line from Johns Hopkins University to the Western suburbs of Baltimore County drawn from lists of dwellings obtained from a commercial mailing service.
- Minnesota: Residents of four contiguous census tracts in the southern part of the city of St. Paul drawn from a list of dwellings provided by the county assessor's office and listings of Hispanic members of a local church.
- Northwestern: Residents of community Areas 6, 8, 34, and 60 in the city of Chicago drawn from census data compiled and maintained by the city of Chicago Department of Planning and Development and commercial lists.
- UCLA: Residents in Los Angeles County within a 15-mile radius from the UCLA Medical Center drawn using random dialing of telephone exchanges corresponding to census tracts in Los Angeles

County within a 15-mile radius from the UCLA Diabetes Center.

At all sites, lists were obtained from Centers for Medicare and Medicaid Services to increase the number of available older people. Near the end of the recruitment period, all sites also were allowed to recruit eligible friends and family members of enrolled participants (n=390). Recruitment included a phone screening questionnaire and brochure distribution. Exclusions included age or ethnicity ineligibility; previous CVD, cancer, or other serious medical conditions; current pregnancy; weight over 300 pounds (a requirement for CT scanning); cognitive impairment; or plans to move in the next five years. Recruitment aimed for balance by age groups.

The final cohort at baseline (n=6814) included 2,624 (39%) Caucasians, 1,894 (28%) African Americans, 1,493 (22%) Hispanics, and 803 (12%) Chinese Americans. The age distribution was 45-54 (n=1,947, 29%), 55-64 (n=1,885, 28%), 65-74 (n=2,017, 30%), 75-84 (n=965, 14%).

As of October 2021, the average age of active participants is 78.5, ranging from 64-105 years. The median age is 77 years.

3.6. MESA Field Center Clinics

Wake Forest University, Winston-Salem, NC: The primary clinic facility for examinations and interviews will be located at the Clinical Research Unit on the main Wake Forest Baptist Medical Center campus. The CRU has a reception area, nine examination rooms, a procedure room, and facilities to process, store, and ship laboratory samples. It also has a dining area and food services for study participants. If required, CT and MRI will be done in Research Radiology scanners also in the main Medical Center. Participants will park in the CRU parking lot or in the hospital parking deck. All areas of the medical center involved in the exam are within easy walking or wheelchair distance of each other.

Columbia University, New York, New York: Exams will take place at the CTSA of Columbia University Medical Center. The CTSA has a reception area, 8-10 examination rooms, a processing/shipping laboratory, and food services for study participants. If required, CT and MRI will be in separate areas on the main campus. CT will be in New York-Presbyterian Hospital; MRI will be in the Neurological Institute. If needed, additional research space at the medical center assigned to General Medicine is also available.

Johns Hopkins University, Baltimore, MD: The clinic exam and spirometry will occur in the cardiology division on Blalock 5 and may also include the Clinical Research Unit (CRU), part of the CTSA at Johns Hopkins Hospital. The brain MRI, brain PET, and lung CT examinations will take place in the Radiology Department at the Johns Hopkins Hospital, which is in the same vicinity as the cardiology division. The MESA field center staff are located in Fells Point, Baltimore, which is around 1 mile from the Hospital.

University of Minnesota, St. Paul, MN: The clinic exam will take place at the University of Minnesota Epidemiology Clinical Research Center in Minneapolis, which is about 10 miles from the MESA study community. Brain MRI exams will take place at the University of Minnesota Center for Magnetic Resonance Research, located approximately 2 miles from the clinic. Lung CT exams will take place at the University of Minnesota M Health Fairview Medical Center, within approximately 1 mile of the clinic.

Northwestern University, Chicago, IL: Participants will have the clinic exam at the Northwestern Department of Preventive Medicine Research Clinic. Following completion of the physical examination, questionnaires, and phlebotomy, a staff member will escort participants to Northwestern Memorial Hospital and the Research MRI Center for their examination (if required), which may be scheduled with a separate appointment.

University of California Los Angeles, Los Angeles, CA: The Examination will be conducted at the research clinic in Alhambra. If required, CT and MRI will be in the Radiology Department at the UCLA campus (25 miles from the clinic). Participants will park at the clinic and be transported by research center van to the Radiology Department and then back to the clinic after completion of the procedures.

4. Human Subjects Considerations

4.1. Informed Consent and Assent

Reliance agreements are established between the University of Washington sIRB and the MESA Field Center Recruitment Site IRBs. With approval by the single IRB for the MESA, written informed consent will be obtained from all study participants. Study participants will meet with a research coordinator by phone or in person to review the exam components in detail. They will be given information about the study both verbally and in easily understandable printed materials. Study information will also be posted on the MESA website. MESA investigators and staff will review potential risks and benefits, individualizing as applicable for each potential participant. After sufficient time to review the MESA study information, as well as ask questions and receive answers about the study, the potentially eligible study participant will be asked to sign and date the consent form indicating their understanding and willingness to participate. Site investigators will be available to answer any questions or provide additional information to prospective study participants.

Prior to Exam 7, MESA MIND established protocols to monitor and assess capacity to consent and obtain written informed consent in accordance local IRBs and state policies at each Field Center. MESA participants with a demonstrated lack of capacity to consent can only assent to participate in MESA MIND and Exam 7 until written authorization is received from a legal authorized representative (LAR) or caretaker, depending upon the state requirements of each Field Center. MESA participants with a lack of capacity to consent include: 1) participants with a prior clinical or research diagnosis of dementia and 2) any participant suspected to be confused or unable understand what is being asked of them during the consenting process. For those without a previous diagnosis of dementia, capacity to consent is ascertained by the University of California, San Diego Brief Assessment of Capacity to Consent (UBACC). Should a participant fail the UBACC, a lack of capacity to consent is established. Incapacitated participants may assent but cannot complete the exam without consent from a LAR or caretaker according to state requirements of each Field Center.

Assent to participate in Exam 7 is required from participants with a clinical or research diagnosis of dementia. This assent is reinforced by the LAR's consent. The participant will review and sign either a consent or Assent Form (based on local Field Center policy), which is a shortened version of the full Informed Consent Form. The LAR will sign the full MESA Consent Form.

The University of Washington single Institutional Review Board will review and approve the MESA Informed Consent Form along with participant-facing materials, the study protocol, and all amendments or changes. The MESA study will comply with the Declaration of Helsinki.

4.2. Potential Risks and Benefits

4.2.1. Confidentiality

Per the MESA protocol, participant health records will not be shared directly with those not involved in participants' clinical care or MESA, except as required by law. Records relating to this study will be kept confidential, and all Personal Health Information (PHI) data will be protected according to the expectations under the HIPAA Privacy Rule. PHI will only be shared with additional consent from the participant. Only study identifiers will be used in study records. A password-protected, encrypted database linking the study identifier to the subject's identifiers will be stored separately at each field center. Only MESA personnel will have access to this database. Paper records, if any, will be stored in a locked cabinet in a secure room. Publication of general study results will not identify individual study participants.

The following groups will have access to participant information from the MESA study: research staff at the field center; Data Coordinating Center at the University of Washington. The following groups will have access to de-identified participant health records from the MESA study: the National Institutes of Health and their representatives; Institutional Review Board at the University of Washington.

MESA data are covered under a Certificate of Confidentiality agreement from the NIH (Section 2012 of 21st Century Cures Act as implemented in 2017 NIH Certificates of Confidentiality Policy). A Certificate of Confidentiality allows researchers to refuse to disclose identifiable research information in response to legal demands. Certificates are issued to researchers to help protect the privacy of human subjects enrolled in sensitive, health-related research. A description of the MESA is available on http://www.ClinicalTrials.gov. This website will not include information that can identify study participants or Personal Health Information. This website may include a summary of the study results.

Geocoded addresses (i.e., latitude/longitude of resident addresses) will be shared with MESA Air, MESA Lung, and MESA Neighborhood ancillary studies using an anonymized ID, void of any personal and health data. These will be used to obtain characteristics of participant neighborhoods, such as air pollution, availability of different kinds of stores, and layout of streets. Only the University of Washington will hold the key to reconnect these neighborhood characteristics to participant identifying information.

As described in more detail below, genetic information, particularly on a scale generated from genome-wide SNP arrays or whole-exome and/or genomic sequencing, is individually identifying, and the risks of re-identification of research participants from unauthorized access to their genomic information is a well-recognized potential privacy risk. Genetic information will only be released to researchers with a MESA-approved ancillary study or through NIH data repositories such as the Database of Genotypes and Phenotypes (dbGaP). These researchers will sign a data use agreement that includes a stipulation that prohibits attempts to re-identify study subjects.

RNAseq datasets derived from clinical research and funded by the NIH will be made available to investigators through a web portal called the Database of Genotypes and Phenotypes (dbGaP). Relevant datasets developed through the MESA will be submitted to dbGaP or similar internationally recognized data repositories. Qualified investigators will be able to receive de-identified genomic and phenotypic data from these dbGaP datasets. dbGaP has two access levels, open and controlled. Open data may be viewed by anyone. Controlled access is for downloading of de-identified participant-level data and requires pre-authorization. Data releases to investigators for approved research purposes and analyses will be stripped of personal identifiers.

Eligible researchers may make an application to view individual-level data submitted to dbGaP. These applications are co-signed by both the investigator and the signing official at the investigators' institution. These

requests will be reviewed by the appropriate NIH Data Access Committee at the appropriate NIH Institute or Center.

Submission of the data access request will constitute agreement and acknowledgment by both the PI and the institutional signing official to the terms of use for the specific dataset(s) requested, which are detailed in the "Data Use Certification" (DUC) documents that are provided on the dbGaP website. The DUC statements outline policies and procedures for using the data, such as limiting use to the project described in the Data Access Request form; not distributing the data beyond those permitted to handle it; not attempting to identify or contact study participants from whom phenotype data and DNA were collected; awareness of the specified principles regarding intellectual property; adhering to policies on the timeframe for publications stemming from the data; and other provisions designed to protect the confidentiality of study participants and to foster scientific advance.

Federal and State laws, including the Genetic Information and Nondiscrimination Act (GINA) which makes it illegal for health insurance companies, health plans, and employers to discriminate against individuals based on their genetic information, afford some measure of protection for participants in the event of unintended disclosure of genetic data.

4.2.2. Physical and Emotional Risks

The procedures used in this study are considered to be low risk and mostly related to discomfort. Incidental findings from exam procedures may have a major impact on participants' health. These findings may cause worry, additional medical testing, and, potentially, cost.

- **Blood Draw**: Risks of drawing a blood sample are discomfort at the site of needle insertion, bruising (black and blue discoloration) or inflammation at the site, and, rarely, faintness. Bruising, if it occurs, is usually painless and disappears within a few days.
- **Arterial stiffness measurement**: This procedure has no known risks. The test is not invasive and involves the same amount of pressure used to measure blood pressure taken with a cuff. Checking the neck artery pulse involves slight pressure.
- **Spirometry**: This lung test can sometimes cause coughing or dizziness. Very rarely, the dizziness may be severe but improves with resting or lying down. Occasionally after receiving the albuterol inhaler, a temporary sensation of "heart racing" and shakiness may develop. This will go away after a few minutes.
- **CT of the lung**: This procedure uses ionizing radiation, which is not necessary for participants' medical care and is for research purposes only. The radiation in this study is of low risk and not expected to measurably increase the risk of cancer.
- **Heart rhythm recorder**: In some people, the adhesive on the patch (Cardea SOLO) may cause skin irritation.
- **Brain MRI**: There are no known risks to having a brain MRI. Earplugs or earphones will be provided to wear during the test because the machine can produce loud noises, which may be uncomfortable. Some people may feel anxious in the scanner if they are claustrophobic.
- **Short Physical Performance Battery**: Risks of this test include shortness of breath and chest tightness, faintness, or heart problems, although they are rare.
- **Cognitive Assessments**: Repeated evaluations of mood and mental status may be slightly frustrating or produce fatigue and boredom.

- **Brain Amyloid PET**: The PiB "tracer" used to bind to amyloid are commonly used investigational drug in Alzheimer's research. [18F] Florbetaben (Neuroceq ®) is FDA approved. Because it will be given in trace amounts, there are no additional drug-related risks associated with it. A needle is used to inject the tracer into a vein in the arm. Insertion of the needle may cause pain or a stinging sensation at the injection site. On rare occasions, the insertion of a needle can cause bleeding, a blood clot, swelling, or infection at the site of insertion. Participants may also experience a tingling or stinging sensation when the compound is injected. Participants may be claustrophobic or uncomfortable lying still for 30 minutes during the procedure, or they may experience anxiety which could increase their blood pressure. PET imaging involves exposure to small amounts of ionizing radiation, which has no expected harmful effects in the doses used for this purpose. However, the possibility exists for a rare reaction to any of the substances or procedures to which participants are exposed.
- **Overnight Sleep Study:** Sleep monitoring may result in some discomfort when the electrode leads (wires) are placed on the head and face. The removal of the sensors may leave a small area of red skin that goes away within a few days. The sleep study equipment may feel awkward and be associated with some difficulty in sleeping during the night of the study.
- **Sleep monitor (wrist):** There is a minor risk of skin irritation from the wrist bands.
- **Activity Monitor (Hip).** Risks associated with wearing the hip activity monitor are minimal; however, participants may feel inconvenienced by wearing this device.
- **Continuous Glucose Monitoring:** Potential risks to these participants due to the proposed research are low. CGM systems are considered minimally invasive, involving a sensor probe placed in the subcutaneous tissue to measure glucose levels in the interstitial fluid. Risks primarily include infection at the device insertion site and skin irritation from the adhesive used to secure the device.
- **Ambulatory Blood Pressure Monitoring:** The 24-hour ambulatory blood pressure monitoring can be uncomfortable because of the repetitive inflation of the blood pressure cuff. Participants may feel discomfort from the blood pressure cuff on their arm when it inflates. During sleep, the cuff may cause sleep disruption. Very rarely, the pressure may damage a nerve in the arm.
- **The following procedures were assessed and do not have any foreseeable risk to participants, beyond the risks of loss confidentiality in the event of a data breech:**
    - **Seated blood pressure**
    - **Anthropometry**
    - **Urine collection**
    - **Collection of personal history/medical history/medications and other questionnaires.**
    - **Completion of home diaries and non-sensitive questionnaires**
    - **Neck measurement**
    - **Pulse oximetry**

Participation in research carries a theoretical risk for some people of being given information that they might prefer not to have heard (for example, some test results) or that may be difficult for them or their health care provider to interpret. This may lead to other tests, which MESA will not pay for.

4.2.3. Genetic risks

Even without personal health identifiers, genetic information is unique to each subject. There is a potential risk that a subject could be identified from genetic data generated by MESA. In addition, the genetic data has ancestry and health information, which the subject may wish to keep confidential. These risks may increase in the future as technologies advance, and more researchers study participant genetic information. The Genetic Information Non-discrimination Act is a federal law that prevents insurance companies from using genetic

information to deny health insurance coverage. The law also prevents employers from getting or using genetic information for employment-related decisions. However, the law does not prevent companies that provide life insurance, disability insurance, or long-term care insurance from using genetic information, and participants will be fully informed of this potential risk through the informed consent process.

4.2.4. Financial Risks

Participants and/or their insurance company(s) will not be charged for procedures related to study participation, including services, supplies, and procedures. They may receive reimbursement to cover travel and related expenses for study visits. The research staff will review the travel reimbursement policy with study participants at the Field Centers. In the unlikely event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment, and follow-up care as needed. If a participant suffers a complication, illness, or injury as a result of study participation, treatment will be provided through clinical services at the Field Center. Care for such injuries will be billed to the participant or their insurance company.

4.2.5. Potential benefits

Most participants in MESA will not benefit directly from MESA-related procedures. Rather, the benefits of the study will primarily accrue to society in general through the advancement of basic science and medical knowledge regarding cardiovascular disease. It is hoped that the knowledge gained from MESA will lead to knowledge from which future patients will benefit. This hope for future benefit justifies asking participants to agree to any risks from participating in MESA.

Although most participants are unlikely to benefit directly from MESA participation, there are some situations where a direct benefit is possible (described below). MESA investigators will review potential benefits as part of informed consent. However, the investigators will avoid exaggerating or unduly emphasizing these potential benefits because doing so could exacerbate therapeutic misconception on the part of potential participants. As the consent forms will make clear, the decision to participate in MESA should not be influenced by an expectation of direct benefit.

MESA participants will receive results of some standard clinical laboratory values, such as lipid profiles, eGFR, and glucose. In addition, participants will receive results or summaries from exam components they choose to complete, such as lung function testing, activity monitoring, CTs, or MRIs. These sources of information may help increase some participants' understanding of their health.

Additional knowledge may be gained from the research analyses of MESA data that could identify causes of the diseases studied by MESA and improve the treatment. However, this knowledge is not expected to benefit MESA participants directly. MESA does not intend for research results to become part of a subject's medical record.

5. Exam 7 Ancillary Study Aims

The MESA contract supports only the core components of the seventh exam. The innovative scientific measures from Exam 7 are supported by grant-funded Ancillary Studies secured by MESA investigators. A summary of the funded Exam 7 Ancillary Studies is provided below.

5.1. MESA-MIND

The Multisite Study of the Vascular Contributions to Alzheimer's disease: The MESA Multisite AD study (PIs:

Timothy Hughes, PhD, Kathleen Hayden, PhD, Jose Luchsinger, MD)
Sites: All Field Centers

Without a means to prevent dementia, the incidence of Alzheimer's disease (AD) is projected to triple in the coming decades. Vascular and metabolic disorders are proposed as potent modifiable risk factors for the development of AD and related dementias, and may provide key pathophysiologic targets for therapeutic intervention. Investigators of this study propose to leverage: (1) brain imaging data and cognitive data obtained from two NIH funded MESA ancillary sub-studies (Heckbert A253 and Hughes A301) and (2) MESA's unique and extensive cardiovascular, metabolic, genomic and transcriptomic data to conduct a thorough assessment of cognitive function and dementia-related pathology using MRI and PET imaging. The first assessment (Visit A, 2019-2021) targeted number of 2,500, equaling 75% all participants (n=3,300) who attended Exam 6 to complete: clinic visits, detailed cognitive testing and brain MRI at all sites. The Visit A MRIs are coordinated with Dr. Heckbert's A253 MRI exam (2017-2018). Now at Exam 7, investigators will reassess cognitive function and collect additional MRIs 3 years later in 2022-23 (Visit B) to examine the trajectory of cognitive decline, cerebrovascular disease and brain atrophy. Assessment of brain amyloid with amyloid PET is conducted at three sites and continues across Visit A and Exam 7. Following cognitive testing, participants are adjudicated for cognitive status, including Mild Cognitive Impairment (MCI), dementia, and their various subtypes. This work provides a rich infrastructure of neurocognitive and neuroimaging data collection in MESA that will be related to subclinical vascular and metabolic measures collected in MESA to better define the vascular contributions to dementia. They measures can also be related to other outcomes in ongoing studies in MESA, a framework for additional ancillary studies, and novel data and manuscripts pertaining to brain aging and dementia risk among underrepresented racial/ethnic groups not already included in current dementia and AD cohort studies.

5.2. MESA 24h-ACT

Leveraging the 24-hour movement paradigm to preserve cognitive function and prevent Alzheimer's disease: The Multi-Ethnic Study of Atherosclerosis (MESA) 24H-ACT Study (PIs: Priya Palta, PhD, MHS, Keith Diaz, PhD, and Kelley Pettee Gabriel, PhD, MS)
Sites: All Field Centers
Linked to: MESA-MIND

The overall goal of the MESA 24H-ACT Study is to quantify the impact of physical activity and sleep via the 24-hour movement paradigm to preserve cognitive function and prevent Alzheimer's disease. Participants who meet eligibility criteria and agree to participate (estimated n=2,125) will be included. As part of MESA 24H-ACT, we propose to augment MESA with an 8-day activity monitor (hip) and sleep actigraphy protocol to objectively measure the 24-hour activity cycle (sedentary behavior, light intensity physical activity, moderate-vigorous intensity physical activity, and sleep). To accomplish these goals, this study will examine the associations of 24-hour activity cycle behaviors, ascertained via device-based measures, cross-sectionally with cognition; markers of cerebrovascular disease, neurodegeneration, and amyloid burden; and the prevalence of MCI and dementia (AIM 1); examine self-reported 24-hour activity cycle behaviors with prospective changes in global cognitive function, cerebrovascular disease and neurodegeneration (AIM 2); and elucidate the bidirectional relationship between impaired cognitive function and 24-hour activity cycles over 12 years. MESA 24H-ACT will address current evidence gaps and provide empirical evidence to accelerate a paradigm shift towards an integrated model that incorporates all 24-hour movement behaviors to optimize brain health across racially/ethnically diverse populations.

5.3. MESA Glucose Homeostasis

Glucose homeostasis and cognitive decline: The Multi-Ethnic Study of Atherosclerosis (PI: Morgana Mongraw-

Chaffin, PhD, MPH, FAHA)
Sites: All Field Centers
Linked to: MESA-MIND

The research will improve understanding of the role that dysglycemia and type 2 diabetes (T2D) play in the development of cognitive function, decline, and Alzheimer's disease risk. Specifically, this proposal focuses on determining which aspects of dysglycemia increase cognitive risk, and related cognitive disparities by sex and race/ethnicity. To achieve this goal, investigators will add highly detailed, continuous measures of glucose regulation in the Multi-Ethnic Study of Atherosclerosis (MESA) to 1) Investigate the antecedent determinants of glucose homeostasis (from continuous glucose monitors at Exam 7 (n=2000) and change over two years (n=1000), 2) Determine whether continuous glucose markers of glucose homeostasis, dysglycemia, and change over time are associated with cognitive decline, incident cognitive impairment (including MCI and Alzheimer's), and Alzheimer's and ADRD biomarkers, and 3) Investigate the heterogeneity in Alzheimer's disease and related dementia pathologies by sex and race/ethnicity. Findings from this research will identify new mechanisms for the development of Alzheimer's disease and Alzheimer's disease related dementias, discover new primary and secondary prevention targets, and have the potential to change clinical care.

5.4. MESA Neighborhood

MESA Neighborhood Study III: Neighborhoods and Aging (PI: Jana A. Hirsch, PhD, MES)
Sites: All Field Centers
Linked to: MESA-MIND

Rising prevalence of Alzheimer's Disease and related dementias (ADRD) necessitates research to identify points of intervention to slow cognitive decline. Given known disparities in ADRD risk across racial and ethnic groups, it is critical that research examine diverse populations and identify sources of disparities. Beyond the role that individual factors (e.g. age, race, gender, and socioeconomic status) play in the progression of ADRD, neighborhood factors (e.g. social and built environments) may explain prevalence of and disparities in cognitive health. This study, *MESA Neighborhoods III,* extends previous multi-level, neighborhood research from the first two ancillary studies. In contrast to the first two neighborhood studies which focused on cardiovascular risk, *MESA Neighborhoods III* will focus on aging and cognitive decline. Specifically, the main *objective* is to identify unique patterns of neighborhood change related to the causes of prevalence and disparities in cognitive decline and dementia. Following the protocol used in the previous MESA neighborhood studies, investigators will collect and process GIS-derived social and built environment neighborhood-level variables and link them to deidentified geocoded addresses through Exam 7. Investigators will also administer surveys during Exam 7 to collect survey-based scales of neighborhood variables previously collected during Exams 2/3 and Exam 5. Investigators will then analyze this longitudinal dataset to identify environmental determinants that increase older adults' risk of cognitive decline and dementia. Investigators will quantify and investigate predictors of disparities in cognitive decline and dementia by socioeconomic position and race/ethnicity.

5.5. MESA Sleep

Longitudinal Relationships Among Sleep, Cognition and Alzheimer's Disease Biomarkers: Discerning Causal Associations, Mediators, and Susceptibility (PIs: Susan Redline, MD, MPH and Shaun Purcell, PhD)
Sites: All Field Centers
Linked to: MESA-MIND

As the population has aged, there has been a staggering increase in the prevalence and morbidity of cognitive

impairment and Alzheimer's disease (AD) related dementias (ADRD): it is estimated that by 2050 88 million people will have ADRD and associated health care costs will exceed $1.1 trillion annually. Combinations of genetic, lifestyle, and environmental factors appear to increase risk for ADRD through age-related changes in neuronal processes that lead to progressive accumulation of amyloid-beta (Aß) and tau. Recent research indicates that healthy sleep and circadian physiology are critical for brain health; and conversely, that disturbed sleep and circadian disturbances may accelerate Aß accumulation and cognitive decline. Although sleep and circadian rhythms become increasingly disturbed with aging, there is large inter-individual variation. Disturbed sleep also contributes to vascular disease, including vascular stiffness and nocturnal blood pressure. Thus, individual differences in sleep/circadian disturbances may increase the prevalence of age-related ADRD risk factors as well as directly contribute to ADRD risk. Thus, there is large potential for using metrics of sleep physiology as ADRD biomarkers to improve identification of at-risk individuals, as well as for targeting modifiable sleep behaviors and physiological processes (sleep, 24 blood pressure) as interventions for preventing or attenuating age-related cognitive impairment. MESA Sleep will leverage the comprehensive sleep phenotyping we performed from 2010-2013 (MESA-SLEEP; Exam 5), along with ongoing and newly proposed data to be collected in the MESA MIND study, which includes state-of-the-art cognitive, neuropsychiatric, and brain imaging studies in a sample of MESA participants studied at 2 time points 2.5 years apart (2019-2023) to efficiently and uniquely address critical research gaps. While the MESA MIND study addresses the role of mid- and later-life vascular disease as a risk factor for ADRD, it does not address the potential contributory or mediating roles of sleep and circadian disorders. Exam 7 components include overnight state-of-the-art polysomnography, 8-day actigraphy, and 24-hour blood pressure recordings, aiming to recruit 1800 of the 2000 participants targeted for that examination. Investigators will generate advanced quantitative metrics of sleep and circadian rhythm to characterize the evolution of sleep disturbances over critical aging periods. These measurements will be incorporated into longitudinal assessments of cognition in order to define the temporal associations between sleep disturbances and cognitive impairment and thus define which sleep disturbances and metrics are antecedent factors for cognitive impairment. Investigators will evaluate whether sleep measured 8-10 years prior to brain imaging, as well as trajectories of sleep change, predict neurodegeneration, cerebral vascular disease, and AD brain biomarkers. Investigators will determine whether associations differ in men and women and individuals of different race/ethnic backgrounds. Investigators also will assess the role of nocturnal hypertension as a mediating pathway linking sleep disturbance and cognition/AD susceptibility. The study has a large potential impact given that sleep disturbances and nocturnal hypertension are modifiable targets. The study also will inform gender-appropriate risk stratification approaches.

5.6. MESA Lung

Precision phenotyping of emphysema in the elderly: The MESA Lung Study (PI: Graham Barr, MD, DrPH)
Sites: All Field Centers

Chronic obstructive pulmonary disease (COPD) and emphysema are, jointly, the third leading cause of death in the United States. COPD prevalence and mortality are increasing, particularly among women and minorities. While the natural history and major risk factors for COPD, defined by spirometry, are reasonably well defined, that of emphysema, and their interaction, is less clear.

The MESA Lung Study found that emphysema, assessed on computed tomography (CT) as percent of emphysema-like voxels in the lung greater than the upper limit of normal is common among older adults in the general population, usually occurs in the absence of spirometric COPD, is a strong correlate of cardiac function, and predicts all-cause mortality independently. In subsequent large-scale unsupervised machine learning of emphysema-like voxels on ~3000 CT from heavy smokers in SPIROMICS, we identified six robust CT emphysema subtypes. Three were equally common in MESA Lung and corresponded, colloquially, to classic

but much less precise descriptions of diffuse (or generalized) emphysema and senile emphysema, in addition to a new obstructive fibrotic emphysema. How these new CT emphysema subtypes co-occur with COPD and progress among the elderly is unknown.

Investigators therefore propose to use novel CT measures among 1800 returning MESA participants in MESA Exam 7 and collect hair samples, nasal brushings and Paxgene tubes to test the following aims: 1) diffuse emphysema is progressive, with high mortality, whereas senile is benign and obstructive fibrotic emphysema is distinct; 2) CT emphysema subtypes have differing environmental risk factors; 3) gene expression profiles of lung-relevant tissue vary between different CT emphysema subtypes.

5.7. MESA Stress Reactivity

Stress reactivity and cognitive decline (PI: Kiarri Kershaw, PhD)
Sites: All Field Centers
Linked to: MESA-MIND

The way people respond to stressful situations (i.e., stress reactivity) varies widely due to differences in environments, preferences, and constraints, but this is rarely accounted for in epidemiologic of stress and health. The bulk of evidence relating stress reactivity to healthy aging come from animal studies and experiments in humans using laboratory stressors. This is a major limitation because laboratory stressors cannot capture the variety, severity, or duration of stressors that individuals face in their daily lives. In this study, we will add to the literature on stress reactivity and cognitive decline by adding more objective indicators of stress reactivity to a longstanding community-based cohort, the Multi-Ethnic Study of Atherosclerosis (MESA). Our overall goal is to use these measures to examine associations of stress reactivity with cognitive impairment and risk of Alzheimer's disease and related dementias in a natural setting. We will achieve this goal by leveraging a wealth of available data on transcriptomics, cognitive function, and biomarkers of Alzheimer's disease severity (Aβ deposition, cerebral small vessel disease, and neurodegeneration) as well as planned data collection efforts through the MESA-MIND ancillary study. Given the higher burden of both stressful experiences and cognitive impairment in racial/ethnic minorities and low-SES individuals, understanding the relationships between stress reactivity and dementia risk has the potential to elucidate mechanisms underlying disparities in dementia risk.

6. Exam 7 Study Visits

An outline of the planned examination and rationale is provided in the following sections.

A single Exam 7 consent form will include study procedures for both the core and ancillary study components. Informed consent and permission to release medical information will be obtained either in clinic or by phone/video conference. Consent is documented by a mailed signature returned to the Field Center or e-signature documented in the data collection software.

Clinic schedules will be tailored to the needs of participants and the arrangements of the clinics. See additional details in section 6.3.

- Blood pressure, anthropometry, oximetry, and urine collection will be measured in a fasting state, before phlebotomy.
- All participants will be scheduled fasting, with initial blood samples to be drawn before 10:00 AM.

The examination will start in May 2022 and will be completed in 22 months.

6.1. Table of Exam 7 Components by Core or Ancillary Study

A brief summary of the funded Exam 7 Core and Ancillary Studies Components is provided below in Table 4. All six Field Centers are included in all ancillary studies. All participants are selected for all ancillary studies unless they meet study-specific exclusion criteria.

Table 4: Exam 7 Components

Title	PI	Components (time)	Total Time	Blood draw/ volume	Radiation
Core	McClelland, Robyn	Consent/Check-in (20m) Seated Blood Pressure (12m) Anthropometry (10m) Phlebotomy/Urine (15m) Medical History (5m) Personal History/Demographics (10m) Medications Inventory (10m)	90 min	70 mL total includes core and all Ancillary Studies.	none
MESA-MIND	Hughes, Timothy	Consent (Core) Informant info (2 min) Anthro, blood pressure (Core) Blood draw (Core) Arterial stiffness (20min) Meds, MedHx (Core) UDS physical exam and SPPB (30 min) Cognitive testing (60 min) Brain MRI (60 min) Brain amyloid PET (120min) Informant interview (no ppt burden)	250 min for all 340 with amyloid PET	26 mL (part of 70 mL total)	3.46 mSv for PiB or 0.646 rem for Florbetaben in up to 600 participants (JHU/COL/WFU)
MESA 24-H ACT Study	Palta, Priya Diaz, Keith Gabriel, Kelley	Consent (Core) Phlebotomy (Core) Activity monitor (hip) (8 days during all waking hours) Sleep monitor (wrist) (8 days during all sleeping hours, or 24 hours)	30 minutes in clinic 30 minutes at home (includes ~3-4 minutes/day to put on/take off activity monitor and fill out wear/sleep and nap logs)	7 ml (from MIND, part of 70 mL total)	none

Title	PI	Components (time)	Total Time	Blood draw/ volume	Radiation
		Daily wear, sleep, and nap logs (20 min of instruction time).			
MESA Glucose Homeostasis	Mongraw-Chaffin, Morgana	Consent (Core) Monitor instructions and application (25 min, wear 7-14 days) At home diary 2 years later (n=1000): Repeated monitor/diary/cognitive testing	25 minutes in clinic 210 minutes at home 2 years later: 210 minutes at home 30 minutes at home for abbreviated remote cognitive testing (only if MESA-MIND is not renewed)	3ml (part of 70mL total)	none
MESA Neighborhood III	Hirsch, Jana	Consent (Core) Questionnaire (25 min)	25 minutes	none	none
MESA Sleep	Redline, Susan; Purcell, Shaun	Consent (Core) Home visit consisting of overnight sleep study (polysomnography) and sleep monitor (wrist) for 8 days Questionnaire (125 ~~5~~ min) Neck measurement 24 hour ambulatory BP monitor At home sleep diary	5 minutes in clinic 90 minutes at home for initial home visit (includes PSG setup, BP cuff measurement, monitor use instructions~~, and questionnaires~~) 24 hours PSG (concurrent with first day of wrist sleep monitor) 24 hours activity monitor (hip) for 8 days 24 hours ABPM (concurrent with last day of wrist sleep monitor)	none	none

Title	PI	Components (time)	Total Time	Blood draw/ volume	Radiation
MESA Lung IV	Barr, Graham	Consent (Core) Blood and urine (Core) Environmental Exposures Questionnaire (15 min) Spirometry exclusion/completion (5 min) Pre-bronchodilatory spirometry (15 min) Post-bronchodilator spirometry (5 min) Pulse oximetry (Core) Non-contrast Lung CT (30 min) Nasal brushing (5 min, N=1000, select all) Hair collection(10 min, N=1350, select all) PAXgene tube (n=300)	86 min	2.5 ml (part of 70 mL total)	3 mSv
MESA Stress Reactivity	Kershaw, Kiarri	Consent (Core) Heart Monitor Patch Application/Instructions (30 minutes, 7 days wear time) Daily Stress Questionnaire (15 minutes for 7 days)	30 min in clinic plus 15 min daily for 7 days	none	n/a

6.2. Description of Exam 7 Components by Core or Ancillary Study

6.2.1. Core Exam Components

- **Blood Pressure:** Resting blood pressure will be measured preferably in the right arm after five minutes in the seated position. An automated oscillometric method (Dinamap) and appropriate cuff size will be used in clinic. Three readings will be taken; the second and third readings will be averaged to obtain the blood pressure levels used in analyses. Home visits will use a mobile blood pressure device (OMRON 10).
- **Anthropometry:** Height and weight will be measured to the nearest 0.1 cm and 0.5 kg respectively. Body mass index (kg/m2) will be used a measure of overall obesity. Girths (neck, waist at the umbilicus and hips at the maximal circumference of buttocks) will be measured to the nearest 0.1 cm using a steel measuring tape (standard 4 oz. tension).
- **Pulse Oximetry:** Resting oxygen saturation will be measured in the seated position using a pulse oximeter with a finger probe. Nail polish will be removed if necessary. Oximetry will be measured off supplement oxygen if used. For participants who use supplement oxygen, supplement oxygen will be restarted immediately if they are short of breath or their oxygen saturation drops below 82%.
- **Laboratory Measurements**: A maximum of 70mL of blood and spot urine will be collected (which includes all core and ancillary studies). Core measurements will include a lipid profile, glucose, creatinine, insulin, HbA1C, and urinary creatinine and albumin. White cells may also be cryo-preserved for future generation of cell-lines and isolation of DNA needed for genetic studies. If samples are lost or damaged, participants will be invited to repeat the biosample collection on a different day.
- **Questionnaires**: Standard questionnaires (similar to those at prior MESA exams) will be used to collect information about demographics, socioeconomic and psychosocial status, physical function, medical, ~~and~~ family, and sleep history, medication use, dietary and alcohol intake, and smoking.

6.2.2. MESA-MIND Exam Components

- **Additional Blood Collection:** A total of 26 mL will be collected for future assay of established and evolving AD biomarkers. The 26 mL of blood will be used to add novel biomarkers from plasma exosomes and pericyte function. This is included in the maximum blood draw volume of 70 mL.
- **Arterial Stiffness Measures:** Assessment of arterial pulse waves and pulse wave velocity will be performed using a brachial and leg cuff-based approach (with a simultaneous, automated recording of the cuff blood pressure) using a Fukuda VaSera.
- **Cognitive Function Testing:** Cognitive tests administered at MESA Exam 5 and repeated for Exam 6 and MESA-MIND Visit A (2019-2021) - which included the Cognitive Abilities Screening Instrument (CASI), Digit Symbol Coding (DSC), and the Digit Span Test (DST) – will be repeated again in 2022-23 for MESA Exam 7 (2022-2023). The reassessment will allow for examination of cognitive trajectory and whether cardiometabolomic and AD biomarkers predict incident MCI and dementia. The

measures will be supplemented by the standardized set of measures (Uniform Data Set; UDS version 3) that are collected nationally and longitudinally by all Alzheimer's Disease Centers to characterize mild AD, MCI and vascular dementia. The UDS includes the following tests: Montreal Cognitive Assessment (MoCA: a brief test of global cognitive function that assesses attention, working memory, short-term memory, language, and visuospatial ability), Story Recall (participants hear a short story and are asked to recall the details immediately after it is read and again 20 minutes later), Benson Complex Figure Test (participants view a complex figure and are asked to draw the figure from memory immediately after exposure and again 20 minutes later), Verbal Fluency (participants list as many words as possible in 60 seconds that begin with a particular letter of the alphabet or that belong to a particular category, such as "animals"), and a clinician-administered physical exam. Frequent breaks will be provided as needed, and participants will be informed that testing can be discontinued at their request. All cognitive assessments will be administered by trained, certified staff under the supervision of ancillary study investigators. Cognitive test administration will be audio-recorded for random quality control checks to correct drift. Participants may complete cognitive assessments in-person, by video call, or by telephone (if in-clinic or video assessments are not possible). Participants with clinical or research diagnosed dementia will not have cognitive function testing.

- **Clinician's Physical Examination:** A clinical neurological exam will be administered by a trained nurse, nurse practitioner, physician's assistant, clinical fellow, a coordinator with medical training, or medical doctor. It will assess basic neurological signs and symptoms of stroke and Parkinsonism.
- **Short Physical Performance Battery (SPPB):** The SPPB is a group of measures that combines the results of gait speed, chair stand, and balance tests in order to assess the lower extremity functioning in older persons.
- **Brain MRI: :** MRI data will be acquired in accordance with multisite protocols including ADNI2, and the NINDS Common Data Elements recommendations. All images will be acquired on a 3T Siemens scanners with a high resolution 20-channel head/neck coil. Sequences include: T1 and T2 (for morphology), T2 FLAIR (to quantify white matter hyperintensities), DTI (to assess microstructural integrity of the white matter), BOLD/fMRI (for resting state brain connectivity), pseudo-continuous arterial spin labeling (pcASL, for quantification of cerebral blood flow), and susceptibility-weighted Images (SWI). The brain MRI protocol is consistent with the Atrial Fibrillation Study MRI protocol.
- **Amyloid PET Imaging:** The subset of study participants at Wake Forest, Columbia and Johns Hopkins Field Centers who agree to participate in the optional CT/PET scans will receive amyloid imaging with low-dose CT for attenuation correction. Fields Centers will use either the [11C] Pittsburgh Compound B (PiB) or [18F] Florbetaben (Neuroceq ®) PET tracer. The total radiation exposure of the PET scan with PiB is approximately 0.347 rems, which includes exposure associated with the head CT completed prior to the PET. For Florbetaben, total radiation exposure is approximately 0.646 rem. Participants will be monitored from the time of tracer injection until after the imaging session is complete for

signs of rare adverse events. Participants who receive the PiB tracer will also be contacted 24-72 hours after the procedure by telephone to inquire about adverse events. Participants with clinical or research diagnosed dementia will not have amyloid PET imaging.

6.2.3. MESA 24H-ACT Exam Components

- **Sleep Monitoring**: A wrist sleep actigraphy device (GeneActiv) will be used to determine sleep duration and quality. Participant instruction and distribution of the wearable devices will occur at the end of the participant's MESA Exam 7 visit, at a home or remote visit. Participants will receive verbal or written instructions and a package that includes the wrist sleep monitor, detailed written wear instructions, a tracking log, and contact information. The monitor will be programmed and standardized by staff at the clinic prior to distribution and initializing data recording. The wrist sleep monitor will be worn for the entire 24-hour period and simultaneously with the activity monitor (described below) for the duration of 8 days. Three days after receiving the device, study staff will call to confirm device wear, address any questions, and trouble-shoot problems. Participants will return the device and related materials to study staff using a pre-paid and addressed envelope or provide it to study technicians at a home visit after 8 days on the wrist during all sleeping hours. Participants may be asked to repeat the sleep monitoring if it captures less than 5 days of data or if another scientific question arises that could be answered with repeated activity monitoring.
- **Activity Monitoring:** The ActiGraph wGT3X-BT activity monitor (ActiGraph, Pensacola, FL) will be used to detect waking behaviors. Participant instruction and distribution of the hip activity monitor will also occur at the end of the participant's MESA Exam 7 visit (or provided at a home visit or mail), along with the wrist sleep monitor. The device would similarly need to be initialized to begin data collection by staff at the clinic using ActiLife6 software prior to device distribution. The hip activity monitor will be worn simultaneously with the wrist sleep monitor (described above) for the duration of the 8 days. Three days after receiving both devices, study staff will call to confirm device wear, address any questions, and trouble-shoot problems. Participants will return the device and related materials to study staff using a pre-paid and addressed envelope or provide it to study technicians at a home visit after 8 days on the hip during all waking hours. Upon receipt of the hip activity monitor, study technicians will download the data using ActiLife6 software and fully charge the battery prior to distributing the device to a new participant. Participants may be asked to repeat the activity monitoring if it captures less than 5 days of data or if another scientific question arises that could be answered with repeated activity monitoring.
- **Sleep and Nap Tracking, including Prompts for Daily Wear:** Each day in the tracking log, participants will take 3-4 minutes to record the times corresponding to when the wrist sleep monitor was replaced with the ActiGraph (waking periods), and when the hip activity monitor was replaced with the wrist sleep monitor (sleep periods). Participants will also record the clock-times corresponding to when s/he took any naps during waking

periods. This log will be integrated into the diaries used by other ancillary studies with wearables.

6.2.4. MESA Glucose Homeostasis Exam Components

- **Continuous Glucose Monitoring:** Participants will be consented and asked to wear a continuous glucose monitor (FreeStyle Libre Pro CGM from Abbott) for up to 14 days. The device may be distributed in clinic, during a home visit, or via mail along with video instructions. Calibration is not needed during wear time. Participants will then be asked to remove the CGM and mail it back to the study site using a prepaid envelope (or return it to study technicians during a home visit). If the CGM is lost or damaged, or if another scientific question arises that could be answered with repeated monitoring, participants may be invited to repeat the monitor. A subset of participants (n=1,000 focused on those with measured amyloid PET) will be asked to repeat the CGM data collection 2 years after Exam 7.
- **Diary Entry:** Participants will be asked to keep a paper diary of their waking, sleeping, and mealtimes. Diary instructions will be distributed along with the CGM. For the subset of participants asked to repeat the CGM collection 2 years after Exam 7 (2024-2025), diary tracking will also be repeated. This diary will be integrated into the logs used by other ancillary studies with wearables.
- **Cognitive Function Testing:** A subset of 1000 participants will be asked to repeat CGM and diary data collection 2 years after Exam 7. All participants complete an abbreviated cognitive assessment by telephone or video call in accordance with standardized and validated MESA-MIND protocols at the same time.

6.2.5. MESA Neighborhood Exam Components

- **Neighborhood Questionnaire:** Participants will be asked to complete a Neighborhood Questionnaire. The survey will include measures from previous MESA Neighborhood surveys (aesthetic quality, walking environment, availability of healthy food, safety, social cohesion, physical disorder, social disorder) and novel questions from PACER (Perceptions About Change in Environment and Resources) regarding neighborhood change and aging supports. The survey will take approximately 25 minutes to complete. The survey can be completed in any mode (in-person, tele-visit/remote, web-based, mail, or phone). Participants will be compensated for completing the survey.
- **Calculated Graphic Information System (GIS) measures using anonymized geocodes:** At no additional burden to participants, GIS measures will be collected and processed at Drexel University. Geocoded addresses (i.e. latitude/longitude of resident addresses) will be shared with MESA Neighborhood ancillary studies using an anonymized ID, void of any personal and health data. These will be used to obtain characteristics of participant neighborhoods, such as availability of different kinds of stores, and layout of streets. Only University of Washington will hold the key to reconnect these neighborhood characteristics to participant identifying information.

6.2.6. MESA Sleep Exam Components

- **Sleep Actigraphy Monitoring (Wrist):** Participants will be fitted for a wrist sleep monitor during the Exam 7 clinic visit or home visit, or mailed the device and given telephone instructions. Devices will be programmed before distribution. The wrist sleep monitor device (GeneActiv) will be worn on the nondominant wrist for 8 consecutive days to improve rhythm assessment and to overlap, as possible, both the night of the polysomnography study and 24-hour ABPM (ambulatory BP). The device looks like a wristwatch, has a patient-activated event marker, a rechargeable battery, and light sensor. These features improve the ability to annotate off times and sleep onset/offset and provide high quality light data to allow analysis of light cues in relationship to sleep-wake patterns. The device can be worn during showering and bathing but should be removed during prolonged periods of immersion (swimming). Instructions on returning the device with use of a pre-paid mailer will be provided (unless retrieved at the time of a study technician home visit). Once the watch is returned to the Field Center, a staff member will download the data using commercial software and electronically transfer the data files to the Sleep Reading Center (SRC) via a secure website. Sleep diaries will be concurrently scanned and sent to Brigham and Women's Hospital to be used when editing each record. Participants may be asked to repeat the sleep monitoring if it captures less than 5 days of data or if another scientific question arises that could be answered with repeated activity monitoring.
- **Sleep Diary Entry:** Participants will be instructed on completing a brief daily sleep log for 8 consecutive days (while using the wrist sleep monitor), when waking up daily, recording bed & wake-up times, recording perceived sleep latency, naps, any unusual events during the day or night, ratings of sleep quality, times when the wrist sleep monitor and BP cuff (first night only) is removed. This information will be used to edit the wrist sleep monitor data, provide subjective reports of daily variation in sleep patterns and identify factors that influence night-to-night variability. This diary will be integrated into the logs used by other ancillary studies with wearables.
- **24-hour Blood Pressure Monitoring:** The ABPM (OnTrak; Spacelabs), a lightweight portable device validated for ambulatory blood pressure (BP) measurement, will be applied for 24 hours on a non-dominant arm (unless there is a contraindication to use that arm, for example, related to lymphedema), typically at the end of the physical activity monitoring period, and overlapping with the last day of wrist sleep monitoring. Specifically, ABPM will overlap with the one night of wrist sleep monitoring but not with the home sleep study (polysomnography). After the initial period of in-home activity monitoring, the ABPM, programmed by the MESA research assistants before use and provided with fresh batteries, will be fit during a return home visit (when other equipment is retrieved). Appropriate cuff size will be determined by a trained research assistant after measuring the participants' mid-arm circumference. Cuffs will be secured, a marker placed to allow ease of replacement, and subjects instructed on how to remove them and check for placement. An initial automated BP recording will be made during placement; placement will be rechecked if the automated reading is >15% different than a manual reading. Participants will be instructed on how to carry the monitors using a

shoulder strap or waist band. Devices will be programmed to measure BP at 30 min intervals. Cloth covers for each cuff will be provided to optimize comfort. The ABPM will be retrieved by the research team during a home visit or returned by mail. Once the ABPM is returned to the Field Center, a staff member will download the data using commercial software and electronically transfer the data files to the Sleep Reading Center (SRC) via a secure website. Diaries will be concurrently scanned and sent to Brigham and Women's Hospital to be used when editing each record. If the study does not have sufficient data, or if another scientific question arises that could be answered with repeated ambulatory blood pressure monitoring, the participant will be invited to repeat the study.

- **Polysomnography:** Polysomnography (PSG) is the gold standard for objectively measuring sleep neurophysiology, sleep-related breathing abnormalities and sleep-related movements, providing information not available from wrist sleep monitor. The Nox Medical A1 PSG unit will be used for this home sleep study, which is fully compliant with American Academy of Sleep Medicine (AASM) standards. The device acquires high resolution signal quality through 32-bit signal processing and 256 kHz sampling on all channels and provides advanced noise reduction and anti-aliasing, thus facilitating generation of quantitative metrics. It is designed to be low burden (the bluetooth wrist oximeter has no wires, allowing unrestrained movement). The system is powered by a rechargeable battery and has 1 GB of data collection capacity per study. The montage includes airflow (via a nasal pressure cannula, DC-coupled for linearization), respiratory effort (high resolution thoracic and abdominal inductance plethysmography bands), finger pulse oximetry (Nonin Wrist-OX2 3150), ECG, bilateral frontal, central and occipital EEGs, EOG, and chin and tibialis EMGs. Should a home visit not be possible, a modified version of the NOX A1 device will be mailed or distributed to the participant at the end of the clinic exam for self-application. This modified version contains the same sensors other than the EEG, EOG and chin EMG gold cup electrodes. Instead, the device will have self-applied electrodes for sleep EEG assessment, which can be readily applied using verbal and written instructions and an optional instructional video.
- Methods for instruction and set up will be similar to what was used in the first MESA Sleep exam. Research assistants will review with the participant the purpose of the test, instructions on wearing study equipment, and then apply the sensors. After the home sleep study, the PSG unit will be retrieved by study personnel or courier. The research assistant will upload the sleep study (PSG) to a secure cloud platform using commercial software and accessed by SRC personnel for quality control analysis, standardized scoring, and off-line processing for quantitative phenotypes. To be minimally acceptable, a PSG study should have at least 4 hrs of continuous, predominantly artifact-free tracing on key channels. If sufficient data is not collected, or if another scientific question arises that could be answered with repeated polysmnography, participants may be invited to repeat the polysomnography.
- **Sleep Questionnaire:** All participants will complete a general Sleep Questionnaire at Exam 7. Those ~~with an in-home sleep study~~who consent to any MESA Sleep monitor (wrist activity, ABPM, or PSG) will complete an additional set of sleep questions ~~during the PSG study setup. It~~, including ~~includes~~ validated questions on sleepiness (Epworth

Sleepiness Scale; PROMIS Sleep Impairment Scale), sleep quality and insomnia symptoms (Women's Health Initiative Insomnia Rating Scale Index, Insomnia Severity Index), restless legs symptoms (International Restless Legs Screening Questionnaire), SDB symptoms and sleep duration/timing (SHHS Sleep Habits Questionnaire) and chronotype (a single item from Horne Ostberg and the UKB chronotype question). Thus, questions assess self-reported sleep duration, quality, sleepiness, snoring, circadian preference, and symptoms of sleep disorders (SDB, insomnia and restless legs syndrome).

- **Neck Measurement:** Research assistants will measure the participant's neck circumference also during the Exam 7 clinic visit or at a home visit during setup of the PSG study using a non-distensible tape measure placed below the laryngeal notch and perpendicular to the long axis of the neck.

6.2.7. MESA Lung Exam Components

- **Safety Forms:** Participants will be asked to complete a questionnaire to ensure safety of spirometry and, if selected, administration of albuterol.
- **Pre- & Post-bronchodilator Spirometry:** Spirometry consists of participants inhaling and exhaling as hard and as fast as they can through the mouth. Participants will also be asked to breathe in and out slowly through the mouth. These actions will be repeated at least three times to ensure valid readings. The setup and procedures will take approximately 15 minutes to complete. For in-person clinic visits, a SensorMedics model 1022 rolling-barrel spirometer will be used for all readings, and the procedure will follow American Thoracic Society guidelines. Home visits will use a portable pocket spirometer. device. A new mouthpiece will be used for each volunteer. Participants with airflow limitation (prebronchodilator FEV1/FVC ratio <0.70 or <LLN) will receive two inhalations of 90 mcg albuterol via MDI and spacer, and will then repeat the spirometry test ("post-bronchodilator spirometry") for up to an additional 5 minutes. Lung function measured by spirometry is a specific, quantifiable marker of obstructive lung disease. It strongly predicts both pulmonary and cardiac events, including incident heart failure. Post-bronchodilator spirometry is necessary to define chronic obstructive pulmonary disease (COPD), the third leading cause of death. Repeat measures over time define of progression of lung disease. Post-bronchodilator spirometry will only be performed at in-person clinic visits.
- **Non-contrast Full-Lung CT Scan:** Non-contrast, full-lung, MDCT scanning (SOMATOM Flash) of the chest will be performed for approximately 30 minutes to assess the pulmonary emphysema using lung density, bronchial wall thickness, and hyperinflation. Participants with a cumulative exposure in MESA of >25 mSv will be excluded from all CT scanning. The youngest participants will be approximately 60 years old; hence pregnancy is not an issue. The estimated average dose across the whole Lung cohort is 2-3 mSv. The dose varies with body size so is lower in thinner men and higher in heavier women. However, since radiation beams are absorbed by adipose tissue, the effective dose to susceptible organs in heavier participants is likely to be as low or lower than in thinner participants.

- **Hair Follicle Collection:** All participants will be selected for hair follicle collection for an additional 5 minutes. Ten hairs are plucked from the scalp, inserted in a vial and frozen at -80C.
- **Nasal Brushing:** All participants will be selected for nasal brushing for an additional 5-10 minutes. A polyester-tipped flexible soft brush is inserted into one of the participant's nostrils via a nasal speculum, then rotated 180 degrees and withdrawn. The procedure will be repeated once. After swabbing, the brush is cut off, the swab is placed in a vial and frozen at -80C.
- **Environmental Exposures Questionnaire:** Data on home characteristics and environmental exposures will be collected. The daily check-in will take up to 15 minutes to complete.

6.2.8. MESA Stress Reactivity Exam Components

- **Heart Monitoring:** Participants will wear a Cardea SOLO ECG heart monitor patch for 7 days. Consent, application of the patch, and delivery of instructions will take approximately 30 minutes to complete at the clinic, home, or remote visit. The device will be returned to sleep study technicians or mailed to the reading center in a pre-paid envelop. If the heart monitor patch is lost or damaged, or if another scientific question arises that could be answered with repeated ECG monitoring, participants may be invited to repeat the monitor.
- **Daily Stress Questionnaire:** Data on daily stressors and positive and negative affect will be collected over the phone or using a self-administered web-based or paper survey for 7 days when participants are wearing the Cardea patch. The daily check-in will take up to 15 minutes to complete.

6.2.9. Follow-up Phone Calls

- Clinic staff contact participants by telephone at regular intervals (Follow-up Calls) to ask questions regarding health since the previous telephone interview, specifically those involving a hospitalization, nursing home admission, or diagnosis of myocardial infarction (MI), angina, congestive heart failure (CHF), peripheral arterial disease (PAD), stroke or transient ischemic attack (TIA). The schedule of follow-up phone calls are displayed in the Fourth Contract Period timeline from section 3.2. The 23rd, 24th, and 25th Follow-up phone calls occur during the Exam 7 period.
- The surveillance phone interview in MESA serves several purposes:
    - To ascertain whether participants have experienced any potential events
    - To update tracking data including address, phone number, email address, contact information and secondary contact information
    - To update participants' vital status
    - To obtain information regarding participants' general health and health care treatment since their last MESA telephone Follow-up call or clinic visit
    - To obtain detailed information about specific medical conditions that participants have been reported (by a physician) to have since their last MESA telephone

Follow-up call or clinic visit

- To obtain detailed information about any procedures or hospitalizations participants have had since their last MESA telephone Follow-up call (not since clinic visit)
- To introduce upcoming exams and schedule an appointment.

6.3. Flexibility of Exam Structure

The seventh examination of the MESA contract will include in-clinic, remote, and home visit options for recording data and performing exam components. Given the age and frailty of participants and the ongoing pandemic, the exam will allow maximum flexibility for data collection. The majority of Exam 7 components can be completed at an in-home visit with a study technician, and many could be completed using a remote visit model (sending exam equipment to the participant and talking with a study technician by phone or video conference). Participant questionnaires can be completed in the clinic, by phone with an interviewer, using a web-based survey, or on paper forms at home. The following table summarizes which components can be completed by each type of exam.

Table 5: Exam Components allowed by each visit type

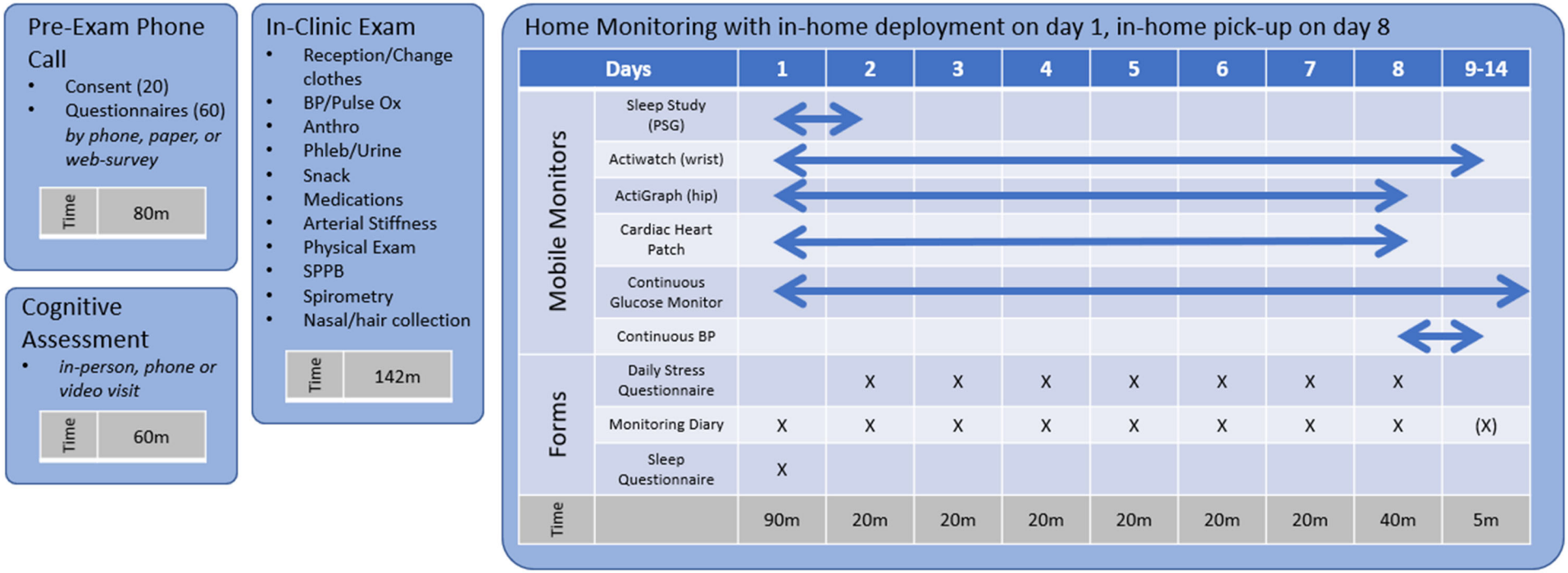
Component	Study	Visit Type			
		In-clinic	Home	Remote	Phone
Clinic Reception and Consent	All	Yes	Yes	Yes	No
Blood Pressures	Core, MIND	Yes	Yes	No	No
Pulse Oximetry	Core, Lung	Yes	Yes	No	No
Height	Core, MIND	Yes	Yes	No	No
Weight	Core, MIND	Yes	Yes	No	No
Waist circumference	Core, MIND	Yes	Yes	No	No
Neck circumference	MIND, 24hAct	Yes	Yes	No	No
Phlebotomy	Core, 24hAct, Glucose, Lung, MIND	Yes	Yes	No	No
Urine Collection	Core/Lung	Yes	No	No	No
Medications	Core, MIND	Yes	Yes	Yes	No
Medical History	Core, MIND	Yes	Yes	Yes	Yes
Personal History/Demographics	Core	Yes	Yes	Yes	Yes
Arterial Stiffness	MESA-MIND	Yes	No	No	No
Clinician's Physical Examination		Yes	No	No	No
SPPB		Yes	No	No	No
Cognitive Testing		Yes	Yes	Yes	No
Brain MRI		Yes	No	No	No
PET imaging		Yes	No	No	No
Informant Interview		Yes	Yes	Yes	No
Sleep Questionnaires	MESA Sleep	Yes	Yes	Yes	Yes
Sleep Study/Diary		No	Yes	Yes	No
Actigraphy/Diary		Yes	Yes	Yes	No
Blood Pressure Monitor/Diary		Yes	Yes	Yes	No
Activity and Sleep monitor/Diary	24hACT	Yes	Yes	Yes	No
Physical Activity Questionnaire		Yes	Yes	Yes	Yes
Spirometry exclusion/completion	Lung IV	Yes	Yes	No	No
Pre and post-bronchodilatory spirometry		Yes	Yes (no BD)	No	No
Environmental Exposures Questionnaire		Yes	Yes	Yes	Yes
Nasal brushing		Yes	No	No	No
Hair collection		Yes	No	No	No
Lung CT		Yes	No	No	No

Neighborhood Questionnaire	Neighborhood	Yes	Yes	Yes	Yes
Continuous Glucose Monitor/Diary	Glucose	Yes	Yes	Yes	No
Cardea SOLO Patch	Stress Reactivity	Yes	Yes	Yes	No
Daily Stress Questionnaire		Yes	Yes	Yes	No

Examples of the different exam models are reflected in the next sections. The order of exam components remains flexible based on the priorities of the study and the needs of the participants.

6.3.1. Exam 7 In-Clinic Visit Option

The in-clinic visit option allows participants to complete consent over the phone, either signing the consent form via REDCap or by mailing the consent form to the participant for signature. Questionnaires can be completed by phone, paper, or web survey based on participant preference (collected during the consent phone call). The MESA-MIND cognitive assessment can also be administered by phone or video visit, but it will not be collected at the same time as the questionnaires. The in-clinic exam includes components in the list below. Home monitoring is scheduled after the in-clinic exam, with study technicians setting up PSG, activity and sleep monitors, cardiac heart patch, and continuous glucose monitor on day 1. After at least 7 days of wear time, technicians return to the home to pick up the monitors and place the ambulatory blood pressure monitor. After 24 hours of wear, it is returned to the clinic by mail or returned during an imaging visit. CT, MRI, and PET imaging can occur before or after the home monitoring period, but the home monitoring devices cannot be worn during the MRI or PET. Estimated total participant burden is 6.2 hours.



6.3.2. Exam 7 Home Visit Option

The home visit option also allows participants to complete consent over the phone, either signing the consent form via REDCap or by mailing the consent form to the participant for signature. Questionnaires can be completed by phone, paper, or web survey based on participant preference (collected during the consent phone call). The MESA-MIND cognitive assessment can also be administered by phone or video visit, but it will not be collected at the same time as the questionnaires.

The home exam includes components in the list below. If feasible, the participant can also be invited to come to the clinic after the home visit for scanning and/or other procedures that are not possible during a home visit.

- Consent
- Anthropometry
- Oximetry
- Seated Blood Pressure
- Blood Collection
- Cognitive Assessment
- Questionnaires
- Spirometry
- Monitor device placement and instructions
    - ABPM
    - Hip activity monitor
    - Wrist activity monitor
    - Continuous glucose monitor
    - Heart Monitor Patch
    - Overnight Sleep Study (PSG)

Home monitoring equipment can be set up at the same time as the home exam. After at least 7 days of wear time, the monitors are returned by mail or picked up by study staff. Those who participate in the home visit will not complete hair, nasal brushing or urine collection, arterial stiffness, SPPB, or a physical exam. Estimated total participant burden is 5.4 hours.

6.3.3. Exam 7 Remote Visit Option

The remote visit option also allows participants to complete consent over the phone or video visit, either signing the consent form via REDCap or by mailing the consent form to the participant for signature. Questionnaires can be completed by phone, paper, or web survey based on participant preference (collected during the consent phone call). The MESA-MIND cognitive assessment can also be administered by phone or video visit. The remote exam includes components in the list below.

- Consent
- Cognitive Assessment
- Questionnaires
- Monitor device placement and instructions
    - ABPM
    - Hip activity monitor
    - Wrist activity monitor
    - Continuous glucose monitor
    - Heart Monitor Patch
    - Overnight Sleep Study (PSG)

Home monitoring equipment will be mailed to the participant, and study staff will guide them through applying the monitors by phone or video visit. After at least 7 days of wear time, the monitors are returned by mail or picked up by study staff. Those who participate in the home visit will not complete blood pressure, anthropometry, pulse oximetry, spirometry, hair, nasal brushing or urine collection, arterial stiffness, SPPB, or a physical exam. Estimated total participant burden is 3.2 hours.

7. Notification of and Referral for Study Findings

One of the benefits of the study to the participants will be the provision of an extensive battery of medical tests at no cost to them. This information will be made available to the participant and their physician if desired. Participants will receive an initial report summarizing results available at the completion of the clinic visit (e.g., height, weight, blood pressure) and additional results reports as they become available, including routine laboratory results (e.g., plasma glucose, lipids, and serum creatinine), and results of additional results or procedures such at CT, MRI, or PET. Cognition results letters will be sent to participants within a year of cognitive assessments. Participants and their health care providers will be immediately notified if potentially serious medical problems are identified during any of the examinations. A CC referral system will notify FC staff based on the urgency of the need for medical attention. Criteria for emergent and urgent notification are provided in the Manual of Operations section entitled, "Notification and Referrals."

8. Exam 7 Pilot Study

The entire Exam 7 protocol as described above, or parts therein, will be piloted in up to 24 volunteers who are not MESA participants. The selection criteria for the pilots are adults who are 45-85 years old who are willing and able to consent to the protocol. The protocol will be piloted at all sites but not all procedures will be done at all sites (e.g., imaging procedures may be dropped at most sites). No specimens will be stored. Alerts and results reporting will follow the main MESA protocol.

9. Data Management

9.1. Field Center Data Management

9.1.1. Field Center Procedures

The following principles and procedures will be followed at the Field Center for data collection:

- Most clinic data will be entered directly via computer or tablet and will not include personal identifiers. Only the tracking form will have the participant's name and address.
- Study records will be stored in locked cabinets in a locked room.
- Only the study personnel will have access to the data and the codes.
- All computerized information will be protected by access codes known only to the principal investigator and certain designated staff members.
- No data will be published with participant names or other identifying information.
- All staff members will be trained to keep participants' information confidential and will be informed of the penalty for breach of confidentiality.

9.1.2. Data Entry and Transmission

Each Field Center will be responsible for entering the clinic data via a computer or tablet device and data entry applications that are connected to the Coordinating Center database. This task will be performed by the technician or interviewer who is collecting the data. The data entry software will be programmed for table lookups, range checks, skip pattern rules, consistency checking, and ID selection from a list of valid ID numbers. Data will reside centrally on the

password-protected CC database, and Field Center personnel will be able to perform updates as needed.

All entered data will be automatically transferred securely to the Coordinating Center database. This transfer will include exam data, events data, tracking data, and follow-up data.

9.2. Confidentiality and Security

The consent form signed by the participant will provide assurance that all individual data collected in the study will be kept confidential to the extent provided by the Privacy Act of 1974 and by the Health Insurance Portability and Accountability Act (HIPAA). A Certificate of Confidentiality will be obtained. Each center that has data with personal identifiers will provide file security so that confidential data are not released. Specifically, participants will be informed that: (1) the only people who will know that they are research participants are members of the research team and, if appropriate, their physicians or health care providers; (2) no individual identifying information will be disclosed to others, except as part of ascertainment of events information, as permitted by the consent form, or if required by law; and (3) when the results of the study are published or discussed in conferences, no information will be included that would reveal their identity.

9.3. Coordinating Center Data Management

9.3.1. Development of the Database Management System.

The Coordinating Center has established an "Intranet" for use by all sites involved in MESA. (Intranet is the term used for the implementation of Internet technologies within an organization, rather than for external connection to the global Internet.) Using the Intranet, all sites will have access to selected Coordinating Center databases for uploading of data and queries to the database. As members of our Intranet, each Field Center and Central Reading Centers and Laboratory will have access to downloadable data files as well as electronic versions of manuals, forms, staff directories, and collaborative manuscripts. Having only one central copy of these documents and files will make it easier to assure that all centers have access to current information. Safeguards will be put in place so that only specific files can be accessed over the Intra- or Internet, and then only by authorized users.

The Coordinating Center developed a series of databases to store and manage data which forms a comprehensive system linked by unique participant ID numbers. There will be one raw database to which data files from Field Centers and Reading Centers and Laboratory will be uploaded. After local cleaning and verification of the data, they will be loaded into the appropriate master database accessible only by Coordinating Center personnel. This master set of databases at the Coordinating Center will not be accessible to anyone on the Intra- or Internet; they will physically reside on a different computer.

A tracking database will be developed for the sole purpose of monitoring data completeness for each individual at each visit. This database will be programmed so that the different sets of data expected from different sub-sets of the cohort at various points in time can be tracked separately. The database will include both Field Center data and Reading Center data. Reading Center data will be tracked to assure that the data have been: (1) collected at the Field Center; (2) sent to the

Reading Center; (3) received at the Reading Center; (4) processed at the Reading Center and sent to the Coordinating Center; and (5) received at the Coordinating Center.

Data on cardiovascular events will reside in a separate database as well. Because of sensitivity issues surrounding medical record data, this database will not be accessible over the Web. However, Field Centers will be able to check on the status of data for a particular event via reports on the Web.

Since the Field Center, Reading Center and Central Laboratory staffs will be allowed to edit and correct data in the raw database, the changes will be tracked in a database such that the original values and history of all the changes are recorded. This change database will record the date, time, who made the change, name of the variable, the form it came from, and the reason change was necessary. Included in this database will be documentation of changes to computed variables.

The Coordinating Center has also developed and maintains a database to track publications and presentations. The database allows quick and easy access to information about publications and presentations for authors, the Publications and Presentations Committee, the Steering Committee, the Monitoring Board and the NHLBI Project Office. Elements of the database include title, authors, manuscript proposal date, date for completion, submission date to journal, status of manuscript with the journal, publication date, and abstract. Information from this database is accessible for viewing on the web to all investigators.

All data sets ready for dissemination to study investigators or staff will be moved to the computer acting as the Coordinating Center web server as compressed files ready to be transferred. Medical record security is a current topic of concern, and the Microsoft SQL Server databases will be encrypted and fully protected with user/password security and "firewall" software that acts as a screening tool, providing an electronic barrier to unauthorized use of a computer system by hackers or other unauthorized users. To maintain privacy, no names, addresses, Social Security numbers or other personal identifiers will reside on an Intra- or Internet-accessible database.

9.3.2. Development of Web Sites

The Coordinating Center has developed and maintains three websites: an external site for the general public, a website for MESA participants, and an internal site for study investigators and personnel.

External Website This external website informs its target audiences about the project, generates project support, and reduces mailing and printing costs. Specifically, the external website includes (1) Project description and rationale; (2) Contact information for project centers and staff; (3) Text of project newsletters; (4) Study component schedules of administration; (5) Study forms and manuals; (6) Calculator tools for assessing cardiac health; (7) List of publications with copies of abstracts; and (8) Search capability.

Participant Website This website is specifically targeted to MESA participants. The purpose is to disseminate up-to-date information about the study, report new findings, and post appropriate

links and documents that participants would find interesting. An archive of MESA Messenger participant newsletters is available on the site.

Internal Website This website provides a way for project staff to facilitate communication, share information, reduce mailing and printing costs, and increase efficiency. Staff are able to both view and contribute documents or files to this website. Study committees and working groups have assigned pages for posting calendars, meeting minutes, and other relevant materials. In addition, the website will allow investigators to submit research proposals for review and receive feedback. The internal website also includes (1) Data files for download, at varying levels of access; (2) Data documentation; (3) Access to P&P database; (4) All study forms and manuals; (5) Study component schedules of administration; (6) E-mail directory of project staff; (7) Calendar of project deadlines; (8) Steering Committee and other reports; (9) Project meeting schedules; (10) Links to other web sites of potential interest; and (11) Search capability.

Passwords are used to maintain the security of this site. One password will be required to access the site, and a second password, which will change frequently, will be required in order to download data.

9.3.3. General Coordinating Center Management

The following principles and procedures will be followed by the Coordinating Center:

- Only MESA Coordinating Center staff will have access to the Coordinating Center's personal computers, thus simplifying security arrangements.
- The Coordinating Center will store MESA data on servers employing fault-tolerant RAID volumes. “RAID” stands for Redundant Array of Inexpensive Disks, which means that all data stored on the server is written across multiple disks. This helps to protect against data loss due to mechanical disk failure. The ZFS file servers create snapshots of the file system periodically throughout the day. This allows staff to restore previous versions of files easily. The Coordinating Center will also maintain incremental system backups on a nightly basis using secure offsite network backup provided by UW Technology. This includes a copy of the data stored outside the seismic area at a secure facility in Eastern Washington. Additionally, backups may be retrieved from this system using the version stored locally on campus servers in less than 10-15 minutes. The last backup of each year is also kept as a permanent archive throughout the study period. System backups are routinely checked to make sure that they are readable and complete. Raw data in a computer-readable form (from data transmissions or data entry at the Coordinating Center) will be archived separately.
- Sensitive data, such as participant names and social security numbers, will be kept in a separate database table with additional security passwords required for access.

10. Quality Assurance and Quality Control

10.1. Overview of Quality Assurance and Quality Control

Activities undertaken to ensure the highest possible data quality for MESA can be divided into two areas: Quality Assurance and Quality Control. Quality assurance activities entail all steps taken prior to data collection to assure accuracy and to minimize errors. Quality control activities are the steps taken after data are collected to examine quality, particularly to measure reproducibility and identify errors.

MESA quality assurance will emphasize training of staff and maintenance of equipment. Quality control procedures will emphasize the technical procedures included in the exam, and will be designed to permit rapid identification of problems early enough in the study to have an effect. Due to the finite resources, both in terms of participant time and burden and Field Center and Central Agencies staff and time, quality control must be concentrated on key study components. The Operations Committee is charged with quality assurance related to training. Equipment maintenance is overseen by appropriate technical committees, such as the MRI Committee, while compliance with maintenance is monitored by the Quality Control Committee. The Quality Control Committee is charged with developing the details of the QC protocol; for monitoring its implementation during the data collection phase; and for quickly identifying and resolving any problems that are identified.

10.1.1. Quality Assurance

Quality Assurance activities are those performed before the data are collected to minimize the number of data errors that occur. Primary steps in assuring good quality of study data are adequate training and periodic observation of study personnel. A highly motivated, conscientious staff may be the best guarantee of data quality. Other key considerations include adequate monitoring of technician performance by supervisory staff at the Field Centers and support units. Such monitoring can identify and correct problems weeks or months before they would become apparent from Quality Control activities such as statistical analyses performed by the Coordinating Center.

Quality Assurance activities in MESA will include: (1) a well-documented, standard protocol to be performed at all sites in an identical manner; (2) centralized training of technicians so that all technicians are trained to perform MESA measurements in the same way; (3) requirements regarding demonstrated proficiency in performing MESA procedures before initial certification of technicians is granted, and requirements for a minimum number of procedures required to maintain certification; (4) routine observation of technicians to verify adherence to protocol; and (5) routine calibration of equipment such as scales and blood pressure devices.

10.1.2. Quality Control

Quality Control activities are those performed after data are collected to identify any errors which have occurred. Quality control in a large study such as MESA has two major purposes: (1) to identify problems in data collection and measurement in time to institute appropriate corrections; and (2) to quantify the quality of data collected over the course of the study so as to provide information necessary to interpret study results. To accomplish the first goal, adequate data must be accumulated to enable valid analyses to be performed within a brief period after initiation of data collection. To accomplish the second goal, sufficient data must be compiled throughout the study to detect any drift or deterioration in data quality over time. Because of finite resources, both in staff and in acceptable burden on participants, each component of a quality control

program must be selected on the basis of assessing the need, feasibility, and overall importance to the main goals of MESA.

Data from the specialized Reading Centers and the support laboratories are among the most important collected by MESA. High-quality data must be obtained from these units in order to fulfill the primary goals of the study. For these reasons, the Quality Control Committee will place special emphasis on quality control of these units.

For the other examination components, the Coordinating Center can provide considerable quality control information by relatively simple analyses of data acquired from all participants. Monitoring of the distribution of individual values and of mean or median values by technician, center, time, subject subgroup, etc. may identify many problems. Because of the large numbers available, this will be a particularly useful way of detecting many problems. Some of this information, such as noting problems with blood processing at a certain Field Center, may be reviewed by a central unit.

The following sections summarize the quality control procedures to be conducted by the individual Central Laboratories and Reading Centers.

10.2. Central Laboratory

All blood and urine samples collected for MESA will be shipped to the Central Laboratory every other week. Shipping schedules will be set up for each Field Center to avoid loss of samples due to arrive on weekends or holidays. Quality control procedures will include:

- Sample monitoring
- Assay monitoring
- Participation in extrinsic quality assurance programs
- Measurement of blind duplicates from Field Centers
- Monitoring of Field Center logs
- Site visits to Field Centers
- Monitoring of local hematology quality control

For all of the central Reading Centers, certain scans will be cycled through the reading process at pre-defined intervals in order to assess whether any drift is occurring in the interpretation of the images.

11. Reading Center and Laboratory Data Management

The Reading Centers will receive data from the clinics transmitted electronically. After receiving the data, Reading Center personnel will retrieve the studies and either send the medium back to the Field Center or store them on site. A list of studies received will be sent to the Coordinating Center for purposes of tracking. Processed data from the Reading Center will be transmitted to the Coordinating Center each week.

The Central Laboratory will receive blood and urine specimens and an inventory list from the clinics every two weeks. A list of samples received will be sent to the Coordinating Center to

add to the Tracking Database. Analysis results will be transmitted to the Coordinating Center every week.

Reading Centers and Laboratories will perform routine backups of all data regularly.

12. Participating Centers Organization, Roles and Responsibilities

12.1. Organizational Structure

A diagram of the organization structure of the study can found at https://www.mesa-nhlbi.org/aboutMESAPersonnel.aspx#chart. A full list of MESA Committees and their charge is available on the MESA website at https://www.mesa-nhlbi.org/aboutMESAPersonnel.aspx#committees

12.2. Participating Organizations

The centers involved in the study and their principal investigators are listed in Table 6. Awards for the original MESA contract were made on January 15, 1999 and additional centers were added through the various exams and ancillary studies.

Table 6: List of Centers and Principal Investigators in MESA

Center	Site	Principal Investigator
Coordinating Center	University of Washington	Robyn McClelland, PhD
Field Center	Columbia University	Steven J.C. Shea, MD, MS
Field Center	Johns Hopkins University	Wendy S. Post, MD, MS
Field Center	Northwestern University	Norrina B. Allen, PhD
Field Center	University of Minnesota	James Pankow, Ph.D.
Field Center	University of California at Los Angeles	Karol Watson, MD, PhD
Field Center	Wake Forest University	Alain G. Bertoni, MD
Central Laboratory	University of Vermont	Russell P. Tracy, PhD
Accelerometer Reading	Columbia University	Keith Diaz, PhD
Arterial Laboratory	University of Pennsylvania	Julio Chirinos, MD, PhD
Brain MRI Reading Center	University of Pennsylvania	Nick Bryan, MD, PhD
Echocardiography Imaging Reading Center	Northwestern University	Sanjiv Shah, MD
Lung CT Reading Center	University of Iowa	Eric Hoffman, PhD
MESA Mind Neurocognitive Operations	Wake Forest University	Tim Hughes, PhD Bonnie Sachs, PhD
Magnetic Resonance Imaging Reading Center	Johns Hopkins University	Joao Lima, MD, PhD
PET Reading Center	University of Michigan	Robert Koeppe, MD
Spirometry Reading Center	Columbia University	R Graham Barr, MD DrPH

		John Hankinson, PhD
Sleep Reading Center	Brigham and Women's Hospital	Susan Redline, MD, MPH

The Project Office is in the Prevention and Population Sciences Program, Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute.

A full list of Field Center investigators is available on the MESA website at https://www.mesa-nhlbi.org/aboutMESAPersonnel.aspx.

The roles and responsibilities of each center are as follows:

12.2.1. Coordinating Center

- Establish a study timeline to guide overall study activities, including planning and oversight of Steering Committee and subcommittee activities.
- Provide leadership and coordination for establishing and maintaining study communications, including the use of video conferencing, meetings, and a central, accessible website.
- Provide administrative leadership and scientific coordination to develop the final study protocol, manuals of operations, and forms, including sample selection, recruitment, certification of field staff, examination, interview, medical record abstraction, and follow-up procedures.
- Develop, implement and maintain a database management system capable of: storage of existing participant data; data entry and weekly transmittal at each of the Field Centers, Reading Centers and Laboratories; generation of reports for use by Field Centers, Project Office and Steering Committee; and summaries of exam to be sent to participants and their physicians.
- Coordinate training and certify Field Center staff in examination procedures and interviews in accordance with protocol.
- Purchase, distribute, and coordinate utilization of appropriate common mechanical and electronic equipment among all centers, including computer hardware and software.
- Develop and maintain manuals of operations describing in detail study activities at each participating center.
- Develop, implement and maintain a system for quality control of data to verify completeness, compare the distribution of values from different Field Centers and different examiners, identify outlying values for separate review, review adherence to schedules for reexaminations and other data collection and analyze laboratory performance on external standards and blind duplicates.
- Provide leadership for the editing, analysis, and publication of study data in collaboration with the Steering Committee and the NHLBI Project Office.
- Provide support for Monitoring Board meetings.
- Produce data sets of MESA data for use by investigators and for distribution to the public, according to NHLBI guidelines.

12.2.2. Field Centers

- Provide individuals with expertise in cardiovascular epidemiology, clinical cardiovascular disease, noninvasive imaging, laboratory measurements, statistics, longitudinal studies management, and related fields who will participate in the development of the protocol, the manual of operations, and the specific forms used for recording interviews, abstracting records, and examination results.
- Examine, and maintain follow-up of enrolled participants.
- Provide trained and certified technicians and imaging centers to carry out data collection procedures and implement quality control procedures as determined by the Coordinating Center.
- Inform participants and their physicians of any important medical findings discovered on examination.
- Enter all data derived from the recruitment interview, clinic examinations, and surveillance phone calls into electronic data collection program and transmit to the Coordinating Center.
- Collect, process, and transmit data to appropriate reading centers and blood samples to laboratories.
- Collaborate with the Steering Committee, Project Office, and Coordinating Center in analyses of data and publication of results.
- Participate in quality control investigations and implement corrections as necessary.

12.2.3. Central Laboratory

- Recommend specific blood and urine analyses to be performed on all participants and other analyses on selected cases and controls.
- With the Laboratory and Steering Committees, develop protocols for biospecimen collection, processing, and analysis of samples at the Central Blood Analysis Laboratory.
- Recommend feedback to participants and their physicians regarding measurements.
- Perform or coordinate analyses.
- Electronically record data derived from the biospecimen analyses and provide measurements to the Coordinating Center.
- Design and implement quality control measures for blood collection and processing at the Field Centers and for analysis of samples at the Central Laboratory.
- Train, certify, and oversee quality control monitoring of Field Center laboratory technicians in blood collection and processing protocols and of CBAL technicians in the analysis of samples.
- Participate in analysis and publication of study results.

12.2.4. Project Office

- Participate in the Steering Committee and its subcommittees in the development of the study protocol.
- Ensure that the study meets its scientific objectives while remaining on schedule and within budget, and work with the Steering Committee to resolve any technical problems that arise.
- Monitor study progress by maintaining close contact with investigators, reviewing study documents, inspecting and accepting contract deliverables, and performing periodic site visits.

- Interpret the contract Statements of Work and any other technical performance requirements for the Steering Committee.
- Assist Contracting Officer in authorizing reimbursement of costs and negotiating any changes in the contract Statements of Work, periods of performance, or delivery schedules.
- Participate in analysis and publication of study results.

12.2.5. Contracting Office

- Participate in the Steering Committee and its subcommittees to ensure that study resources are used within funding allotments and in accordance with contractual requirements.
- Provide Project Officer with an interpretation of contractual requirements.
- Monitor the study expenditures and deliverables. Recommend appropriate action to Project Officer and, upon Project Officer's approval, provide authorization for any required action.
- Assist Project Officer in negotiating any funding and/or contractual changes. Upon Project Officer's approval, provide authorization for funding and/or contractual changes.

12.3. MESA Monitoring Board

The MESA Monitoring Board has been appointed by the Director, NHLBI, to advise the Institute on the design and conduct of the study and on the analysis and interpretation of results. Meetings of the Board will be held approximately annually. Members of the Board are listed on https://www.mesa-nhlbi.org/aboutMESAPersonnel.aspx#board.

13. Study Policies

13.1. Publications and Presentations

The policies governing proposals for data analysis, presenting MESA data, and publication are found at https://www.mesa-nhlbi.org/Publications.aspx.

13.2. Ancillary Studies

The MESA investigators and NHLBI encourage ancillary studies (sub-studies that are supported by other than contract funds) to enhance the scientific contributions of the study. Policies and conditions for proposing ancillary studies, collaborating, and monitoring ancillary study activities are provided on https://www.mesa-nhlbi.org/ancillary.aspx.

14. Appendix A: MESA Amyloid Ancillary Study

Study Title: Interplay of Myocardial Fibrosis and Cardiac TTR amyloidosis in Age related cardiac remodeling in MESA.

Cardiovascular events, such as atrial fibrillation and heart failure result from long-term interactions among diverse risk factors that cause subclinical pathology and clinical disease manifestation.

Recently, enormous advances in imaging and treatment modalities, reveals a higher prevalence of cardiac amyloidosis. Cardiac ATTR is characterized by accumulation of misfolded transthyretin (TTR) protein in the extracellular space of the myocardium leading to heart failure and cardiomyopathy. In addition, myocardial fibrosis have been recognized as the cause and the consequence of heart failure and other cardiovascular diseases. In this application, we hypothesize that longitudinal changes in extra-cellular volume (ECV) in absence of amyloid is attributed to progressive myocardial fibrosis. Since there are no well-established predictors for cardiac ATTR amyloidosis we also aim to examine antecedent markers for predicting future amyloidosis. As a result, we expect the study to establish pivotal importance of fibrosis and amyloidosis in cardiac remodeling to create opportunity for novel strategies to prevent cardiovascular events.

Specific Aims:

Aim 1: Identify the determinants of progressive myocardial fibrosis phenotype by magnetic resonance imaging (MRI) at MESA exam 7.

Aim 2: Identify the determinants and antecedent markers of MESA cardiac TTR amyloidosis by leveraging all datasets collect at all exams, augmented by MRI phenotypes of amyloidosis and SPECT obtained at Exam 7.

Aim 3: Determine how much progressive myocardial fibrosis predicts the extent of cardiac amyloidosis by leveraging exam 5 and 7 datasets.

The MESA Amyloid Ancillary Study plans to recruit 800+ MESA participants MESA participants from Exam 5 including those who underwent MRI with gadolinium based contrast (Dotarem or Gadavist) irrespective of whether they underwent T1 mapping at MESA 5 or not. MRI and SPECT/CT can be completed at same visit or separately. MESA Amyloid visits will occur during the same time period as Exam 7 clinic visits or after.

14.1. Study Principal Investigators

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14.2. Informed Consent

Written consent for the MESA Amyloid study will be obtained at the beginning of the scheduled appointment, following local institutional policies of social distancing and screening procedures for COVID-19. This should be done in a private area with ample time as needed to answer any questions asked by the participant. The content of the consent form complies with guidelines from the National Heart, Lung, and Blood Institute, the MESA Steering Committee, and the requirements of each field center's IRB. The content is designed to inform the participant of the purpose and procedures of the study and the voluntary nature of their participation. The form makes the participant aware of the right to withdraw from the study, to not participate in a procedure, or to decline to answer any question(s) without penalty.

14.3. MRI Protocol

14.3.1. Inclusion/exclusion criteria

Inclusion Criteria:

1. MESA participant at Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University, or University of California, Los Angeles.
2. Completed a cardiac MRI at MESA Exam 5.

Exclusion Criteria:

3. Contraindications to MR, including those with pacemakers, aneurysm clips, cochlear implants or other implanted electronic devices.
4. Participants with a history of occupational exposure to small metallic projectiles will be further evaluated with orbital radiographs as needed.
5. Do not give gadolinium-based contrast if allergic to gadolinium contrast (Dotarem or Gadavist), abnormal kidney function or if eGFR <45ml/min. Perform MRI without contrast.
6. Unable to lie flat for the MRI exam.
7. Unable to consent due to cognitive impairment.
8. Is a recipient of an organ transplant.

14.3.2. MRI Visit Procedures

The initial visit or contact includes administration of informed consent and a screening questionnaire. Once the initial contact or visit is completed, the participant will be scheduled for MRI. Screening questionnaires will be reviewed for verification and to ensure that no interval change in health status has occurred. Once eligibility is confirmed and written consent is obtained, point-of-care fingerstick testing for creatinine will be performed if not done within the past 30 days.

After the peripheral upper extremity IV is placed, the participant will rest quietly for 5 minutes, after which blood pressure will be taken twice using an automatic blood pressure cuff. The participant will then proceed to heart MRI. The participant will receive one Gadolinium based contrast agent (either Dotarem or Gadavist). The scan is estimated to take 45-50 minutes, and

total table time 60 minutes. After completion, the participant will undergo a brief exit interview to assess for symptoms potentially related to gadolinium-containing contrast (Gadavist or Dotarem) administration. The participant will then receive reimbursement and conclude the study. Of note, if upon testing for creatinine at the follow-up visit show that the participant is ineligible for contrast but still safe to proceed to MRI, the participant will proceed with non-contrast MRI.

All MRI images will be acquired and transmitted de-identified (without any individually identifiable participant information) to the JHU MRI Core Lab in DICOM format. All exams must be appropriately labeled and accompanied by a completed MRI Completion Form (MCF). All images will be stored in DICOM format. Images will be saved on the local PACS system as well as transmitted to Johns Hopkins through Ambra.

14.3.3. Potential Risks

- **Finger stick or catheter placement**: temporary discomfort from the needle stick, bruising, infection, or fainting.
- **MRI risks**: Because the MRI machine acts like a large magnet, it could move iron-containing objects in the body. Participants with metal in their body, such as a fragment in the eye, aneurysm clips, ear implants, spinal nerve stimulators, or a pacemaker, will be excluded.

  MRI may cause claustrophobia. Participants may feel discomfort by the loud banging noise or instructions to not swallow during the study. Participants will be given earplugs.
- **Risks associated with any Gadolinium based contrast including but not limited to Dotarem or Gadavist**: Gadolinium-based contrast can have the following rare but serious risks.
  - Very rarely, a life-threatening allergic reaction with breathing difficulty and low blood pressure.
  - Participants with reduced kidney function will not receive gadolinium containing contrast. The risk of a condition called nephrogenic systemic fibrosis or nephrogenic fibrosing dermopathy (NSF) is very low in people with normal or mild to moderately reduced kidney function. NSF has been reported to occur between 2 days and 18 months following injection of gadolinium-based contrast. There is no known treatment for NSF. Some people have even died from this. Signs and symptoms of NSF may include: burning, swelling, hardening or tightening of the skin, blood vessels and internal organs (heart, lungs, liver); yellow spots on the white part of the eyes; joint swelling and stiffness; pain in the hip bones or ribs; muscle weakness. NSF has been reported in people with severe kidney disease, but is very rare (approaching zero) otherwise. NSF has not been reported in people with normal kidneys.

  A few side effects of gadolinium containing contrast injection (Dotarem or Gadavist) such as mild headache, nausea, and local pain may occur. Rarely (less than 1% of the time) low blood pressure and lightheadedness occurwhich can be treated immediately with intravenous fluids. Very rarely (less than one in one thousand), a person is allergic to gadolinium-based contrast. These effects are most

commonly hives and itchy eyes, but more severe reactions have been seen that result in shortness of breath.

Studies have shown that gadolinium containing contrast agents may accumulated in various parts of your body, including bone, brain, and other organs. The amount accumulated increases with the number of times any Gadolinium containing agent such as Gadavist or Dotarem is administered. There is no evidence currently that this is associated with any adverse health effects.

14.4. SPECT/CT Protocol

14.4.1. Inclusion/exclusion criteria

Inclusion Criteria:

1. MESA participant at Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University, or University of California, Los Angeles.
2. Completed a cardiac MRI at MESA Exam 5.

Exclusion Criteria

1. Do not give gadolinium-based contrast if allergic to gadolinium contrast (Dotarem or Gadavist), abnormal kidney function or if eGFR <45ml/min. Perform MRI without contrast.
2. Unable to lie flat for the SPECT/CT or MRI scan.
3. Unable to consent for the study due to cognitive impairment.
4. Is a recipient of an organ transplant.

14.4.2. SPECT/CT Visit Procedures

The average SPECT/CT scan appointment is approximately 3.5 hours. This includes a 150-minute waiting period after the radiotracer is injected and 30 minutes of imaging time for the SPECT/CT scan. The cardiac MRI scan can be completed during the 150-minute waiting period. If scan quality is determined to be unacceptable, the scan must be repeated while the participant is still present. Due to limited budgets, there are no funds for rescanning during a repeat appointment.

SPECT/CT acquisition and reconstruction will be performed locally at MESA centers using study-specific protocols. 18 mCi of 99mTc-PYP/HDP will be injected, followed 150 minutes later by SPECT/CT and planar chest imaging (scan time: ~ 20 minute SPECT/CT and ~ 7 min planar). Digital images of the SPECT/CT data will be electronically transferred to the Nuclear Reading Center at Brigham and Women's Hospital (Boston, MA). The images will be analyzed by visual and detailed semi-quantitative approaches using regions of interest on the myocardium, lungs, and blood pool. Attenuation corrected SPECT/CT images will be used.

Clinic Visit procedures include:

1. The participant arrives and completes registration, including written consent, at the front desk following current institutional policies.
2. MESA staff measures and records the participant's weight.
3. MESA staff inserts an intravenous access for injection of the radiotracer.
4. MESA staff asks the nuclear technologist to inject the radioactive tracer required for the SPECT/CT scan. After the injection, all staff should maintain 6 feet of distance from the participant whenever feasible.
5. The participant is seated in a separate room for the duration of the 150-minute wait-period.
6. During the wait-period, MESA staff administers the questionnaires.
7. Walk the participant to the scanner table 10 minutes prior to the scan acquisition time. This can be as early as 2.5 hrs after injection.
8. The nuclear technologist completes the SPECT/CT image acquisition which will take approximately 20 minutes.
9. The nuclear technologist completes the Planar image acquisition which will take approximately 7 minutes.
10. The nuclear technologist fills out SPECT/CT Procedure Completion form. MESA staff enters data from the form into the data entry system, ensuring that the nuclear technologist's assigned MESA staff ID number is recorded.
11. MESA staff addresses any remaining participant questions or concerns before they depart.
12. MESA staff or nuclear technologist transfers SPECT/CT scan images to the BWH-NRC through WebPax software as detailed in the study Manual of Operations.

14.4.3. Potential Risks

- **Radiation Risks:** The estimated mean effective radiation dose from this ^{99m}Tc-PYP SPECT/CT study is 4.3 mSv. The annual average effective total natural background radiation dose worldwide is 3.1 mSv, related to both cosmic rays and terrestrial radionuclides. Therefore, the anticipated radiation dose associated with ^{99m}Tc-PYP SPECT/CT in this study will be comparable to natural background radiation for 16.6 months.