CARDIA Study

Year 30 Exam ▪ Exam IX

PROTOCOL

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1. Introduction

The Coronary Artery Risk Development in (Young) Adults (CARDIA) Study was initiated in 1984 by the National Heart, Lung, and Blood Institute (NHLBI) to assist in providing a better understanding of the trends and determinants of coronary heart disease (CHD) in the United States (US). The study began by focusing on young adults - persons 18 to 30 years of age at the time of the Year 0 (Y0) baseline screening, undertaken between March 1985 and June 1986. A random selection of 5,115 black and white men and women identified by each of the four CARDIA field centers constituted the cohort. The selection procedures, study populations, and methods used are described elsewhere(The CARDIA Coordinating Center, February 1985 #1798;The CARDIA Study Steering Committee, February 1985 #1805) and the consequent baseline results have been reported[1,2].

Subsequent exams were held at Y2 (June 1987-June 1988), Y5 (June 1990-June 1991), Y7 (June 1992-June 1993), Y10 (June 1995-June 1996), Y15 (June 2000-June 2001), Y20 (June 2005-August 2006), and Y25 (June 2010-August 2011). The contents and methods used in these prior examinations are described in the previous CARDIA manuals of operation (MOOs) and protocols.[3-17] Follow-up examinations at Y2, Y5, Y7, Y10, Y15, Y20, and Y25 achieved high retention, collected a rich set of high-quality data and stored specimens bearing on the risk factors and possible causes of cardiovascular disease (CVD), and led to 589 peer-reviewed publications (as of January 2015).

This document describes the protocol for the Y30 Exam (also referred to as Exam IX) undertaken from June 2015, with initial target end-date of May 2016, on the CARDIA cohort, whose members are now 48-60 years old. We propose to re-examine at least 72% of those surviving (3,445 participants study-wide) at a time when risk factors and subclinical abnormalities are prevalent and clinical events continue to emerge. This document provides the rationale, objectives, and methods for the Y30 Exam for the CARDIA cohort. Abnormalities in cardiac structure and function, 25-year trajectories in cardiac structure and function, and possible differences in these abnormalities and trajectories between white and black adults are major focuses for this exam cycle. The impact of timing and varying levels of risk factors acquired throughout young adulthood and middle-age on the occurrence and progression of myocardial dysfunction and early clinical events, and the elucidation of the basis for racial disparities in the development of CVD risk factors, subclinical cardiac abnormalities, and potentially early clinical events are further objectives for Y30.

Y30 core exam data, as well as findings in separately-funded ancillary studies, will allow examination of the antecedents and prevalence of subclinical atherosclerosis in diverse populations, analyses of the role of predisposing genetic traits in the presence of behavioral and physiologic risk factors in order to detect genotype-by-environment interactions, and determination of how these differ in men and women, and in blacks and whites. These findings will increase understanding of the 30-year antecedents of middle-aged risk factors and subclinical disease. This knowledge will be important in designing preventive medicine policies and interventional studies that address the growing epidemic of obesity and reduce the public health burden of CVD, and that are tailored to specific population subgroups and settings where they will be most effective.

2. Study Objectives

The Y30 Exam offers a unique opportunity to examine the evolution of lifestyle and risk factor profiles from young adulthood into middle age, and their effect on both incidence and progression of subclinical disease, and the incidence of clinical events. Of particular importance in this context is the emerging epidemic of obesity and its role in the pathogenesis of diabetes mellitus (DM), high blood pressure (HBP), dyslipidemia, and CVD. More generally, we will assess how phenotypic expression of genetic

traits, environmental exposures, and ethnicity operate in conjunction with inflammatory markers and other intermediaries to influence atherosclerosis and its early manifestations.

A large body of data has been collected over these 30 years regarding important influences on cardiovascular health (CVH), including the social and physical environments, behaviors, genomics, biological risk factors and biomarkers, healthcare access, and risk factor management. Furthermore, we have collected substantial data on the presence and extent of subclinical disease, including carotid intima-media thickness (IMT), coronary artery calcification (CAC), abdominal aorto-iliac calcification, and measures from echocardiography. As of January 2015, there have been 356 deaths reported, with substantial numbers of clinical endpoints ascertained at examinations, e.g., hypertension and DM, and 272 CVD endpoints, including myocardial infarction (MI), stroke, and chronic heart failure (HF). These endpoints, plus available data and stored specimens, provide a rich platform for addressing critical unanswered questions regarding CVD prevention and CVH promotion.

We will continue follow-up contacts, endpoints surveillance, analyses and publications, ancillary studies activities, training of new investigators, and conduct the Y30 Exam on at least 3,445 participants (estimated as 72% of those still alive at exam start). The cohort (ages 48-60 at Y30) is increasingly experiencing clinical and subclinical disease, and offers a unique opportunity to examine the evolution of lifestyle and risk factor profiles from young adulthood into middle age, and their effect on both disease incidence and progression. Of particular importance in this context is the continuing epidemic of obesity and its health outcomes. Likewise, preliminary evidence in the CARDIA cohort has suggested the importance of cardiac structure and systolic and diastolic function as critical indicators of subclinical and impending clinical CVD, with potentially important sex and race differences. The long-term trajectories and associations of antecedent risk factors and risk markers have not been elucidated, however.

Core Contract Major Objectives

The Y30 objectives are to:

1. **Determine abnormalities in cardiac structure and function that exist in a community-based sample of mid-to-late middle-aged adults; to utilize novel measures to better understand myocardial dysfunction; to examine 25-year trajectories in cardiac structure and function; and to assess whether these abnormalities and trajectories differ between white and black adults**: CARDIA will conduct echocardiography during the Y30 Exam to enable assessment of cardiac structure and function, including changes in cardiac structure and function with aging. CARDIA will continue to incorporate novel approaches to echocardiography as well as assessing measures that will enable evaluation of change from the Y5 and Y25 echocardiography exams.
2. **Assess the impact of timing and varying levels of risk factors acquired throughout young adulthood and middle age on the occurrence and progression of myocardial dysfunction and early clinical events**: CARDIA will study the impact of traditional and novel risk factors acquired throughout young adulthood on the development of subclinical abnormalities in mid-life. Predictors of changes in left ventricular structure and function (measured by echocardiography) will be examined, as well as other subclinical abnormalities such as microalbuminuria. As clinical events continue to accrue, CARDIA will also continue to address the associations of these risk factors with clinical events.
3. **Use a comprehensive approach to elucidate the basis for racial disparities in the development of cardiovascular disease risk factors, subclinical cardiac abnormalities, and potentially early clinical events:** CARDIA will assess the basis for differences between blacks and whites in the development of subclinical abnormalities. Racial differences in predictors and their varying consequences on the development of subclinical abnormalities will be explored. Potential predictors of interest include

psychosocial characteristics, lifestyle behaviors, and biological attributes. As clinical events continue to accrue, CARDIA will continue to address racial differences in clinical abnormalities.

4. **Continue cohort follow-up for cardiovascular events, including heart failure, coronary heart disease, stroke, and atrial fibrillation:** CARDIA will continue to ascertain and validate important clinical endpoints. As the cohort has aged, the number of clinical events has increased. We will explore the relationships of risk factor and subclinical disease trajectories with the incidence of clinically important events, and racial differences in these events.
5. **Provide a platform for ancillary studies particularly to the cohort Year 30 examination, a training ground for new investigators, and data sharing:** CARDIA will continue to replenish and further enrich its rich repository of data and specimens to allow additional in-depth ancillary studies on how changes that occur in young adulthood contribute to the development of cardiovascular and other abnormalities in mid-life, and how these changes differ by race, sex, and other factors. By doing so, CARDIA will continue to nurture a vibrant training and data sharing environment.

Major Y30 Exam Ancillary Studies' Objectives

The following scientific objectives relate to ancillary studies designed and funded as integrated components of the Y30 examination.

Cognitive Assessment (1 R01 HL122658-01)

Increase understanding of cognitive function in mid-life and identify risk factors extending from early adulthood. CARDIA will assess cognitive function at the Y30 examination, repeating three tests performed at the Y25 Examination and administering four additional tests. This assessment will enable the determination of 5-year changes in cognitive function and the predictors of these changes. The relationship of 30-year trajectories of key vascular, metabolic and lifestyle factors to midlife cognitive function and 5-year change will be determined, as well as related genetic risk scores. The relationship of brain region volumes and characteristics measured by magnetic resonance imaging (MRI) to level and change in cognition in adulthood will also be assessed.

Pulmonary Function Assessment (R01 HL122477)

Identify subclinical manifestations of COPD and other lung disease and explore why heart and lung disease co-exist. CARDIA will assess pre- and post-bronchodilator spirometry in order to evaluate factors in young adults that predict incident chronic obstructive pulmonary disease (COPD) and/or restriction, to determine whether incident COPD and incident restriction are associated with distinct cardiac structural and functional changes, and to determine the lung structural and intrathoracic vascular changes associated with incident COPD and incident restriction. This assessment will also enable longitudinal studies in comparison to the lung function measurements made at Y0, Y2, Y5, Y7, Y10, and Y20.

Other CARDIA Objectives

In addition, objectives from previous examinations that will continue to be addressed are:

- **To measure physiologic correlates of blood pressure and hypertension:**
  - Compare relationships between physiologic measures and blood pressure (BP) in blacks and whites;
  - Determine relationships between physiologic correlates of BP and other cohort characteristics; and
  - Identify and quantify subclinical conditions associated with elevated BP, such as early renal dysfunction and structural vascular changes.
- **To assess the levels and determinants of obesity, body fat distribution, and weight change.**

- **To continue the follow up to:**
  - Identify correlates of smoking cessation and other changes in smoking behavior.
  - Identify correlates of changes in blood lipid and lipoprotein levels.
  - Study the relationship of levels and changes in blood lipids and lipoproteins to the early stages of both cardiovascular and non-CVDs.
- **To continue to evaluate individual and group-level correlates of risk factor awareness, treatment, and control, including factors related to health care access and utilization.**

Secondary objectives for this exam are:

- **To develop and apply effective methods of follow-up during these middle-aged adult years to re-examine at Y30 Exam at least 72% of the surviving participants.**
- **To assess risk factors for CVD and other endpoints in these participants with high accuracy and precision.**
- **To continue developing and using the most appropriate analytic methods, especially longitudinal data analysis methods, for example utilizing both cross-sectional and cohort data to assess age-related trends in risk factors during young and middle adulthood.**

3. Exam Components

Exam components for all CARDIA core exams, including Y30, are listed in Table 1. (Ancillary studies and substudies are not included in this table.)

[TABLE 1 BEGINS NEXT PAGE]

Table 1. Schedule of components by CARDIA exam

	Year/Exam[1]								
	1985	1987	1990	1992	1995	2000	2005	2010	2015
	0	2	5	7	10	15	20	25	30
BLOOD PRESSURE									
Resting	X	X	X	X	X	X	X	X	X
Standing and Reactivity	-	X	-	-	-	-	-	-	-
LABORATORY MEASURES									
Genetic									
Stored DNA	-	-	X	-	X	X	X	X	X
Stored Cells for Cell Immortalization	-	-	-	-	-	X	-	-	-
Plasma									
Lipids	X	X	X	X	X	X	X	X	X
Lipoproteins	X	X	X	X	X	X	X	X	-
Apoproteins	X	X	-	-	-	-	-	-	-
CBC	X	-	-	-	-	-	-	-	-
Lp(a)	-	-	X	-	-	-	-	-	-
Fibrinogen	-	-	X	-	-	-	X	-	-
ApoE Phenotype	-	-	-	X	-	-	-	-	-
Stored Plasma	-	X	X	X	X	X	X	X	X
C-Reactive Protein	-	-	-	X	-	X	X	X	-
Interleukin-6	-	-	-	-	-	-	X	-	-
Serum									
Cotinine	X	-	-	-		-	-	-	-
SMAC 12	X	-	-	-	-	-	-	-	-
Fasting Insulin	X	-	-	X	X	X	X	X	X
Fasting Glucose	X	-	-	X	X	X	X	X	X
Oral Glucose Tolerance Test	-	-	-	-	X	-	X	X	-
Stored Serum	X	X	X	X	X	X	X	X	-
GGT	X	-	-	-	X	-	-	-	-
Fasting Creatinine	X	-	-	-	X	X	X	X	X
Uric Acid	X	-	-	-	X	X	-	-	-
Urine									
Creatinine	-	-	-	-	X	X	X	X	X
Albumin	-	-	-	-	X	X	X	X	X
Stored Urine									X
ANTHROPOMETRY									
Height	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X
Skinfolds	X	X	X	X	X	-	-	-	-
Chest Circumference	-	X	X	-	-	-	-	-	-
Waist Circumference	X	X	X	X	X	X	X	X	X

[1] year of study indicates when original data collection occurred; assay or coding may occur later

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	Year/Exam[1]								
	1985	1987	1990	1992	1995	2000	2005	2010	2015
	0	2	5	7	10	15	20	25	30
Hip Circumference	X	X	X	X	X	-	-	X	X
Thigh Circumference	-	-	-	X	-	-	-	-	-
Elbow Breadth	X	X	-	-	-	-	-	-	-
Shoulder Breadth	-	X	-	-	-	-	-	-	-
Sitting Height	-	X	-	-	-	-	-	-	-
Toenails	-	X	-	-	-	-	-	-	-
Eye Color	-	-	X	-	-	-	-	-	-
Skin Reflectance	-	-	-	X	-	-	-	-	-
MEDICAL HISTORY									
Medical History	X	X	X	X	X	X	X	X	X
Illicit Drug Use	X	X	X	X	X	X	X	X	X
Death Certificate	-	X	X	X	X	X	X	X	X
Mortal Events	-	-	-	X	X	X	X	X	X
Safety Questionnaire	-	X	-	-	-	-	-	-	-
Interim Hospitalization	-	X	X	X	X	X	X	X	X
Chest Pain/Palpitations	-	-	X	-	X	-	-	-	-
History of Lung Problems	X	X	-	-	X	X	X	-	-
Oral Contraceptive History	-	-	-	-	X	-	-	-	-
Women's Reproductive Health	-	-	-	-	-	X	X	X	X
Sleep Habits	-	-	-	-	-	X	X	-	-
Tobacco	X	X	X	X	X	X	X	X	X
Alcohol	X	X	X	X	X	X	X	X	X
Weight History	X	X	-	-	-	-	-	X	X
Sociodemographics	X	X	X	X	X	X	X	X	X
FAMILY HISTORY QUESTIONNAIRE									
Family History	X	-	X	-	X	-	-	X	-
PHYSICAL ACTIVITY/FITNESS									
7-Day Physical Activity	X	-	-	-	-	-	-	-	-
Physical Activity Questionnaire	X	X	X	X	X	X	X	X	X
Graded Exercise Test and ECG	X	-	-	X	-	-	-	-	-
Baecke Questionnaire	-	-	X	-	X	-	-	-	-
Household Chores	-	-	-	X	X	-	-	-	-
Sedentary Behavior Questionnaire	-	-	-	-	-	-	-	X	X
Neighborhood Env. Questionnaire	-	-	-	-	-	-	-	-	X
NUTRIENT INTAKE QUESTIONNAIRES									
CARDIA Diet History	X	-	-	X	-	-	X	-	-
Food Frequency	-	X	-	-	-	-	-	-	-
OBESITY QUESTIONNAIRES									
Knowledge, Attitude, Behavior	-	-	X	-	-	-	-	-	-
Self Image	-	-	-	X	-	-	-	-	-
Weight Change	-	-	-	X	X	-	-	X	X

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Table 1. Schedule of components by CARDIA exam

	Year/Exam[1]								
	1985	1987	1990	1992	1995	2000	2005	2010	2015
	0	2	5	7	10	15	20	25	30
Dietary Practices, Behaviors, and Attitudes	-	-	-	X	X	X	X	X	X
Self-Efficacy for Eating Behavioral Scale	-	-	-	-	X	-	-	-	-
Binge Eating Disorder	-	-	-	-	X	-	-	-	-
Weight Control	-	-	-	-	-	-	-	X	-
Dietary Habits	-	-	-	-	-	-	X	-	X
Beverages Questionnaire	-	-	-	-	-	-	-	X	X
PSYCHOSOCIAL QUESTIONNAIRES									
Cook-Medley	X	-	X	-	-	-	-	-	-
Life Events	X	X	-	-	-	-	-	-	-
Social Support A	X	X	-	-	-	-	X[2]	-	-
Social Support and Conflict	-	-	-	-	-	X	X	-	-
Framingham Type A	X	X	-	-	-	-	X	-	-
John Henryism	X	-	-	-	-	-	-	-	-
Type A-B Interview	X	-	-	-	-	-	-	-	-
Karasek Job Strain	-	X	-	-	X	-	-	-	-
CES-Depression	-	-	X	-	X	X	X	X	X
Anger-In Expression	-	-	X	-	-	-	X	-	-
Speilberger Trait Anxiety	-	-	X	-	-	-	-	-	-
Discrimination	-	-	-	X	-	X	-	X	X
Chronic Burden	-	-	-	-	-	X	X	X	X
Quality of Life	-	-	-	-	-	X	X	X	X
Social Network	-	-	-	-	-	X	X	X	-
Caregiving Stress	-	-	-	-	-	-	X	-	-
Goal Striving Stress	-	-	-	-	-	-	X	-	-
Subjective Standing	-	-	-	-	-	-	X	-	-
Neighborhood Cohesion	-	-	-	-	-	-	X	-	X
Geocoding	-	-	-	-	X	X	X	X	-
COGNITIVE FUNCTION QUESTIONNAIRES									
Rey Auditory-Verbal Learning Test	-	-	-	-	-	-	-	X	-
Digit Symbol Substitution Test	-	-	-	-	-	-	-	X	-
Stroop Test	-	-	-	-	-	-	-	X	-
PULMONARY FUNCTION									
Testing	X	X	X	-	X	-	X	-	-
Questionnaire	X	X	-	-	X	X	X	-	-
ECHOCARDIOGRAPHY									
LV Mass	-	-	X	-	-	-	-	X	X
Systolic, Diastolic Function	-	-	X	-	-	-	-	X	X

[2] subset of items from this questionnaire only

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Table 1. Schedule of components by CARDIA exam

	Year/Exam[1]								
	1985	1987	1990	1992	1995	2000	2005	2010	2015
	0	2	5	7	10	15	20	25	30
CORONARY CALCIUM	-	-	-	-	-	X	X	-	-
ABDOMINAL CT									
Abdominal Adipose Tissue, Abdominal Muscle, and Hepatic Steatosis	-	-	-	-	-	-	-	X	-
CAROTID IMT	-	-	-	-	-	-	X	-	-
GENETIC STUDIES									
Genotype by Environmental Interaction	-	-	-	-	-	X	X	X	-

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4. Study Design

4.1. Population

Of the 5,115 participants, between the ages of 18-30, enrolled by CARDIA field centers in 1985-86, 45.5% were men, 51.5% were black and 48.5% white. It is anticipated that approximately 3,445 (~72% of those surviving) will be examined during the Y30 Exam at the four field centers: 780 in Birmingham, 798 in Chicago, 883 in Minneapolis, and 984 in Oakland. Participants will be invited to participate in the Y30 Exam during its entire period which extends approximately from June 2015 through May 2016.

4.2. Informed Consent

All participants will go through an informed consent process prior to initiating the Y30 Exam. Each field center has a consent form approved by their intuitional review board (IRB), which while sharing some common consent sections, vary slightly due to individual IRB regulations. Each form explains the procedures of the Y30 Exam with accompanying risks and benefits. After the participant has read and had any questions answered about the consent form, they are asked to individually check or initial each consent activity as follows (wording and format varying slightly by field center):

- Participate in the CARDIA Y30 Exam to measure weight, cholesterol, BP, and other physical factors related to heart disease, including echocardiography.
- Donate blood and urine samples to be frozen, stored, and used for future research.
- Allow CARDIA investigators to extract DNA and RNA from blood samples to be analyzed for genetic information related to heart disease, obesity, BP, DM and other conditions affecting people in midlife, understanding that the information will be kept confidential at all times.
- Allow genetic/DNA samples and data to be released, for research purposes only, to other qualified non-CARDIA investigators.
- Allow researchers from private or non-profit organizations to have access, in a way that cannot identify the participant, to specimens, DNA or other CARDIA information in order to develop diagnostic laboratory tests, medications, or other therapies that could benefit many people.
- Specify whether the participant wishes to be informed of genetic findings for which a known treatment to prevent or lessen an important disease exists.

Consent is sought for two ancillary studies: 1) Lung Function Decline and Cardiopulmonary Disease Risk from Young Adulthood to Middle Age (CARDIA Lung Study) (Kalhan, PI) and 2) Determinants of midlife cognitive function in the CARDIA study (CARDIA Cognition Study) (Sidney, Yaffe, PIs); and one substudy: Brain MRI Substudy (Launer, PI), at the same time as the core consent. Field center consent form configuration:

BHM—one consent form for CARDIA core exam; one consent form for each ancillary study; and one consent form for the Brain MRI Substudy

CHI—one consent form for CARDIA core exam; and one consent form for each ancillary study

MIN—one consent form for CARDIA core exam; one consent form for each ancillary study; and one consent form for the Brain MRI Substudy

OAK—one consent form for CARDIA core exam; one consent form for each ancillary study; and one consent form for Brain MRI Substudy

4.3. Data Collected

The Y30 Exam measures numerous risk factors, including BP; anthropometry; blood concentrations of glucose, insulin, lipids (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides), and creatinine; urine concentrations of albumin and creatinine; dietary habits, physical activity; smoking behavior; alcohol use; sociodemographic factors; and psychosocial factors in order to assess cohort changes and trends. In order to enhance comparability across exams, these measurements will use the same data collection and analysis techniques that were previously used, whenever possible, as described for key measures in Section 5. Data on socioeconomic status (SES), alcohol intake, healthcare utilization, medication use, recent hospitalizations, and medical diagnoses are collected using the same methods and questionnaires as described in previous years' exam manuals. Y30 will enrich data on environmental exposures by continuing to measure weight control practices, sugar-sweetened beverage intake, and sedentary behavior. Neurocognitive functioning again will be assessed at the Y30 Exam, making it possible to study changes that occur in neurocognitive functioning with aging, as participants will be age 48-60 years at Y30. An echocardiography examination again will be conducted at each of the four field centers to measure several indicators of cardiac structure and function, enabling an examination of longitudinal changes in cardiac structure and function. Brain structure and function again will be assessed with MRI, in the same subset of participants as at Y25. In the event a participant cannot or will not participate in the follow-up MRI, a replacement will be identified in the non-MRI sub-cohort. All reasonable attempts will be made to ensure the new participant is from the same sex-race group as the participant from the original sample. If matching is not possible then a new participant should be identified on a rolling basis.

In addition to the measurements mentioned above, numerous aliquots of serum and plasma,

DNA isolation, RNA isolation, and urine are set aside. These will be inventoried with the previous exams' specimens for future studies of associations and longitudinal trends in new analytes and potential risk factors as they are discovered.

4.4. Comparability Studies

A series of comparability studies will be conducted to ensure that Y30 measures are consistent with measures from previous years. An explanation of these studies follows.

Echocardiography—A comparability study will be conducted to ensure agreement among readers for the CARDIA Y30 echocardiography readings. This study will be performed at the Echocardiography Reading Center (ERC) at the Johns Hopkins University (JHU) School of Medicine. A random sample of 100 CARDIA Y25 2D echocardiography images will be re-read by all three CARDIA Y30 ERC readers. The random sample will be stratified by field center (25% from each), gender (50% of each), and BMI (30% > 30 kg/m^2) and selected by the laboratory of Dr. Christopher Cox at the JHU Bloomberg School of Public Health using a random number generator. The purpose of this comparability study is to examine inter-reader variability for 2D echocardiography measurements from Y25 between Y25 and Y30 ERC readers. The variables that will be re-read and compared are the nine main echo parameters used in analysis.

Specifically, they are:

1. Two Dimension - Four Chamber LV End Diastolic Volume
2. Two Dimension - Four Chamber LV End Systolic Volume
3. Two Dimension - Four Chamber LV Ejection Fraction
4. MMODE Aorta and Left Atrium - Aortic Root
5. MMODE Aorta and Left Atrium - Left Atrium Internal Dimensions
6. MMODE LV and RV - LV End Diastolic Volume

7. MMODE LV and RV - LV End Systolic Volume
8. MMODE LV and RV - LV Ejection Fraction
9. MMODE LV and RV - LV Mass

The results will be presented to the Design & Analysis and Quality Control (QC) Subcommittees in July 2015. The Y30 echocardiography machine and software are essentially the same as Y25, with minor upgrades to user interfaces only. Therefore, no comparability study is needed to compare machine or software between the two exams.

Laboratory Assays—Comparability studies will be conducted for the following assays: serum glucose, serum insulin, serum creatinine, plasma lipids (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides), urine albumin, and urine creatinine (in the form of the urine/creatinine ratio). For serum glucose, serum insulin, serum creatinine, and plasma lipids, 50 samples from each sex-race group – 10 from each sex-race-specific quintile of the stratified random sample, supplementing with high and low values – will be selected. Samples for urine creatinine/albumin ratio will include the same person for urine creatinine and albumin (50 samples from each sex-race group – 10 from each sex-race-specific quintile of the stratified random sample), supplementing with 10 from the top 10% of the ratio.

5. Rationale and Methods

We will conduct the Y30 examination of the Coronary Artery Risk Development in Young Adults (CARDIA) cohort between June 2015 and June 2016 and follow the cohort for events ascertainment through June 2018. A large body of data has been collected over the past 25 years regarding important influences on cardiovascular health (CVH), including the social and physical environments, behaviors, genomics, biological risk factors and biomarkers, healthcare access, and risk factor management. Furthermore, we have collected substantial data on the presence and extent of subclinical disease, including carotid intima-media thickness (IMT), coronary artery calcification (CAC), abdominal aorto-iliac calcification, and measures from echocardiography. We will supplement those data with data collected during the Year 30 exam and continued follow-up. Below, we describe the specific rationale and methods for collecting these data elements. We begin with a focus on the major objectives, then we include the rationale and methods for collecting other data elements, including those to be collected using resources provided by the integrated ancillary studies.

CARDIA is in an increasingly powerful position to make an important impact on our ability to prevent CVD and promote CVH, and the current investigative team is well position to take full advantage of this opportunity. Of particular importance in this context is the continuing epidemic of obesity and its health outcomes. Likewise, preliminary evidence in the CARDIA cohort has suggested the importance of cardiac structure and systolic and diastolic function as critical indicators of subclinical and impending clinical CVD, with potentially important sex and race differences. The long-term trajectories and associations of antecedent risk factors and risk markers have not been elucidated, making the data to be collected during the Y30 exam of substantial importance.

5.1. Rationale and Methods for Major Scientific Objectives

5.1.1 Scientific Objective 1

To determine abnormalities in cardiac structure and function that exist in a community-based sample of mid-to-late middle-aged adults; to use novel measures to better understand myocardial dysfunction; to

examine 25-year trajectories in cardiac structure and function; and to assess whether these abnormalities and trajectories differ between white and black adults.

Background. HF is a complex syndrome of clinical signs and symptoms indicating that the heart cannot meet the circulatory demands of the body because of altered or inefficient pump function. HF is caused by several underlying processes and diseases. The American College of Cardiology (ACC) and American Heart Association (AHA) have defined stages to classify the natural history of HF (Table 2)[18].

Table 2. Stages of chronic heart failure according to ACC/AHA guidelines[18]

Stage	Definition	Examples
0	No risk factors for HF	
A	At risk for HF but with no structural heart disease or symptoms of HF	Hypertension, CAD, DM, obesity
B	Structural heart disease without signs or symptoms of HF	Prior MI, echo or ECG evidence of LV remodeling including LVH or reduced EF, asymptomatic valvular disease, +/- diastolic dysfunction
C	Structural heart disease with prior or current symptoms of HF	Shortness of breath, fatigue, reduced exercise tolerance, edema
D	Refractory HF requiring specialized interventions	Symptoms at rest despite maximal medical therapy

Abbreviations: CAD denotes coronary artery disease; ECG, electrocardiographic; EF, ejection fraction; HF, heart failure; LV, left ventricular; LVH, LV hypertrophy; MI, myocardial infarction.

Data from Olmsted County[19] suggest that the prevalences of HF stages are 47% (Stage 0), 28% (Stage A), 23% (Stage B), 4% (Stage C), and 0% (Stage D) among 45-54 year olds, with a rightward shift among older age groups. Compared with Stage 0, men with Stage B HF, or asymptomatic structural heart disease, had a hazard ratio (HR) of 4.0 (95% CI, 1.3-11.9) for death over 5.5 years' median follow-up, but power was limited to address this issue in women (HR 0.9, 0.3-2.7)[19]. This study sample was small and exclusively white, with few events and limited covariates. Further work is therefore needed to define potential race/ethnic and sex differences in incidence, prevalence, and prognosis of important preclinical findings (i.e., Stage B HF) in middle-aged adults.

HF affects about 5.7 million Americans currently, including more men than women and more blacks than whites, with 670,000 new cases and over one million hospitalizations annually[20]. The lifetime risk for HF after age 40 is estimated to be 20%-30% in whites[21]. HF prevalence appears to be stable or increasing and occurring at somewhat earlier ages than in past decades, especially in blacks[20]. Indeed, Bibbins-Domingo et al.[22] recently described that over the first 20 years of CARDIA, the cumulative incidence of HF before age 50 years was 1% in blacks and <0.1% in whites (P=0.001; mean age at onset 39 years). Among blacks, independent predictors (measured at Y0) of HF occurring 15 years later included higher diastolic blood pressure (DBP) and body mass index (BMI), lower HDL-cholesterol, and kidney disease. Depressed left ventricular (LV) systolic function at Y5 was independently associated with the development of HF on average 10 years later (HR for abnormal systolic function, 36.9, 95% CI 6.9 - 198; HR for borderline systolic function, 3.5, 1.2 to 10.2)[22]. These early CARDIA data indicate an urgent need to understand the natural history of development of myocardial dysfunction (systolic and diastolic) and its association with potentially modifiable risk factors in younger adults.

Hypothesis: We will observe important changes in LV systolic and diastolic function at Y30 compared to Y5 and Y25, in part related to physiologic changes that typically accelerate around age 50-55 years and

that are related to longitudinal levels and trajectories of BP, body weight, and inflammation. Additional specific hypotheses are listed in the Echocardiography section below (5.3.).

Preliminary Findings. Non-invasive echocardiographic measurements of cardiac structure and function, including LV mass (LVM), LV geometry, LV ejection fraction (EF)/fractional shortening (FS), left atrium (LA) size, and valvular assessment, provide important information and serve as valuable prognostic indicators of future clinical CVD events, particularly HF. Analyses of echocardiograms from Y5 and Y10 (half the cohort) identified that BMI and BP are risk factors associated with early findings of higher LVM, abnormal geometry, and change in LVM in young adults[23]. The presence of diastolic dysfunction at Y5 was associated with substantially increased risk for a composite endpoint of all-cause mortality, MI, HF, and stroke in young adults. Using a validated algorithm to define diastolic dysfunction from standard echo measures (including mitral inflow velocities), we observed that severe diastolic dysfunction (SDD) was present in 1.1% and abnormal relaxation (AR) was present in 9.3% of the Y5 cohort. After multivariable adjustment, the HRs for the 15-year composite endpoint in participants with SDD and AR were 4.3 (95% CI, 2.0-9.3) and 1.6 (1.1-2.5), respectively[24]. Clearly, assessment of LV diastolic function, particularly through tissue Doppler methods, is useful. At Y25, CARDIA participants underwent echocardiographic examinations that included a novel measurement of LV myocardial strain by speckle tracking echocardiography (STE). Preliminary results indicate that this modality has promise to refine regional and global estimation of systolic and diastolic function. CARDIA Y25 echo findings indicated: 1) good correlations between LV volumes and EF measured by STE and standard measures, with greater precision using STE due to better definition of myocardial borders; 2) smoking in young adulthood is associated with reduced diastolic function 25 years later; and 3) baseline DBP and change in DBP, baseline BMI and change in BMI, male sex, and antecedent C-reactive protein (CRP) levels are associated with preclinical evidence of reduced LV systolic function (greater longitudinal systolic strain even in the setting of preserved EF) 25 years later. These findings strongly suggest dynamic changes during middle age in LV systolic and diastolic function that will now be followed by repeat measurement at Y30. Additional important information from prior and repeated echo measurements includes data on the population-based prevalence of hypertrophic cardiomyopathy, other cardiomyopathies, mitral valve prolapse (MVP), and valvular regurgitation.

Methods. With the ERC, we have developed and implemented the protocol to assure that high-quality imaging and image interpretation are obtained. See Section 5.3. for details. The Y30 echo evaluation includes direct comparison with measurements obtained at Y5 and Y25 of LV size and function; pulsed and color flow Doppler of the mitral, aortic, and tricuspid valves; and tissue Doppler imaging (obtained at Y25) to assess LV diastolic function. We are identifying the longitudinal determinants of LVM, LA diameter, LV geometry, EF/FS, and both long-term (Y5 to Y30) and short-term (Y25 to Y30) changes. We are also identifying the long-term and short-term progression and determinants of LV diastolic function (available from Y5 echo data, and more refined measures from Y25 tissue Doppler imaging) and short-term changes in LV myocardial strain (from speckle tracking images). We are examining the influences of race, sex, and a healthy lifestyle, with particular focus on widely prevalent findings of elevated BP and obesity. Further, we are examining the associations with clinical and subclinical coronary artery disease (CAD), now increasingly prevalent in CARDIA. We are studying longitudinal associations of these echo measurements with Y0, Y7, and Y20 graded exercise treadmill (GXT) performance; Y15, Y20, and Y25 CAC; Y20 carotid IMT; and other vascular and peripheral artery assessments measured over time in CARDIA. We are also examining cross-sectional correlations of echo measures with other measures of vascular health and disease at Y30. Finally, we are studying the natural history, progression, and correlates of valvular heart diseases (e.g., MVP, mitral and aortic regurgitation), hypertrophic

cardiomyopathy, and asymptomatic LV systolic dysfunction, and relate estimates of right ventricular (RV) pressure from tricuspid regurgitation velocity to prior and current assessments of obesity, pulmonary function, and tobacco use.

The sophisticated echocardiographic measurements obtained at Y25 provide an important baseline to assess the prognostic significance of these measures during the coming years as the CARDIA cohort ages and begins to have an increasing burden of HF and other CVD events. Acquisition of similar echocardiographic measures at Y30 provides important information regarding rates of change in these measures and their associations with antecedent, potentially modifiable risk factors, as well as subsequent clinical events, thereby informing potential avenues of CVD prevention and CVH promotion.

5.1.2 Scientific Objective 2

To assess the impact of timing and varying levels of risk factors acquired throughout young adulthood and middle-age on the occurrence and progression of myocardial dysfunction and early clinical events.

Rationale. Several prospective studies on CVD have shown that baseline CVD risk factors measured during young adulthood strongly predict long-term CVD events despite potential changes in subsequent risk factor status[25-29]. For example, the Precursor Study showed that serum cholesterol levels measured during medical school predicted incident CHD morbidity over the next 31 years[26]. Most of the higher serum cholesterol levels at baseline did not exceed clinical thresholds defining abnormal elevation. The Chicago Heart Association Detection Project in Industry observed similar BP and serum cholesterol associations with long-term outcomes[25,28,29]. These studies suggest that higher levels of CVD risk factors, although not clinically elevated, at young ages are associated with a higher risk for CVD outcomes in later years.

Longitudinal studies have reported that risk factor levels measured in the remote past are more predictive of subsequent CVD events than concurrent risk factor levels. For example, the Honolulu Heart Study found that cholesterol levels measured 10 years before baseline were more predictive of 12-year CHD incidence than those measured at baseline[30]. The Framingham Heart Study found similar results for remote antecedent BP levels[31].

Similar findings were observed for subclinical disease measures in CARDIA. Loria et al. reported that while Y15 risk factor levels (e.g., BP and LDL-cholesterol) are significantly associated with Y15 CAC, these relationships disappear with an adjustment for baseline (Y0) risk factor levels[32]. Conversely, the Y0 risk factor levels measured in young adulthood, although low, are significantly related to Y15 CAC with or without adjustment for the Y15 levels. Baseline risk factor levels also predicted CAC equally as well as averaged 15-year levels. CARDIA Y25 data confirmed these findings. Furthermore, Pletcher et al. demonstrated that pre-hypertension prior to 35 years of age is strongly associated with CAC later in life[33]. Similar findings were also observed for non-optimal lipid profiles[34]. These results demonstrated that higher risk factor levels in young adulthood, although not clinically high, may impact the development of subclinical CVD years later.

Hypotheses. It remains unclear why remote measurements are more predictive of CVD events than current assessments. One explanation is that risk factor levels at young ages may more appropriately reflect long-term exposure to elevated risk factor levels while medical or lifestyle interventions may have altered risk factor measurements obtained closer to events. Long-term exposure to elevated risk factor levels may cause irreversible end-organ damage. Risk factors (e.g., BP and cholesterol) may subsequently be lowered by medication to ideal levels, but the risk of CVD may still be higher than in those who have always had ideal risk factor levels. It is also possible that young adulthood is a more vulnerable period—when exposure to higher risk factor levels may be more likely to lead to end-organ

damage. These issues have never been clearly addressed and require sophisticated statistical and epidemiologic approaches, as well as specialized data resources. The risk factor data, echocardiography data, other subclinical data, and clinical CVD data collected during the 30 years of follow-up in CARDIA offer a unique opportunity to assess the impacts of the timing and levels of risk factors on cardiac function and subclinical and clinical CVD.

We list three hypotheses, illustrative of the many hypotheses that can be generated to approach this objective.

1. Cumulative levels of risk factors (e.g., BP, lipids, number of cigarettes, fasting glucose, and BMI) are associated with the occurrence and progression of subclinical atherosclerosis, myocardial dysfunction, and early CVD events, independent of medications and other relevant covariates (e.g., age, sex, race, education, and income).
2. Exposure to elevated risk factors during young adulthood is associated with the occurrence and progression of subclinical atherosclerosis, myocardial dysfunction, and with early cardiovascular events. The relationships are stronger than, and are independent of, the relationships between the concurrent risk factor levels and the above outcome variables.
3. After a risk factor for a subclinical or clinical disease endpoint has been abnormally high for a sufficient period, complete reversibility is lost, in that reduction of the risk factor level does not reduce the risk to that associated with persistently normal levels. Figuratively, "a point of no return," for example due to organ damage, will be reached.

Methods. Several general methods will be used. The outcome variables include continuous variables (e.g., LVM index [LVM [gm]/height [m]$^{2.7}$], end systolic volume, end diastolic volume, myocardial strain, EF, and estimated glomerular filtration rate (eGFR)), ordinal and dichotomized variables (e.g., diastolic dysfunction, left ventricular hypertrophy (LVH), and CAC), and failure time (HF, CHD, and CVD events). We will rely heavily on cross-tabulations, frequency counts, and standard regression methods, particularly Cox proportional hazards regression models and consideration of prediction from predictors measured at different examinations or combinations of examinations. In this section we propose several innovative statistical methods, providing some examples to illustrate the ideas. The methods apply to continuous outcomes (e.g., LVM index) using the identity link function, as well as other types of outcomes using nonlinear link functions, such as logistic regression.

1. Cumulative exposure to each risk factor over 30 years of follow-up. We will estimate the cumulative exposure to each risk factor during the 30 years of follow-up by calculating the "area under the exposure curve" of the risk factor levels measured nine times during the 30 years. The missing values will be imputed using the multiple imputation method based on a mixed effect regression model with all the information collected over time. The relationship between the cumulative exposure to each risk factor and the outcome variable will be analyzed using spline regression, adjusting for other relevant risk factors. The main purpose of this approach is to see whether there is a threshold of cumulative exposure to the risk factor beyond which the impact of the exposure to the risk factor on the outcome will be accelerated.

2. Cumulative exposure during different time periods. In this approach, we will divide the 30-year period of follow-up into three distinct 10-year time periods (Y0 to Y10, Y10 to Y20, and Y20 to Y30). For each period, we will calculate the cumulative exposure to each risk factor during that period. The relationship between the cumulative exposure during each period and the outcome of interest, adjusting for other risk factors, will be examined. In the final analysis, we will enter all three cumulative exposures (over the three periods) in the same model to assess the relative importance of the exposure to the risk factor during the different time periods. The results will help to understand the impact of the

risk factor exposures during different time periods on the outcome of interest. Since the cumulative exposure in each period is adjusted for the cumulative exposures in the other periods, we will note whether a given period is more vulnerable than others. We may repeat the analyses using different time periods (e.g., six 5-year time periods).

3. Nonparametric/Semiparametric Extensions. We will conduct semiparametric or nonparametric analysis to characterize the detailed dynamic effect of the time-dependent risk factors acquired throughout young adulthood and middle age on the various outcomes of interest. For continuous outcome Y, we will employ the varying coefficient model[35]:

$$Y = \beta_0(t) + \beta_1(t)X(t) + \varepsilon \quad \text{or} \quad Y = \beta_0(t) + \int_0^t X(u)du \times \beta_1(t) + \varepsilon,$$

where *t* is the study follow-up time and $X(t)$ is a time-varying risk factor level at time *t*. In the first model, $\beta_1(t)$ measures the effect on outcomes of risk factor level at time *t* while in the second model it measures the effect of the cumulative exposure up to time *t*. Both varying coefficient models provide the information about the critical time(s) or period(s) at which the risk factor level is important. Kernel smoothing and the sieve method will estimate the varying coefficient. In addition, *t* can also be the participants' age, and we may employ multiple imputations before fitting the varying coefficient model. It should be noted that these time varying models can also be applied to binary outcomes using the logit link function.

4. Timing and levels of risk factors and cardiovascular events. The varying coefficient model has been generalized for exponential-family distributed outcomes, longitudinal outcomes, and survival outcomes.[36-40] For example, to study the association between exposure or cumulative exposure and time to a cardiovascular event of interest, we will employ the following models:

$$h(t \mid X(u), 0 \le u < t) = h_0(t)\exp\{X(t) \times \beta(t)\},$$

$$h(t \mid X(u), 0 \le u < t) = h_0(t)\exp\{\int_0^t X(u)du \times \beta(t)\},$$

$$h(t \mid X(u), 0 \le u < t) = h_0(t)\exp\{\int_0^t X(u)\beta(u)du\},$$

or

where *h(t)* is the hazard function at time *t*, *t* is the study follow-up time or age, *X(t)* is a time-varying risk factor level, and $\beta(t)$ is the effect of the risk factor (in the first and third models) or the effect of the cumulative risk factor level up to time *t* (in the second model).

The first model examines the time-varying effect of the time-varying exposure at time *t*. The second model studies the effect of cumulative exposure up to time *t* on the hazard function. The third model studies the effect of risk factor level at different times between [0,*t*] on the hazard function at time *t*. Kernel smoothing and the sieve method will be used to fit the models[40].

Summary. Taken together, these proposed approaches, and others under consideration, will allow greater understanding of the potential importance, timing, and critical exposure periods of risk factor levels from young adulthood to middle age and the effects of these cumulative exposures on subclinical

end-organ and clinical CVD events. The new knowledge generated may identify the presence of critical points for intervening to prevent CVD and promote optimal CVH.

5.1.3 Scientific Objective 3

To utilize a comprehensive approach to elucidate the basis for racial disparities in the development of cardiovascular disease risk factors, subclinical cardiac abnormalities, and potentially early clinical events.

Rationale. Racial disparities continue to exist in CVD risk factors and outcomes in the US. Compared to whites, biological, behavioral and environmental factors that increase CVD risk are more common among blacks, including lower prevalence of ideal CVH, lower life expectancy, higher CVD death rates, higher rates of HF and stroke hospitalization, higher prevalence of hypertension and lower rates of control, and higher prevalence of DM and obesity[41]. While blacks have only slightly higher rates of ischemic CHD, they have dramatically higher rates of other types of CVD. Of particular concern are the increasing rates of stroke in young adults, especially among blacks[42-46], the excess HF documented in blacks in CARDIA[22], the greater rate of sudden cardiac death and lower survival in blacks than in whites[47], and the 3-times greater incidence of end-stage renal disease (ESRD) in blacks than in whites[48]. Besides race, health disparities also exist related to lower educational attainment, income, poverty, geographic location, and other factors[41].

Although these differences in disease rates by race may signal important differences in disease processes, the reasons for racial disparities in CVD are unclear. In some analyses, greater risk for clinical events among blacks is explained by a greater burden of CVD risk factors in blacks. For example in the Multi-Ethnic Study of Atherosclerosis (MESA) Study, blacks had the highest incidence of HF, and this finding was related to their greater prevalence of hypertension, DM, and lower SES[49]. The mechanisms of HF also differed by race/ethnicity, with interim MI having the least impact among blacks, and LVM increase having the greatest impact on Hispanics and whites. In the Atherosclerosis Risk in Communities (ARIC) Study, the proportion of CVD risk attributable to traditional risk factors was higher in blacks than in whites and became more pronounced over time, with the contribution of DM and hypertension being especially high in blacks and in women[50].

On the other hand, others have found persistent disparities unexplained by adjustment for risk factors. For example, in the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) clinical trial, among hypertensive patients with ECG LVH, blacks had substantially more incident HF clinical events than non-blacks, independent of the greater burden of HF risk factors, and while similar at baseline, blacks at Y2 had greater LVM, relative wall thickness, and lower LV midwall shortening in multivariable analyses[51]. In MESA, in comparison with whites, asymptomatic blacks and Hispanics had the least favorable patterns of subclinical myocardial function on MRI, after control for hypertension and LVM[52]. In the CARDIA Study, black race was significantly and independently related to relative LV wall thickness at Y25[53] and with maintenance of early repolarization over 20 years[54].

An independent relationship of race with CVD outcomes after adjustment for CVD risk factors could be due to biological, genetic, behavioral, environmental, or other factors. Biological differences may include, for example, differences in atherosclerotic plaque composition[55,56]. Biological differences in turn could be due to genetic differences between populations, as has been proposed for several CVD outcomes. For example, analyses of ancestry markers (admixture) have concluded that genetic effects may be important in determining CAC and carotid IMT among blacks in MESA[57]. In the HyperGEN Study, a chromosome 11 region containing myosin-binding protein C, mutations of which are linked to familial hypertrophic cardiomyopathy, demonstrated linkage with LV contractility in blacks[58], while a promoter polymorphism in the GNAQ gene has been associated with decreased survival time in black HF patients[59]. In CARDIA, African ancestry was inversely related to forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC), relationships that were replicated in the Health, Aging, and Body

Composition (Health ABC) Study and Cardiovascular Health Study (CHS) cohorts[60]. In several cohorts including CARDIA, the presence of sickle cell trait was associated with an increased risk of chronic kidney disease (CKD), decline in eGFR, and albuminuria compared with non-carriers, suggesting an association with the higher risk of kidney disease in blacks[61]. While the physiologic effects of genetic risk factors on racial disparities in health are still poorly understood, there is some evidence that genetic information can be successfully incorporated into disparity research to reduce measurement and selection bias[62].

Although physiologic and genetic differences between racial groups exist, race is a social construct that defies an exclusively biological definition and includes social, historical, cultural, and environmental dimensions that are highly correlated with self-reported race and with genetic markers of ancestry[63]. Differential behaviors such as dietary and physical activity patterns, psychosocial factors such as coping with stress, racial discrimination, hostility, and environment differences, such as in the built environment and social cohesion, may result. Longer, earlier exposure to risk factors unaccounted for in analyses of middle-aged and older individuals is one source of unmeasured confounding in typical studies of incidence of clinical events, and in many cross-sectional studies of subclinical disease. For example, many studies have used LVM as a proxy for long-term exposure to elevated BP, ignoring differences in susceptibility to BP expressed by LV remodeling.

Addressing the basis for racial disparities in health outcomes is thus a complex undertaking. Diez-Rouz's review of conceptual approaches to the study of health disparities suggests that there are several models (e.g., genetic, fundamental cause, pathways, and interaction), but that it is important to consider the integration of multiple approaches to addressing questions of causation[63]. With its long-term follow-up beginning in early adulthood with a broad set of biologic, genetic, behavioral, and environmental measures, CARDIA is uniquely able to address these issues. As shown in Figure 1, we have developed a conceptual model focusing on myocardial dysfunction and HF, but adaptable to other CVD endpoints. The model integrates Vasan's model of the role of hypertension in HF[64], with more recent data on HF risk factors[65-72], with the ACCF/AHA HF staging system[73], and with conceptual approaches recommended by Diez-Rouz. The model flows from health to disease, and acknowledges the complex construct that is race/ethnicity. For example, adverse physical (e.g., air pollution and built) and social environments constrain behaviors, and these two sets of influences interact with the genome to perturb biology, represented by the biological risk factors and disease processes; race is associated with these three influences. Race may also affect more downstream processes, including healthcare access and quality, which influence risk factor and disease detection and management, and treatment effectiveness and adherence, which affect clinical events.

In addition to 30 years of data on biological risk factors, CARDIA will have collected a wealth of data on psychosocial, behavioral, socioeconomic, and environmental factors including depressive symptoms, experience of discrimination, income, education, and healthcare access, with longitudinal measurements of many of these. For example, CARDIA has observed racial differences in health care access and associations with risk factors and their control[74,75]; associations of racial discrimination with health behaviors, including smoking, alcohol consumption, and physical activity[76]; and relationships between fast food consumption and neighborhood fast food availability among low-income participants[77].

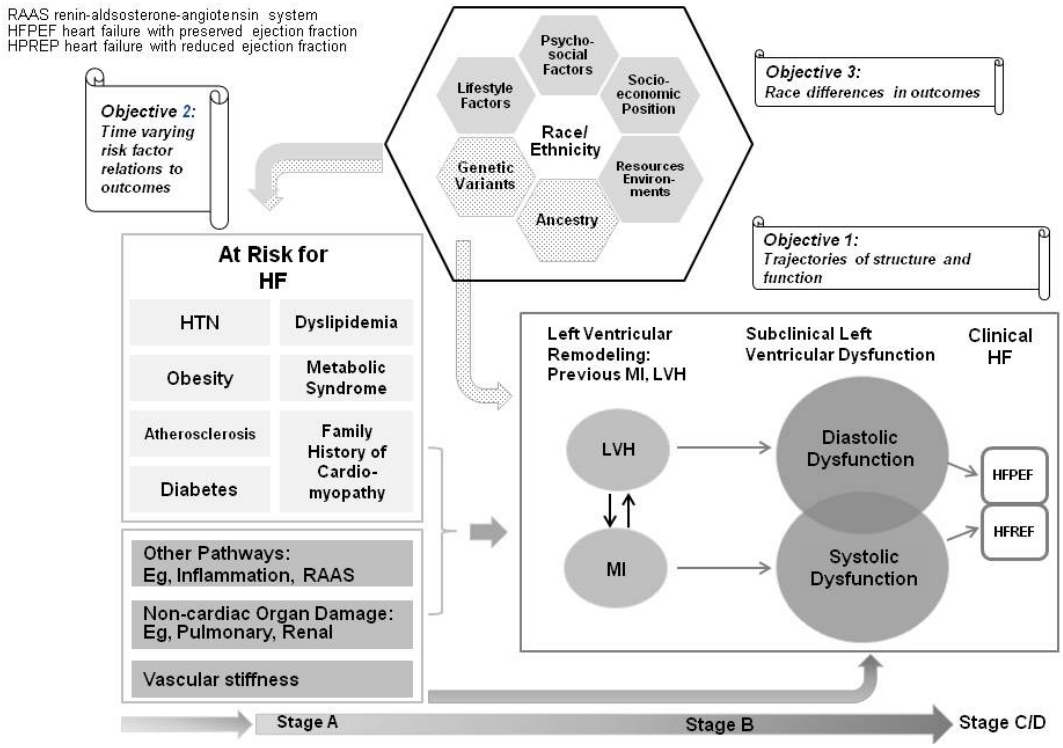
Racial disparities in several CVD risk factors, including obesity, DM, and hypertension, have emerged in CARDIA (Table 3), in some cases early. At Y0, the mean BMI of black women was highest and already in the overweight range. The prevalence of obesity has increased markedly in all groups since then, with blacks having substantially more obesity as well as DM at Y25. Other race-related differences in risk factor exposure are present for dyslipidemia (most prominent in white men) and for smoking. The higher BP present among blacks observed at Y5 was not entirely explained after controlling for a number

of potential risk factors[78], and both race and geographic differences in BP have increased over time[79], with substantial differences in hypertension prevalence present at Y25. Moreover, blacks had higher prevalence of several abnormal ambulatory BP derived phenotypes at Y5 than whites, including higher nighttime systolic blood pressure (SBP), nocturnal hypertension and nondipping[80]. Racial discrimination and responses to unfair treatment were related to BP in blacks at Y5[81], while socioeconomic position indicators[82], psychosocial factors[83], and fitness and physical activity[84] have all been related to the incidence of hypertension in CARDIA.

Table 3. Year 25 prevalence of CVD risk factors and subclinical disease by race-sex group				
	Black men	**White men**	**Black women**	**White women**
Hypertension (%)	40	25	47	16
Obesity (%)	45	35	63	31
Diabetes (%)	17	8	16	6
Dyslipidemia* (%)	36	46	17	14
Smoking (%)	27	13	19	12
Coronary artery calcified plaque >0 (%)	35	48	18	17
Visceral adipose tissue (mm^3, mean (SD))	131 (74)	170 (80)	115 (58)	113 (66)
LV mass (gm)/ht(M)$^{2.7}$ (mean (SD))	43.2 (12.9)	39.5 (9.6)	42.4 (13.0)	35.7 (9.8)

* Dyslipidemia defined as high total and/or low HDL cholesterol

Figure 1: CARDIA Model for Development of Heart Failure:
Race/ethnicity factors with pathologic associations progressing to CVD and early death



Black-white differences in subclinical disease seen to date in CARDIA, with the greatest prevalence of CAC in white men and little difference among women, do not mirror the racial disparities in clinical events noted in national mortality data[41]. With the notable exception of MI, there is a large preponderance in blacks of the hypertension-related clinical outcomes, including HF, ESRD, and stroke. These mirror the greater onset in blacks of early-onset CKD[85,86], albuminuria[87], and lower small artery elasticity in blacks[88]. Through Y20, cases of clinical HF occurred almost exclusively in blacks[34]. The risk factors at Y0 for HF, listed in Section 5.1, included higher diastolic BP. Most of the participants with hypertension at Y0 were not being treated.

As described in Section 5.1.2., CARDIA has shown the importance of long-term exposure to risk factors. The time-averaged cumulative exposures to non-optimal lipid levels between age 20 and 35 years was significantly related to CAC prevalence at Y20[34], and the association was similar across race and sex, and strongly graded. There was a graded association between Y20 CAC prevalence and exposure to "pre-hypertension" before age 35, especially systolic pre-hypertension[33]. Nearly 20% of the cohort developed pre-hypertension before age 35, with greater odds in black, male, overweight, and lower annual income and education participants.

Hypotheses. We list four hypotheses related to racial disparities in the development of, exposure and susceptibility to CVD risk factors as they related to disparities in subclinical and early clinical events:

1. Racial differences in CVD risk factor levels are partially but not fully explained by lifestyle/habits (physical activity, diet, smoking), socioeconomic position, psychosocial factors, and resources and environment at Y0 and their trajectories over 30 years, as well as by ancestry and genetic variants.
2. Greater baseline and cumulative levels of BMI, BP, and insulin resistance in blacks than in whites are the primary factors associated with greater increases in blacks of LVM, LV systolic strain defined by speckle tracking, and diastolic dysfunction defined by tissue Doppler over 25 years from the Y5 to the Y30 CARDIA Examination, and during the 5-year period from Y25 to Y30.
3. Greater Y0 and cumulative levels of several CVD risk factors (BP, smoking, lipids), lower levels of physical activity, and higher incidence of hypertension and DM in blacks than in whites partially, but do not fully, explain the higher cumulative incidence of early clinical events in blacks than in whites over 30 years and during the 5-year period from Y25 to Y30.
4. Blacks have a greater sensitivity than whites to any given level of BP for hypertension-related outcomes including HF, stroke, and subclinical LV dysfunction.

Methods.

Echocardiography. Repeat echocardiography assessment at Y30 will enable longitudinal assessment of LV and LA structure and function. See Section 5.3. for details. We will use echo-derived measurements, including LVM, LA dimension, tissue Doppler assessment of LV diastolic function, LV myocardial strain (particularly longitudinal strain) by STE, LV geometry, EF/FS, and valvular assessment. Because of the age of the cohort members, CARDIA is well positioned to assess alterations of cardiac function as data from other cohort studies suggest that during the age range of the 50s, metabolic and vascular alterations appear to accelerate leading to the greater frequency of events occurring in the 60s and 70s. The use of speckle tracking analysis will additionally enable unique assessment of longitudinal myocardial function, LA function through LA strain measurements, and LV diastolic function.

Other variables. Data collection will include repeated assessments of selected key variables, including sociodemographic, lifestyle/behavioral, and psychosocial factors related to subclinical and clinical outcomes. Data collection is described in detail in subsequent sections. We will employ previously used standardized instruments, including smoking, alcohol intake, physical activity, health-related quality of life, income, employment, education, health care access, discrimination, depression, and self-reported

neighborhood environment characteristics related to physical activity and dietary habits. Through previously-funded ancillary studies, we have geocoded participants' home addresses through Y25 and anticipate ancillary study applications for Y30 geocoding that will provide an additional level of environmental factors such as neighborhood-level socioeconomic conditions, physical activity and nutrition resources, etc. Methods and questionnaires are available on the CARDIA public website: http://www.cardia.dopm.uab.edu/

We will obtain anthropometric measures, body weight, height, and hip and waist girth, as done at earlier exams. BP will be measured using the *OmROn* (Omron Corporation; Kyoto, Japan) HEM907XL sphygmomanometer three times after a five-minute seated rest period. Fasting blood will be obtained for assay of lipids, glucose, and creatinine; urine will be obtained for albumin/creatinine excretion. DM and hypertension are defined using a combination of physiologic variables (e.g., standard cutpoints for glucose) and medication use (e.g., DM medications).

Clinical events. We will continue to collect and adjudicate clinical events as in Section 5.10.

Previously-collected data. In addition to the wealth of environmental, social, behavioral, and biological risk factor data collected over the past 25 years, and described briefly above, we have also collected substantial imaging data on adiposity (dual-energy x-ray absorptiometry) at Y20, abdominal computed tomography (CT) at Y25, and subclinical disease (chest CT [for CAC and pericardial fat] at Y15, Y20, and Y25; carotid ultrasound for IMT at Y20). We will continue current efforts to understand the etiology of racial disparities in these measures and examine the degree to which these measures account for racial differences in clinical events.

Statistical approach. Mediation analysis is the fundamental approach to the study of race differences, wherein the unadjusted race difference in an outcome variable is compared to the race difference after adjusting for variables that are thought to explain or mediate the race variable. In this analysis, the statistical predictive power of the added covariates is noted and interpreted for a fuller understanding of other predictors and of the nature of the mediation. In mediation regression analyses dealing with race differences in CVD risk factors (H1), the CVD risk factor level will be the dependent variable, while predictors are as listed in the hypothesis above. Note that smoking will be dealt with both as a risk factor and as a predictor of other risk factors. CARDIA has collected a wide array of variables within each class of predictors. Addressing H2, the dependent variables in the mediation analysis are echo variables with the primary hypothesized mediators, BMI, BP, and insulin resistance, having been measured repeatedly. The proposed Y30 echo provides an opportunity to examine effects of these variables on changes in the race effect at an age (mean 50 years at Y25, mean 55 years at Y30) when acceleration of subclinical cardiac abnormalities is expected. In H3, we hypothesize that the mediation analysis will identify important mediators, including baseline and cumulative levels of BP, and thus help to explain the race effect. H4 will be tested by studying effect modification in models that are similar to the mediation models above. However, the effect modification models will include race x covariate interaction terms. For example, if blacks have greater sensitivity to BP than whites, the black race x BP interaction term will be positive.

Summary. We hypothesize "a point of no return," for example, due to organ damage, will be reached in these hypotheses. We anticipate that a web of connections will be evident among risk factors, lifestyle/habits, socioeconomic position, psychosocial factors, and resources and environment early in the study, which will augment any ancestry or genetic variants that link risk factors. These results will also track (that is, people will tend to retain their ranking as they age). However, adjustment for potential mediators will not affect the race differences equally over time, and a point of no return will be suggested by a change in the mediation effect of potential mediators at a point in time.

5.2. Pulmonary Function Measurement (CARDIA Lung Study)

The ancillary study *Lung Function Decline and Disease Risk from Young Adulthood to Middle Age (CARDIA Lung)* (R01 HL122477; PI Kalhan) adds pre- and post-bronchodilator lung spirometry to the Y30 Exam and takes advantage of existing Y25 thoracic CT scans (collected under the ancillary study *Longitudinal Changes in Pericardial Adiposity and Subclinical Atherosclerosis* R01 HL098445; PI J Carr) to analyze lung and thoracic vasculature. The scientific rationale and general methods are described below. Operational details are integrated into the Pulmonary Function chapter of the exam MOO to facilitate review and implementation.

COPD is the 3rd leading cause of death in the US[89]. The major risk factor is smoking, but only a minority of smokers develops COPD. Markers that predict risk of COPD, therefore, would be valuable to target risk reduction strategies, identify subclinical disease, and test disease-modifying therapies. A limited number of prospective studies are able to study markers that predict incident COPD. Our group has investigated the predictors and consequences of lung function decline in CARDIA taking advantage of lung function measured in the Y0, Y2, Y5, Y10, and Y20 core examinations. We found that low baseline lung function predicts airflow obstruction at Y20 and that those with baseline airflow obstruction are most vulnerable to the adverse effects of smoking[90]. Markers of systemic inflammation at Y7 were associated with both lower absolute and accelerated decline in lung function, in high-intensity smokers Y7 CRP was associated with prevalent COPD at Y20[91], and blood markers of endothelial dysfunction (soluble intercellular adhesion molecule-1 (sICAM-1) and P-selectin) at Y15 were similarly associated with lower absolute and greater decline in lung function[92]. Our ability to define early-life predictors of incident COPD, however, has been limited by the young age of the cohort: At Y30, the cohort will be at an ideal age (mean age 55) to study incident COPD[93-96].

Although COPD is defined clinically by a forced expiratory volume in 1 second/FVC ratio (FEV1/FVC) less than 0.70, many individuals, including smokers, have "restriction" characterized by low FVC, low FEV1, and normal ratio[97-99]. Both COPD and restriction are associated with CVD and poor health outcomes[100-106]. We found an association between FVC decline and incident hypertension in CARDIA[107], suggesting that declining lung function in young adults is a cardiovascular risk factor, but the lung and heart structural and functional abnormalities that evolve throughout middle age to explain this association have not been investigated. We have recently documented a divergence in cardiac changes between those who have declining FEV1/FVC ("subclinical COPD") and those who have a declining FVC but preserved FEV1/FVC ("subclinical restriction"). Subclinical COPD is associated with decreased left heart chamber volumes; subclinical restriction is associated with left heart hypertrophy and early diastolic dysfunction. We hypothesize that this divergence may be explained by early emphysema in those with subclinical COPD leading to loss of lung vasculature and reduced left-sided preload while those with subclinical restriction have increased lung density and increased left-sided filling pressures.

The specific aims for the CARDIA Lung Study are:

Specific aim #1: To evaluate factors in young adults that predict incident COPD and/or restriction.

Hypothesis: Serologic markers of systemic inflammation (CRP and fibrinogen) and endothelial dysfunction (sICAM-1 and p-selectin) at Y7 of CARDIA are associated with decline in lung function and predict incident COPD and/or incident spirometric restriction from peak lung function to Y30.

Specific aim #2: To determine whether incident COPD and incident restriction are associated with distinct cardiac structural and functional changes.

Hypothesis: Incident COPD and accelerated decline in the FEV1/FVC (subclinical COPD) are associated with reduced left heart chamber volumes but not hypertrophy whereas incident restriction and

accelerated decline in FVC (subclinical restriction) are associated with left heart hypertrophic changes and diastolic dysfunction.

Specific aim #3: To determine the lung structural and intrathoracic vascular changes associated with incident COPD and incident restriction.

Hypothesis: Incident COPD and accelerated decline in the FEV1/FVC (subclinical COPD) are associated with increased emphysema, enlargement of the pulmonary artery (increased right heart afterload), and decreased diameter of the pulmonary vein (decreased left heart preload) whereas incident restriction and accelerated decline in FVC (subclinical restriction) are associated with increased lung density and enlargement of the pulmonary vein (increased left heart preload) but not the pulmonary artery.

We will measure lung function in all willing participants at CARDIA Y30 via pre- and post-bronchodilator spirometry using standard methods[108] that are consistent with prior CARDIA exams. We will also evaluate lung structure on existing Y25 cardiac CT scans to determine the lung structural changes that influence cardiac alterations in both COPD and restriction.

With its enrollment of young adults and robust follow-up, CARDIA provides a unique opportunity to evaluate early-life predictors of COPD and spirometric restriction. By seeking to identify predictors of lung dysfunction beyond cigarette smoking, this investigation fulfills a public health need. By testing the overall hypothesis that phenotypic differences seen in COPD and restriction, including their distinct cardiovascular manifestations, evolve concurrently in a subclinical period from young adulthood into middle age and progress together as the diseases worsen, these investigations represent an innovative approach to the study of lung disease.

5.3. Echocardiography Measurement

Rationale

Structural and functional alterations of the heart and blood vessels represent the most common obstacle to human longevity and quality of life[109-112 24,113]. However, the mechanisms by which genetic endowment and aging interact with environmental factors to predispose individuals to CV morbidity and mortality remain incompletely understood[109-111]. It is well known that age is associated with left ventricular (LV) hypertrophy, but less clear is the degree to which this remodeling is age-specific relative to the influences of obesity, hypertension, smoking, and central vascular stiffness among other known determinants of LV hypertrophy[23,114-126]. Moreover, a large body of evidence from recent work has implicated intrinsic myocardial dysfunction in association with aging[120-124,126,127], and aging-related cellular[118,128,129] and molecular[130-134] processes have been proposed as potential mechanisms of systolic and diastolic dysfunction[123,124,135-137], over and above the effect of atherosclerosis and other pathogenic factors known to impair myocardial performance and predispose human beings to untoward cardiovascular outcomes including HF.

HF is a major health problem in the US and Europe. It afflicts approximately five million patients in the US and each year 800,000 new patients are diagnosed as having HF[138-143]. During the last 10 years, the number of annual hospitalizations due to HF increased from 500,000 to around 900,000. HF causes or contributes to 300,000 deaths each year, and its prevalence increases with age. In this regard, approximately 6-10% of individuals older than 65 years are diagnosed as having HF[140,141]. Due to this high prevalence, associated high rate of hospital readmissions, and costly treatment, HF has become the leading clinical condition in terms of health expenditures in the US[138,142].

Most previous studies indicate that CAD is the main cause of HF in western industrialized countries, being responsible for over 60% of all cases in the US[144-146]. In addition to hypertension and LVH[147], DM has also been implicated as an important pre-disposing condition to the development of HF[146].

Moreover, in several large prospective studies, asymptomatic LV enlargement, hypertrophy, and dysfunction have been associated with a higher risk of developing HF[148-151] and are currently classified as subclinical stages of HF[151], heralding poor prognosis[24,148-152]. These alterations predispose worse outcomes in patients who suffer any other type of cardiovascular complication highlighting their importance as markers of cardiovascular morbidity and mortality. This unfavorable evolution highlights the need to define earlier stages of myocardial dysfunction in individuals of different ethnic background and exposure to diverse environments. Finally, in addition to alterations of LV morphology and function, other important parameters derived from echocardiography imaging such as valvular function, aortic root, and atrial size and function allow for a comprehensive evaluation of the heart and large vessels. In this regard, the importance of left atrial size has been well documented in several studies[153,154] including CARDIA[112,155,156]. Together with measurements of right ventricular systolic pressure, they further highlight the power of echocardiography to study cardiac structure and function.

Hypotheses

1. Left Ventricular (LV) Mass
    a. Change in LVM over 25 years (from Y5 to Y30) is related to change in BP, BMI, and DM.
    b. Specific stresses on the cardiovascular system may influence change in LVM, including peak exercise BP (from GXT Test, Y7).
    c. Lifestyle factors shown to influence LVM in cross sectional analyses may influence change in LVM. These factors include physical activity, alcohol use, and smoking.
    d. Rates of change in LVM may differ by race and gender.
2. Left Ventricular (LV) Function
    a. Age-related changes in LV diastolic function (which reflect abnormalities of LV filling) as assessed by LV inflow Doppler velocities will be greater in individuals with increases in BP and increases in weight, as opposed to individuals with unchanged or lower BP and less weight gain (between Y5, Y25, and Y30).
    b. Disease Prevalence
    c. Individuals with identified possible or definite MVP will have progressive changes in MVP grade.
3. Right Ventricular (RV) Function
    a. Changes in RV area have independent and additive correlation with changes in LV volume and mass, as markers of adverse cardiac remodeling in response to cardiovascular risk factors.
4. Cross-sectional
    a. Elevated insulin levels will be associated with increased increments in LVM.
    b. Change in linear dimension and area of the LA (as well as the derived left atrial volume) is a marker of adverse cardiac remodeling and occurs disproportionately in those with worse cardiovascular risk factors, regardless of gender and ethnicity. Changes in left atrial volume (measured or derived) correlate with changes in markers of LV diastolic function.
5. Speckle tracking
    a. Impaired relaxation, indexed as a shorter early filling wave defined by circumferential and/or longitudinal strain rate by speckle tracking, during the Y5 Examination will be related to the development of symptomatic HF and atrial fibrillation in the following 25 years (Y5 to Y30) of clinical follow-up.

b. LV hypertrophy by echocardiography in Y5 will be related to the development of diastolic dysfunction, defined as impaired circumferential and longitudinal early diastolic strain rate by speckle tracking analysis, measured during the Y25 and Y30 Examinations.
c. The magnitude of LVH defined by echocardiography in the Y5 Examination will be inversely related to the magnitude of circumferential and longitudinal strain and strain rate by speckle tracking analysis measured during the Y30 Examination.
d. Obesity in early adulthood is related to subclinical myocardial dysfunction.

6. 3-Dimension (3D)
    a. Endpoints for risk factor trajectories
    b. Predictors of subclinical and clinical disease development
    c. Reproducible parameters which can be measured serially in population studies in order to quantify change in LV structure and function.
    d. Accurately quantify incipient cardiac functional phenotypes in a population-based sample.
7. 2D vs 3D
    a. To determine the comparability of 2D and 3D cardiac volume and STE measurements with regard to specific values and reproducibility to provide an assessment of the translational value of these findings in clinical practice.

Method of Measurement

The primary priorities of the echocardiography examination are to obtain M-mode, two-dimensional, Doppler, and STE images (from parasternal long-and short-axis, apical four-chamber, two-chamber, and three-chamber views) of the LV, LA, RV, right atrium, and aortic root. A secondary priority is to compare the measurements from Y5 and Y25 with Y30. New 3D technology will be added in Y30 to evaluate global wall strain.

We plan to evaluate: LV function, LV volumes, LVM, LA size, LA volume, LV dimensions, aortic root dimensions, LV % FS, RV volumes, RV function, mitral peak flow velocity in early diastole, mitral peak flow velocity in late diastole, ratio of late-to-early diastolic mitral peak flow velocity, early diastolic mitral flow velocity integral, late diastolic mitral flow velocity integral, ratio of late-to-early diastolic mitral flow velocity integral, and tricuspid regurgitant flow velocity. Speckle tracking will be used to evaluate circumferential and longitudinal strain. 3D assessment will be used to evaluate 3D cardiac volume and function including LVM, LV volumes, LV EF, RV mass, volumes, RV EF, 3D global circumferential and longitudinal strain.

2D measurements will be made using a Digisonics workstation. Speckle tracking and 3D analysis will be performed using a *Toshiba UltraExtend* workstation.

5.4. Blood Pressure

HBP, or hypertension, is a serious public health problem that afflicts approximately 29% of American adults[157]. An additional 28% of American adults have pre-hypertension[157]. Hypertension is a major risk factor for heart attacks, congestive HF, stroke, and kidney disease[158]. These risks are also increased in adults whose BP level is not hypertensive but is above optimal[159]. More than one-half of all heart attacks and greater than three-fourths of all strokes occur in patients with hypertension. In addition, most patients who develop kidney failure and require dialysis are also hypertensive. Although long-term management can reduce the risks associated with HBP, many of the risks have not been eliminated completely[160].

Hypertension is multifactorial; genetic, neural, humeral, vascular, cardiac, renal, nutritional, and psychosocial factors all play interdependent roles[158]. The hypertensive population is also heterogeneous

with respect to the degree of response to manipulation of environmental factors and medications, and the tendency to develop BP-related complications. Major progress has been made in improving understanding of the causes of hypertension and in developing effective therapeutic programs to improve its control[161]. A substantial contribution to this body of information has been made from results of work with CARDIA data. Some examples from the CARDIA Study include: higher BPs in blacks that increased over time was due to obesity, physical activity and alcohol intake levels[78]; benefits of plant food and adverse effects of meat intakes on BP[162]; psychosocial factors (e.g., job strain) assessed early in CARDIA are associated with increased risk of hypertension[163]; BP reactivity to psychological stress predicts hypertension[164]; and, increased risk of hypertension development in young blacks with depression[165].

During examinations prior to the Y20 Exam, BP was measured using the Hawksley random zero sphygmomanometer. The OmROn HEM907XL was used at the Y20 and Y25 Exams and will be used again during the Y30 Exam. Each participant sits in a quiet room for five minutes prior to having three BP measurements taken from the right arm. The second and third BP readings are averaged for analyses. Pulse is taken by standard count methods using the radial pulse.

5.5. Obesity and Anthropometry

After marked increases in recent decades in the US and in many other countries[166,167] (Ng 2014; Rockholm 2010), the prevalence of obesity (BMI ≥ 30.0) may be leveling off in US children, adolescents, and adults[168]. The prevalence remains high, however, with 35% of US adults being obese[168]. Multiple investigations have shown that obesity and, in some cases overweight, increase the risk of morbidity from hypertension; dyslipidemia; type 2 DM; CHD; stroke; gallbladder disease; osteoarthritis; sleep apnea and respiratory problems; and endometrial, breast, prostate, colon and other cancers; and all-cause mortality[169-171]. Moreover, work in CARDIA has shown that the duration of obesity is related to a number of health outcomes, including DM and subclinical CVD[172-174]. Therefore, any effort to prevent and control weight gain in young adulthood and the onset of obesity may have an important impact on the health care system.

There are three primary reasons for including body size measurements in CARDIA:

1. They provide a standardized basis for examining the association between physical size, stature, or ponderosity, and risk for developing CHD, hypertension and other diseases of interest (e.g., chronic obstructive lung disease, DM).
2. Repeated measures of body habitus over time permit investigation of whether changes in body size directly contribute favorably or unfavorably to an individual's risk profile (i.e., tracking of secular and aging trends of known CVD risk factors).
3. Because the data will be collected using standardized methods employed in other observational studies besides CARDIA, the representative nature of the CARDIA data set can be determined using existing data and ethnic groups. Given the patterns of weight gain in the American population and the high prevalence of overweight and obesity, including central fat patterning, it is important to continue obtaining body size measurements on this cohort.

Previous body size measurements have been reported, either independently or collectively, and they have been correlated with a specific risk factor or clinical manifestations of disease. These measures also represent important indicators of growth and nutritional status. Although numerous additional anthropometric and/or body size measurements have been identified, this study focuses on indices particularly relevant to the risk of developing CHD, systemic arterial hypertension, dyslipidemia, and type 2 DM. Measurements were limited to those that are the most relevant and that can be performed

using standardized methodology within and between field centers because of time limitations and other practical constraints of the study.

Specifically, the anthropometric and body measurements included in studies such as the National Health and Nutrition Examination Survey (NHANES), Framingham, and the Lipids Research Clinics Prevalence Survey served as a model for the proposed measurements[175-177]. In addition, the age group initially involved in CARDIA suggested the need for monitoring growth and development, and subsequently the aging process, which may have been unrelated to environmental influences such as dietary or exercise patterns.

1. Height and Weight—Among all the possible measurements, height and weight are universally accepted as primary indices of body size. Weight-for-height standards are available for both men and women from studies such as the Build and Blood Pressure Study and the updated *Metropolitan Life Insurance* tables. Based on standards such as these, relative weight can be determined. In addition, several ratios of weight/height, such as the BMI (Quetelet's Index[1]) or the Ponderal Index[2] have been positively correlated with risk factors for CVD, including hypertension, hyperlipidemia and DM.

BMI is a reproducible, easily measured estimate of obesity that has been used throughout the epidemiologic literature. The World Health Organization[178] and US guidelines[169] recommend the following classifications to define underweight, normal weight, overweight and obesity: <18.5 kg/m^2 (underweight), 18.5 to 24.9 kg/m^2 (normal weight), 25.0-29.9 kg/m^2 (overweight), and >30 kg/m^2 (obese). BMI indicating overweight or obesity is associated with CVD risk factors such as hypertension, DM, and dyslipidemia in the expected directions. Further, obesity is associated with elevated mortality from cardiovascular and other chronic diseases[179].

Prior studies in CARDIA and other cohorts with longitudinal follow-up have demonstrated that duration and age of onset of obesity are associated with the development of CVD[172,173,180,181]. Weight gained early in adult life may be a sensitive indicator of increased risk for CVD. On the other hand, changes in weight or BMI over time, particularly decreases in BMI[182] and weight cycling[183], have been associated with increased risk of all-cause, cardiovascular and CHD mortality, independently of baseline BMI and other risk factors. For these reasons it is important that CARDIA include measures of height and weight during each examination.

Nevertheless, it is well known that there are numerous limitations to indices of relative weight[184,185]. For example, height and weight tables do not reflect the variations across different ethnic, socioeconomic and/or occupational groups nor do they indicate differences in body composition or fat distribution. Thus, in order to determine body composition and fat distribution more accurately, waist measurement is also performed in CARDIA.

2. Waist Girth—Central or upper body obesity is associated with insulin resistance, hypertriglyceridemia and reduced HDL-cholesterol and with the future development of DM, MI, angina pectoris, stroke, and all-cause mortality[186-188]. Visceral (intra-abdominal) adipose tissue shows the strongest link to risk factors for CVD and type 2 DM[189-193]. Waist circumference is a

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[1] Quetelet index is calculated as: weight divided by height squared (W/H^2 [kg/m^2]). Conversion [w(pounds)/h (inches)^2] x 703 (1 lb=0.45 kg) (1 in.=2.54 cm=0.0254 m).

[2] Ponderal index is computed as weight (in kg)/ht^3 (in meters).

measure of central adiposity that correlates well with visceral fat[194-198]. Among nearly 400 CARDIA participants who participated in an ancillary study in which visceral fat was measured, the correlation of waist circumference with visceral fat is 0.44 for black men, 0.64 for white men, 0.62 for black women, and 0.66 for white women.

3. Hip Circumference—Hip circumference was measured during the first five CARDIA exam cycles (through Y10) but was not assessed at the Y15 and Y20 Exams. Hip circumference was measured in Y25 and is included again in the Y30 Exam because of data showing the association of waist-hip ratio (WHR) with cardiovascular risk[199] and the value of ratios such as WHR in adjusting for ethnic differences in body shape[185].

5.6. Laboratory Measures

5.6.1 Glycemia

DM has long been recognized as a major risk factor for CHD, and prospective studies have demonstrated that type 2 DM is strongly associated with the incidence of MI and stroke as well as mortality from CHD and CVD[130-137]. DM is associated with lower HDL cholesterol and higher triglyceride concentrations than normoglycemia, and altered hemostatic factors[138-142,145,146]. Higher mean BP and greater prevalence of hypertension have been observed in patients with DM, with obesity accounting for only a portion of these differences[151-154]. Further, elevated insulin concentrations have been associated with higher triglycerides, BP, and uric acid and lower HDL cholesterol in non-diabetic populations[155,157-159]. Fasting insulin concentration or insulin response to a glucose challenge is an independent risk factor for incident CVD in persons with or without DM. Type 2 DM may be preceded by long periods of hyperinsulinemia[160-163]. CARDIA has measures of fasting glucose and insulin at most of the previous exams, but as the cohort enters middle age with concomitant weight gain and lifestyle changes, the assumption that fasting glucose and insulin rise linearly may not be correct. CARDIA can examine the effects of aging as well as having unique data to assess the impact of glucose and insulin in young adulthood on incident subclinical disease (CAC and echocardiographic changes) as well as progression of subclinical disease by gender and by ethnicity.

At Y30, glycemia chemistries will be measured as follows: insulin by a sandwich immunoassay performed on a *Roche Cobas 6000 Analyzer* with detection by chemiluminesence and glucose by a hexokinase method on a Roche Cobas 6000 Analyzer. These methods utilize the same chemistries as those used in the Y25 Exam, but now are performed on a newer model instrument, the *Roche Cobas 6000 Analyzer*. The glucose method is standardized with National Institute of Standards and Technology (NIST) standards. Prior standardization with NIST standards has occurred and is compared with the current exam year.

5.6.2 Lipids

As in all previous exams, we will measure lipids, including total cholesterol, HDL cholesterol, and triglycerides, and will estimate LDL cholesterol. Elevated blood lipids as well as low HDL cholesterol are well established risk factors for CVD morbidity and mortality[176]. Cholesterol concentrations tend to increase with age in Western populations[177,182-184]; however, national surveys suggest that total cholesterol has been declining over the past three decades in the US population[200]. This decline is being balanced by the secular increase in mean body weight, and continued measurement of lipids will allow us to track this trend in CARDIA participants. Given the increase in HMG-CoA reductase inhibitors (statins) since the Y15 Exam, continued tracking of lipids as part of the Y30 Exam is a critical component of CVD risk assessment. In addition, the women attending the Y30 Exam will be 48-60 years of age, and a moderate proportion of them either will be entering the peri-menopausal transition, in the early stages of menopause, or post-menopausal when lipids start to rise as a consequence of endogenous

estrogen deficiency. In addition to the traditional roles of lipids in CVD risk, with the publication of the Adult Treatment Panel III recommendations from the National Cholesterol Education Program[190], the importance of low HDL cholesterol as a component of the metabolic syndrome has become more evident. Linked with the increase in the average BMI of both CARDIA participants and the US population over the past 30 years, lipids also are key predictors of impaired glucose tolerance and type 2 DM.

At Y30, lipid assays are using the same methods as used in previous CARDIA exams, as follows: quantitative determination of total cholesterol and triglycerides in plasma samples and cholesterol in lipoprotein fractions are performed by in vitro enzymatic tests using Roche reagents on a Roche Double Modular P Analytical Automated Analyzer. All the methods for lipids are standardized to the Centers for Disease Control and Prevention (CDC) Reference Methods. Determination of cholesterol in HDL particles is performed after precipitation of apo B-containing particles by use of dextran sulfate Mg2+ designated comparison method reagent formulated by the CDC. Cholesterol in LDL particles is calculated by the Friedewald equation. In samples with triglycerides >400 mg/dL, where the Friedewald equation is inaccurate, LDL-cholesterol is measured by a homogeneous enzymatic method using Roche reagent on a Roche Modular P Analyzer. A comparison between the values obtained by this method and those obtained by the CDC reference method has demonstrated an average bias of -1.4%. The laboratory inter-assay precision on QC samples is consistently <1.1%, <1.0%, and <1.1% for the high-value QC samples and <1.4%, <2.0%, and <1.5% for the low-value QC samples for total cholesterol, triglycerides, and HDL-cholesterol, respectively. Additionally, a large number of aliquots of fresh-frozen samples selected to have a normal and a high concentration of total cholesterol, triglycerides, and HDL-cholesterol have been prepared in the laboratory and stored at -80 °C. These samples are analyzed multiple times at the beginning of each month to monitor the assays for long-term analytical drifts.

5.6.3 Serum Creatinine

Creatinine reflects kidney function and is both a predictor and a consequence of hypertension[191,192]. Previous reports have suggested that impaired renal function is both a predictor of CVD and an intermediate outcome of the risk factors that CARDIA has been tracking[193,194]. Longitudinal trends in serum creatinine will also be examined for their role in CAC prevalence and progression as well as echocardiographic measures.

Creatinine is measured in serum and urine by an enzymatic method on the *Roche Cobas 6000 Analyzer*. This method has the same chemistry as the method used in the Y25 Exam, but now is performed on a newer model instrument, the *Roche Cobas 6000 Analyzer*. This method is standardized with NIST standards. Prior standardization with NIST standards has occurred and is compared with the current exam year.

5.6.4 Urinary Albumin Excretion

The kidneys in both blacks and whites are susceptible to BP-related target-organ damage even at BP levels within the “normal” or “pre-hypertension” (<140/90 mm Hg) range[194]. Urinary albumin excretion has been proposed as a sensitive and reasonably specific test to identify impaired renal function prior to gross creatinine elevations. A small amount (<20 µg/min) of albumin is normally excreted by the kidney. When urinary albumin excretion exceeds 200 µg/min, it is usually detectable by dipstick methods. Mildly increased urinary albumin excretion, or albuminuria, in the range of 20 to 200 µg/min often is undetectable by such methods. The albumin excreted in the urine over a 24-hour period is considered the “gold standard” for assessing albumin level and defining albuminuria[195]; however, this measure is difficult to perform in observational cohort studies. “Spot” urine collections indexed to creatinine excretion have also been used in other studies[115]. High correlations have been reported between the albumin:creatinine ratio and the 24-hour urinary albumin excretion for men (0.949) and women (0.942).

Albuminuria is a risk factor for kidney failure[196], stroke[197], and cardiovascular and all-cause mortality[201], particularly in diabetic and hypertensive patients. Urinary excretion of albumin above 20 μg/min correlates with a higher prevalence of BP-related target-organ damage (i.e., greater LVM and GFRs) in cross-sectional studies and portends a higher incidence of cardiovascular morbidity and mortality, even in non-diabetics[115,202,203]. CARDIA reported that men and blacks had higher "spot" urinary albumin excretion than women and whites. The strongest correlates of albuminuria occurred in people with either of these conditions[204].

Urinary albumin excretion (as calculated from spot measurement of urinary albumin and urinary creatinine) again will be measured at Y30 using a nephelometry-based assay from Dade Behring on a BN-II instrument comparable to methods used at the Y10, Y15, Y20, and Y25 Exams. The Y10 value will be used as a baseline to establish change, or to focus on albuminuric or normoalbuminuric participants. Having a fifth time point (Y10, Y15, Y20, Y25, and Y30) will permit assessment of linear versus nonlinear trends as well as secular versus aging trends in urinary albumin both overall and in high-risk subgroups.

5.6.5 Hemoglobin A1c and Oral glucose tolerance test (PRF Women Study)

The ancillary study *Pregnancy-Related Risk Factors and Progression to Glucose Intolerance in CARDIA Women* (PRF Women Study) (R01 DK106201; Erica Gunderson, PI) adds HbA1c and OGTT assessments to the Y30 Exam.

Specific Aims: Women enter pregnancy much heavier and gain more weight during pregnancy than previous generations. In 2010, 53% of U.S. women were overweight or obese before pregnancy, and 48% exceeded gestational weight gain (GWG) recommendations. Maternal overweight or obesity is the most common high-risk obstetric condition [205] and related to increased maternal and newborn morbidity and mortality. [206,207] Overweight or obese women are less likely to return to their preconception weight, [208,209;] despite their lower average GWG and larger newborn size than normal weight women. [209] Randomized Interventions to modify GWG, especially in overweight or obese women, have been largely ineffective. [210-212] The American College of Obstetrics and Gynecology recommends modification of maternal weight before conception, as well as advice to control weight gain during pregnancy to prevent adverse perinatal outcomes (APOs) and future obesity [207]. Yet, pregnancy-related risk factors, other than BMI and weight gain, have rarely been linked to risk of APOs, or longer term maternal diseases. Our team previously linked pre-pregnancy metabolic status with subsequent GDM risk, independent of BMI, lifestyle behavior and socio-demographics. Research that encompasses the spectrum of pregnancy-related risk factors from preconception, prenatal and through post-delivery across successive births is necessary to assess individual risk and timing of effective prevention strategies.

In 2009, the Institute of Medicine (IOM) [213] and Agency for Health Research and Quality (AHRQ)[213] stated the need for research focusing on GWG and long-term health outcomes in women; particularly type 2 diabetes. A major barrier is the lack of longitudinal data covering all portions of women's reproductive cycles.[214] The American College of Obstetricians and Gynecologists recommends that women achieve optimal weight before conception and receive advice and monitoring of GWG within recommended ranges. [207] The national shift to preconception care for obese women and others at high risk for APOs, but clear targets, other than weight gain remain unspecified due to limited data. [215] Very few studies have the ability to measure risk factor changes for successive births in relation to progression to glucose intolerance in women during midlife. A better understanding of the pregnancy-related risk factor changes in women can not only reduce APOs, but lessen the burden of maternal obesity and glucose intolerance in mid to late life.

We propose an ancillary study to the NHLBI funded CARDIA Study, a multi-center longitudinal study of 5,115 black and white women and men (18-30 y) enrolled in 1985-1986, and re-examined up to 9 times

through 2011. Exams included measurements of body weight, waist girth and metabolic risk factors every 2-5 years (both before and after pregnancies), glucose tolerance every 5 years via 2-h 75 g OGTTs (1995-2011) and HbA1c (2005-2011). During the 25-y follow up, 1,338 CARDIA women reported 2,xxx births (birth weight, gestational age, APOs). In 1997, CARDIA women also recalled GWG for earlier births and released medical records for validation of APOs. The proposed study coordinates with the Y30 CARDIA exam (2015-2016) to collect self-report of GWG and pregnancy medical record data for 612 births after 1997, and glucose tolerance testing via 2-h 75 g OGTTs and HbA1c (not funded by CARDIA). This study leverages the high quality CARDIA data on childbearing, and preconception to post-delivery cardiometabolic risk factors. This study combines existing data with new data collection to accomplish the following specific aims.

Aim 1. To characterize the contribution of pregnancy-related weight changes (preconception, gestational and post-delivery weight gain) to subsequent risk factor development [obesity, central obesity, glycemia, insulin resistance, dyslipidemia] **for glucose intolerance in women**; **H1a**: High preconception gain, GWG (excess vs. non-excess) pattern for successive pregnancies, and post-pregnancy weight gain will exert independent adverse effects on risk factors for glucose intolerance in women;

Aim 2. To evaluate the impact of pre-pregnancy risk factor trajectories (BMI, waist girth, dyslipidemia, insulin resistance, blood pressure) in relation to: 2a. Excess GWG, and 2b. Adverse perinatal outcomes (APOs: GDM, LGA, preterm births); **H2**: Risk factor trajectories before pregnancy will lead to excess GWG, and increased risk of APOs; Lastly, pre-pregnancy BMI will act as an effect modifier of this association.

Aim 3. To evaluate the independent associations of pre-pregnancy BMI, GWG and post-delivery weight change on the progression to glucose intolerance (pre-diabetes & diabetes) **in women during midlife. H3**: Pre-pregnancy BMI and the pattern of excess GWG (including % above recommendations) across successive pregnancies will elevate the risk of progression to glucose intolerance. The associations will be similar among women with normal pregnancies and those with one or more adverse perinatal outcomes;

Impact: The study goal is to evaluate pregnancy-related changes in cardiometabolic risk factors and their lasting effects on progression to glucose intolerance in Black and White women from before to after pregnancy. The impact of pregnancy-related risk factor changes, specifically preconception risk, GWG, and APOs, will be evaluated in relation to future glucose intolerance. The major impact of this study is to elucidate the relative importance of preconception risk factors, and optimal time periods for modification of risk factors associated with higher risk of progression to glucose intolerance in women during midlife.

5.7. Cognitive Function (CARDIA Cognition Study)

Ancillary study 1 R01 HL122658-01, CARDIA Cognition Study, adds cognitive assessments to the Y30 Examination, enabling a better understanding of cognitive function in mid-life. CARDIA will assess cognitive function at Y30, repeating three tests performed at the Y25 Examination and administering four additional tests. This assessment will enable the determination of 5-year changes in cognitive function and the predictors of these changes. The relationship of 30-year trajectories of key vascular, metabolic and lifestyle factors to midlife cognitive function and 5-year change will be determined, as well as related genetic risk scores. The relationship of brain region volumes and characteristics measured by magnetic resonance imaging (MRI) to level and change in cognition in adulthood will also be assessed.

Abundant evidence now indicates that cardiovascular and metabolic risk factors influence the risk of cognitive impairment and dementia[201-203]. BP, lipids, hypertension, and obesity are inter-related, chronic and highly *modifiable* risk factors that arise in young and middle-aged adults and are linked to cognitive performance and underlying brain changes in later life[204,216]. While most studies (trials and observational) have focused on risk relationships during mid-adult to late-life periods[217-219], the neuropathology associated with cognitive impairment/dementia often develops over decades[220]. The progressive, chronic, and long term natural history of these risk factors across the life span may mean that earlier intervention would be more effective.

The influence of cardiovascular and metabolic risk factors on cognition in young to middle-aged populations has not been well studied, particularly at a population level. Preliminary findings from the baseline (Y25) cognitive evaluation in CARDIA already suggest a critical role for these risk factors with reports of significant associations between mid-life cognitive function and the cumulative burden of SBP and DBP over 25 years as well as with the American Heart Association's ideal CVH components[221,222]. These processes involve metabolic, inflammatory, and other pathophysiologic aberrations and their interactions that develop over long periods of time with a risk and time course that is potentially modified by genetic susceptibility. Evaluation of long-term exposures to the risk factors that initiate these processes could provide a better window into etiologic processes than a single measure of exposure. Furthermore, there is an increasing shift towards understanding the continuum of cognitive aging and as such, clarifying when changes in cognition begin to diverge even as early as mid-life.

The rich array of repeated metabolic and inflammatory measures in CARDIA provides a unique opportunity to evaluate the role of cardiovascular and metabolic dysregulation in early life on subsequent cognitive function and brain health. The use of a lifecourse approach to study risk factors will allow for investigation of long-term exposures, from early life onward, in order to determine the potential effect of risk factor modification at various times.

The cognitive ancillary study will be conducted at all four CARDIA field centers, and all CARDIA participants will be invited to enroll. The cognitive evaluation which will occur during the core CARDIA exam will consist of a brief, five-minute questionnaire on subjective memory[223], TBI history[224,225], family history of dementia, and current cognitive activity[226], and then interviewers will administer a battery of neurocognitive tests. These measures will provide information on cognitive functioning and its association with risk factors (behavioral, biochemical, and genetic) and subclinical CVD in a white, and black middle-aged population. An expanded neurocognitive battery with cognitive measurements repeated from Y25 as well as the addition of four more cognitive tests at Y30 will provide the opportunity to identify associations of established CVD risk factors, lifestyle, and subclinical CVD with change in cognitive functioning over time.

The cognitive measures listed below have been chosen for their broad and efficient coverage of major cognitive domains. Additional factors considered in measurement selection included a) comparability to measures used in previous cohort studies, b) feasibility with respect to the standardization of administration and scoring procedures, c) evidence of sensitivity in normal samples including a wide age range and across race/ethnic groups, d) appropriate for people with low levels of education, and e) relatively easy to administer and objectively scored.

The measures to be administered include three instruments repeated from Y25: the Rey Auditory Verbal Learning Test (RAVLT), the Digit Symbol Substitution Test (DSST) of the Wechsler Adult Intelligence Scale-Revised (WAIS-R), and the Stroop Test, and three additional measures at Y30: the Montreal Cognitive Assessment (MoCA), Category Fluency Test, Letter Fluency Test, and the Rapid Estimate of Adult Literacy in Medicine-Short Form (REALM-SF). These measures tap the following cognitive domains: verbal learning and memory, psychomotor speed and attention, executive function, global

cognitive function, letter and category fluency, and literacy. This test battery is estimated to take, on average, 35-40 minutes for middle-aged adults.

Rey Auditory Verbal Learning Test: This test is a measure of verbal learning and memory. The RAVLT is a brief, easily-administered pencil and paper measure that assesses immediate memory span, new learning susceptibility to interference, and recognition memory. It consists of five presentations of a 15-word list (List A), followed by a free recall of a second word list (List B), and a sixth recall trial of List A. The RAVLT provides measures of immediate memory, efficiency of learning, effects of interference, and recall following short and long delay periods[227].

Stroop Test: This test is considered to measure selective attention, cognitive flexibility and processing speed and it is used as a tool in the evaluation of executive function. In this task, a person is asked to name the color of words when the word is printed or displayed in a color different from the color it actually names; for example, if the word "**green**" is written in the color **blue.** The cognitive mechanism involved in this task is called directed attention, the individual has to inhibit or stop one response in order to say or do something else[228].

Digit Symbol Substitution Test (Part D): The DSST is a measure of psychomotor speed and sustained attention. In this task, the participant is asked to translate numbers (1-9) to symbols using a key provided at the top of the test form. This is a test of processing speed and attention[229,230].

The Montreal Cognitive Assessment: The MoCA is a test of global cognitive function with components of attention, executive function, memory, language, visuospatial skills, calculations, and orientation[231]. Scores range from 0-30. The MoCA was named by a National Institute of Neurological Disorders and Stroke (NINDS) panel to identify tests to harmonize neuropsychological assessment of vascular cognitive impairment[232].

Category and Letter Fluency Tests: The category and letter fluency tests measure verbal production, semantic memory, phonemic fluency, and language[233]. Higher scores indicate better performance, and each test requires about one minute.

Rapid Estimate of Adult Literacy in Medicine: The REALM-SF is an assessment of literacy in which the participant reads common health-related words aloud. The instrument includes seven items and has stable model coefficients and 1 underlying linear factor. REALM-SF and REALM instrument scores were highly correlated in development ($r = 0.95$, $P < 0.001$) and validation ($r = 0.94$, $P < 0.001$) samples. There was excellent agreement between REALM-SF and REALM instrument grade-level assignments when dichotomized at the 6th grade (development: 97% agreement, $K = 0.88$, $P < 0.001$; validation: 88% agreement, $K = 0.75$, $P < 0.001$) and 8th grade levels (development: 94% agreement, $K = 0.78$, $P < 0.001$; validation: 84% agreement, $K = 0.67$, $P < 0.001$). REALM-SF and Wide Range Achievement Test scores were highly correlated ($r = 0.83$, $P < 0.001$) in field testing validation.[234]

5.8. Psychosocial Measures

Accumulating evidence suggests that psychosocial factors play a role in the etiology of CVD risk factors and outcomes. Individuals who are less educated, have lower income, or occupy jobs of lower prestige are more likely to develop adverse cardiovascular risk factors and outcomes relative to their higher SES counterparts[235]. In addition, several studies indicate persons who report greater exposure to chronic stressors, experiences of discrimination, and elevated depressive symptoms are at higher risk of developing various cardiometabolic disorders[236-239].

Recent findings in our cohort indicate that supportive and negative social relationships predict longitudinal changes in BMI and waist circumference[240]; depressive symptoms predict incident

hypertension in blacks[165]; perceived discrimination is associated with more screen time in black men and white women[241]; and a poor childhood psychosocial environment is related to higher mean carotid IMT in whites[242]. Moving forward, we will take advantage of the repeat assessments of several of these psychosocial measures to examine how cumulative exposure to these indicators influences CVD risk. We can also use the available genetic data to conduct gene-environment analyses, exploring interactions with psychosocial measures such as SES, depressive symptoms, and discrimination, with lifestyle variables including diet and physical inactivity.

In addition to individual-level psychosocial risk factors, another important area is contextual and multilevel analyses. There is increasing evidence that neighborhood quality is significantly associated with health, independent of individual risk factors and SES[243]. The long follow-up and high level of mobility in this cohort makes CARDIA well-positioned to make important contributions to the neighborhood effects literature. Recent studies in CARDIA using neighborhood-level indicators have shown that women living in more socioeconomically deprived and less cohesive neighborhoods have higher odds of CAC[244]; fast food restaurant availability predicts fast food consumption[77]; housing instability is associated with incident hypertension in white women[245]; and neighborhood socioeconomic deprivation is associated with lower physical activity in blacks[246]. In the future we will also assess the longitudinal impact of other neighborhood-level factors on CVD risk including racial residential segregation, exposure to crime, and the recent mortgage foreclosure crisis.

Eight-and-a-half-minutes of time (exclusive of sociodemographic characteristics) are dedicated to psychosocial assessment, with all being assessed via self-administered questionnaire. The assessments being collected are noted below with exam years where previously used:

1. Sociodemographic Questionnaire (Form 3) – Y0, Y2, Y5, Y7, Y10, Y15, Y20, Y25, Y30
2. CES-Depression (Form 36) – Y5, Y10, Y15, Y20, Y25, Y30
3. Quality of Life SF-12 (Form 65) – Y15, Y20, Y25, Y30
4. Chronic Burden (Form 64) – Y15, Y20, Y25, Y30
5. Discrimination (Form 44) – Y7, Y15, Y25, Y30
6. Neighborhood Cohesion (Form 56) – Y20, Y30

5.9. Brain Magnetic Resonance Imaging (CARDIA Brain MRI Substudy)

Epidemiological studies have established strong relationships between cerebrovascular disease, morphologic brain changes (infarcts, small vessel ischemic lesions, and atrophy) and cognitive decline and dementia. In numerous large studies of cerebrovascular disease[247-252], MRI has been shown to provide a safe, non-invasive examination of brain morphology and pathology. They have documented the extent of, and consequences from small vessel ischemic disease (SVID), which primarily reflects small vessel pathology secondary to age, hypertension, and other etiologies of small vessel disease such as DM. While the general relationships between cerebrovascular disease, cerebral ischemic lesions, and functional/cognitive impairment are known, details are lacking, particularly those related to the initial disease. Previous studies based on older imaging technology were relatively insensitive to subtle changes of early disease. In ARIC, CHS, Framingham, and Age Gene/Environment Susceptibility-Reykjavik Study (AGES-RS), most participants were over 60 years old at initial imaging, past the age when the earliest expressions of cerebrovascular disease are reflected by MRI. Previous studies also lack early physical, functional and risk factor measures that are needed to document early disease associations. CARDIA offers the opportunity to look more closely at the earliest stages of disease and its known and possible unknown predictors. This is a critical stage of this debilitating process since there is no treatment to reverse vascular brain insults.

New MRI technology now allows more sensitive and specific evaluations. With higher field strength magnets and advanced radiofrequency technology it is now possible to examine the brain at 1 mm isotropic spatial resolution, approximately an order of magnitude better spatial resolution than previous studies. This allows the focused study of specific brain regions that may show early disease-related changes. Newer pulse sequences such as FLAIR provide greater sensitivity to SVID. Furthermore, this greater spatial resolution and disease sensitivity will be very important for the younger age group in CARDIA.

Importantly, new physiological and functional magnetic resonance (MR) methods, particularly arterial spin labeling (ASL) and BOLD (Blood Oxygen Level Dependent), non-invasively measure regional cerebral blood flow (CBF)[253,254], vascular reactivity and brain connectivity[255]. This offers CARDIA the opportunity to evaluate not just morphology as in all previous studies, but to directly evaluate cerebrovascular function as well as changes in CBF in response to a physiological task or stress. Critical hypotheses about blood flow and the morphologic and functional consequences will be addressed on a population basis for the first time. Hence this proposed CARDIA Brain MRI Substudy is a novel, detailed investigation of the earliest morphologic and physiologic phases of this disease process. This information could prove critical in prevention and early management of cerebrovascular disease, before irreversible damage and functional consequences.

A defined subset of 719 CARDIA participants at three field centers (Birmingham, Minneapolis, and Oakland) will be re-imaged with brain MRI on designated 3T MR scanner facilities. In the event a participant cannot or will not participate in the follow-up MRI, a replacement will be identified in the non-MRI sub-cohort. All reasonable attempts will be made to ensure the new participant is from the same sex-race group as the participant from the original sample. If matching is not possible then a new participant should be identified on a rolling basis.

The Brain MRI Substudy protocol takes 45-50 minutes per participant and includes 1.0 mm isotropic 3D FLAIR, PD, T2, and T1 weighted images, a 2D axial resting ASL blood flow sequence, diffusion tensor imaging sequence (DTI-30 direction), and breath-hold and resting state BOLD sequences to evaluate vascular reactivity and brain connectivity.

In addition to improved scanning capabilities, new computerized image analytical techniques allow more rigorous, quantitative analysis of the improved data. Contemporary, statistically sophisticated voxel-based analysis improves accuracy and precision in defining cerebrovascular disease, perhaps as much as improved MR methods. Ischemic lesions as identified by MRI generally correspond to classical cerebral infarcts plus the more extensive and common leukoaraiosis (ischemic white matter disease, or SVID)[256], now accepted as a non-necrotic, ischemic effect on myelin that is secondary to the effects of aging, hypertension, and other small vessel pathologic processes[257]. Early reports of this vasculopathy depended on anecdotal observations that were quickly superseded by semi-quantitative, human observer scoring systems that are not directly comparable, have limited reproducibility, and restricted dynamic ranges[258-260]. The CARDIA automated, digital image analysis methodology reflects the evolution to automatic, quantitative computerized digital image analytical techniques that are very reproducible and offer a greater precision and dynamic range than previous scoring systems[261,262].

The image analysis methodology uses an expert trained Support Vector Machine (SVM) to classify all brain tissue into either normal or abnormal (ischemic) gray or white matter and assigns the tissue type to all voxels, each of which is assigned to one of 92 anatomic regions of interest (ROIs) of the cerebrum[263-265]. Ischemic tissue is further classified into necrotic (traditional infarct) vs non-necrotic (i.e., leukoaraiosis) tissue. In addition, CBF is calculated from the ASL sequence for each voxel as will vascular reactivity by resting and breath-hold BOLD imaging[266-268]. Voxels are assigned to ROIs that are organized in an anatomically hierarchal system that is collapsed into three for this analysis – the

separate volumes of cerebral gray and white matter and basal ganglia (gray and white matter). Abnormal tissue from these ROIs forms the basis for our secondary outcomes (ischemic lesion volume); the sum of the three is the primary outcome (gray, white matter, and total brain volume). CBF and BOLD reactivity will be additional secondary outcomes, reflecting the physiological substrate of cerebrovascular disease.

5.10. Brain Magnetic Resonance Imaging, Transcranial Doppler, and Cognitive & Gait Assessment (Cerebral Small Vessel Disease Study)

The ancillary study *Cerebral Small Vessel Disease in Motor and Cognitive Decline* (Cerebral Small Vessel Disease Study) (R01 NS085002; Farzaneh Sorond, PI) adds a brain MRI, Transcranial Doppler, and cognitive & gait assessment to the Y30 Exam. Below is an excerpt from the Cerebral Small Vessel Disease in Motor and Cognitive Decline Protocol v2; for the entire document, contact Dr. Sorond at farzaneh.sorond1@northwestern.edu.

OBJECTIVES. Cerebrovascular disease is an important cause of mobility disability and dementia. Intracranial small vessel disease (SVD) rather than large strategic cortical lesions appear to be the major factor in the clinical expression of age related motor and cognitive decline [269]. Brain parenchymal changes such as cerebral white matter hyperintensities (WMH), lacunar strokes and cerebral microbleeds have been identified as the surrogate radiographic manifestations of SVD. However, current evidence suggests that once established, radiographic SVD is irreversible. Therefore, any treatment has to be directed at the vascular changes that precede radiographic SVD. Yet, very few studies have examined pre-clinical cerebral vascular measures and their relationship to clinical and radiographic outcomes of cerebral SVD.

This study is designed to identify vascular biomarkers of cerebral SVD (measured by Transcranial Doppler ultrasound (TCD): vasoreactivity [VR], dynamic cerebral autoregulation [dCA], neurovascular coupling [NVC], and pulsatility index [PI]) across the SVD spectrum in a high risk population. Specifically, we will examine the central hypothesis that functional changes in cerebral small vessels (measured by TCD) are strongly linked with radiographic SVD in high risk individuals.

Our specific aims are as follow:

Aim 1: Test the hypothesis that vascular biomarkers measured by TCD are associated with mobility and cognition in individuals with vascular risk factors.

Aim 2: Test the hypothesis that vascular biomarkers measured by TCD are associated with radiographic SVD in individuals with vascular risk factors.

Aim 3: Test the hypothesis that vascular biomarkers (TCD measures) mediate the relationship between vascular risk and radiographic SVD in high risk individuals.

BACKGROUND. The global population is aging. In 2010, 13% of the US population was over the age 65 and by 2020 this number is projected to increase to 16%, translating to 54 million older adults living in the US (US census Bureau, 2010). Cognitive and mobility impairments are the two most common causes of disability in older adults. These two geriatric disorders can lead to disabling forms of dementia and falls. Therefore, to effectively prevent these conditions, we need to identify individuals at risk in the pre-clinical state.

Evidence from epidemiological and clinico-pathological studies has established a strong link between vascular disease and age related clinical outcomes of cognitive and gait impairment. Slow gait speed has been strongly associated with increased blood pressure [270,271], increased risk of cardiovascular death [272], and an increase in incident ischemic stroke risk [273]. Vascular contribution to cognitive impairment and

dementia has been well established in a number of prior studies and summarized in a recent statement from the American Heart Association[274]. Moreover, various brain structural changes attributed to cerebral small vessel disease and measured by magnetic resonance imaging (MRI) have been associated with disorders of cognition, gait, and falls[275-296]. These MRI changes which include white matter hyperintensities (WMH), lacunar strokes, and cerebral microbleeds (CMB) have become accepted as surrogate measures of cerebral small vessel disease (for detailed review see [297]). Therefore, vascular contributions to mobility and cognitive impairment are significant, yet we know very little about the pre-clinical vascular changes which lead to radiographic small vessel disease (SVD) and the associated clinical outcomes.

Among the vascular pathologies affecting the aging human brain, cerebral SVD is the most common degenerative vascular disorder, much more frequent than cerebral macrovascular disease (for detailed review see[269]). While we have made significant radiological advances in imaging the brain structure and its large vessels, our ability to image or to functionally assess the small vessels of the brain has been limited. Recent studies have shown that measures of peripheral large vessel disease such as aortic pulse wave velocity [298-300] and carotid intima media thickness and stiffness [301] are both associated with surrogate MRI measures of small vessel disease, gait speed and cognitive function (for detailed review see [302]). These studies have led to the hypothesis that increased central arterial stiffness exposes the cerebral small vessels to abnormal flow pulsations and contributes to the pathogenesis of cerebral SVD. However, because none of these studies actually measured cerebral small vessel function, we know very little about cerebral small vessel structure and function in relation to motor and cognitive decline. A few recent studies have started to use TCD to examine the relationship between cerebral small vessel function and MRI changes. These studies have shown that TCD derived pulsatility index[303,304] and vasoreactivity[305,306] are cross-sectionally associated with WMH. Very few studies, all limited to cross-sectional analyses, have also examined the relationship between cerebral small vessel TCD measures and cognitive and mobility impairment. We have shown that cerebral vasoreactivity and neurovascular coupling are both associated with gait speed in an elderly cohort[307,308]. A few studies have also shown impaired vasoreactivity in patients with carotid stenosis[309,310]. Only one study using positron emission tomography (PET) in 27 hypertensive participants has shown reduced cerebral blood flow 3 years prior to the onset of cognitive decline [311]. There are no data from population based longitudinal studies linking pre-clinical cerebral small vessel function (as measured by TCD; not MRI surrogate measures or peripheral vascular measures) to subsequent cognitive and mobility impairment. To alter the course of cerebral small vessel disease we need a better understanding of the changes affecting these vessels in the preclinical state where interventions would be most effective.

As the tools to measure cerebral small vessel function become increasingly more sophisticated we are now well poised to advance our understanding of the physiological changes underlying cerebral SVD by directly studying cerebral small vessel function. The introduction of transcranial Doppler ultrasound (TCD) to measure cerebral blood flow velocity [312] provided a powerful tool for non-invasive assessment of cerebral vascular responses to various physiological challenges such as motor or cognitive activation, or change in blood pressure and end-tidal carbon dioxide which we know are regulated at the level of arterioles or resistance vessels of the brain [313-319]. Since the mechanisms underlying each of these vascular responses are likely different, they have been traditionally distinguished by different labels. Changes in cerebral blood flow velocity in response to sudden changes in blood pressure is referred to as dynamic cerebral autoregulation (dCA) [313,314,320]. Changes in cerebral blood flow velocity in response to changes in end-tidal CO_2 is referred to as cerebral vasoreactivity (VR)[315,316]. Changes in cerebral blood flow velocity in response to a motor or cognitive task is referred to as neurovascular coupling (NVC) or functional hyperemia[318,319]. In addition to these three functional measures, the resting cerebral blood flow velocity waveform can be also used to calculate a pulsatility index (PI) which is a measure of

cerebrovascular compliance [321]. Using these approaches, TCD, which can provide continuous non-invasive beat-to-beat measurement of the cerebral blood flow velocity in the basal cerebral arteries with a high temporal resolution, has become the most commonly utilized tool to study cerebrovascular function in humans.[322]

In this study, we propose to use TCD to characterize cerebral small vessel function and its relationship to vascular risk factors, radiographic SVD as well as gait and cognitive function across the spectrum of age and vascular risk factors in a cohort of community dwelling individuals agreeable to participate in our study. These study participants will undergo a full TCD assessment, a full cognitive and mobility battery and a brain MRI study. Findings from this study will extend our preliminary work (see below) and help us characterize small vessel function across the spectrum of age and vascular risk factors and understand its relationship with gait and cognition (Aim 1) as wells as radiographic small vessel disease (Aim 2), and help us examine possible mechanisms linking vascular risk factors to age related cognitive decline and mobility impairment (Aim 3).

The preliminary data described below establishes the relationship between our TCD measures of small vessel function, cognitive impairment, slow gait and radiographic small vessel disease in an elderly cohort late in the disease when neurological changes are not reversible. In the next phase of our study proposed in this protocol, we want to understand these relationships across age and vascular risk factor spectrum, before significant clinical cognitive and mobility impairment has manifested. This knowledge will allow us to identify pre-clinical vascular measures which would lead to novel diagnostic and therapeutic targets to alleviate the devastating consequences of age related cerebral small vessel disease.

Preliminary Data:

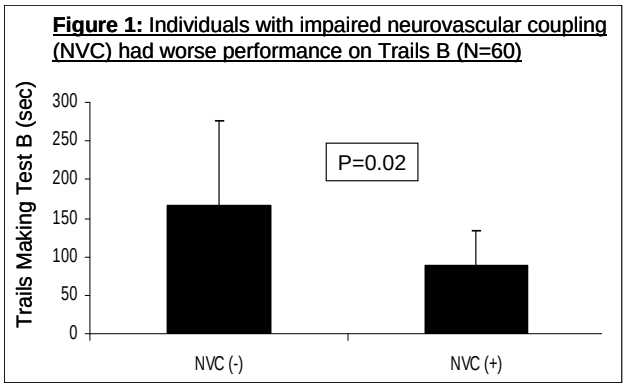
VR is related to mobility. In a multivariate linear regression analysis using data from MOBILIZE Boston Study (MBS) cohort we found that cerebral vasoreactivity (VR) was cross-sectionally related to gait speed (p=0.039). The MBS cohort is a population based cohort which is centered at the Hebrew Rehabilitation Center (Hebrew SeniorLife) in Boston and started recruitment in 2005 included 765 community dwelling participants living in the Boston area. At recruitment, they were >70 years old, without significant cognitive impairments (they had to be able perform all cognitive tasks and fill in monthly fall calendars) and had to be able to ambulate 6-meters without personal assistance [323]. Of the cohort, 465 individuals underwent detailed TCD assessments described in this protocol.

Using the MBS data, we showed that individuals in the lowest quintile of VR had lower gait speeds as compared to those in the highest quintile (p=.047). We also found that compared to individuals in the highest quintile of cerebral VR, those in the lowest quintile had a higher fall rate (p=0.029) [308].

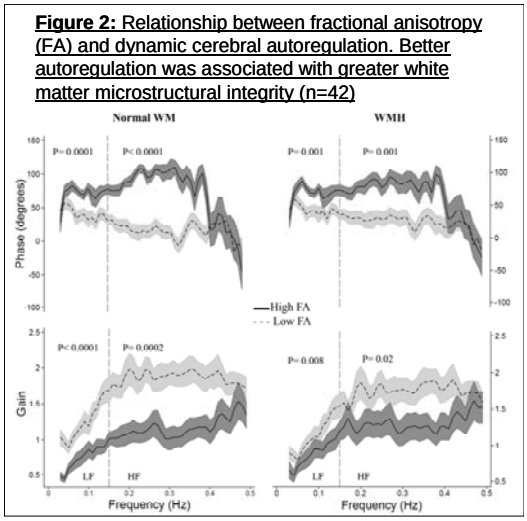
dCA is related to cognition. Using the MB data we also found that the autoregulatory gain (dCA gain of the blood pressure to blood flow transform) was cross-sectionally associated with adjusted Trail Making Test B performance (p=0.041).

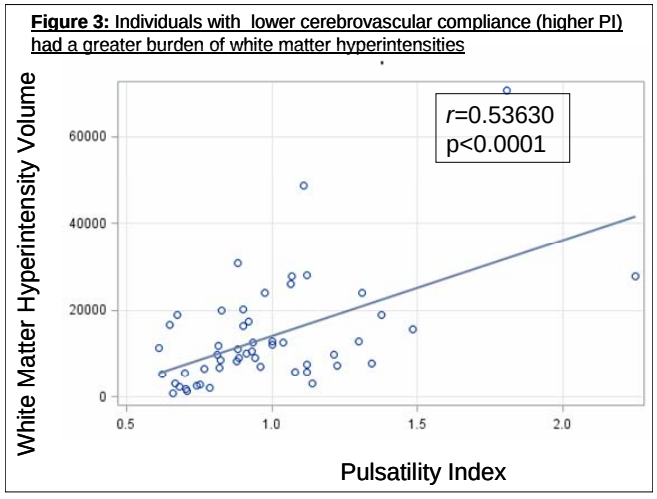
NVC is related to clinical outcomes. In a subset of 40 participants from the MBS cohort with MRI measures, we found that while neurovascular coupling (NVC) and WMH were both significantly associated with gait speed, higher NVC seemed to suppress the negative relationship between WMH and gait speed [307]. In other words, individuals with higher NVC were able to maintain a faster gait speed despite a higher burden of WMH. None of these 40 participants had any lacunes or CMB at MBS1, therefore, we do not have pilot data for these MRI measures. Given the high baseline cognitive status of the MBS participants, we did not see a cross-sectional relationship between NVC or VR and cognition in the MBS1 data. However, in a pilot study of a 60 individuals (not from the MBS cohort) with significantly

impaired Trails B scores we have found a strong relationship between neurovascular coupling and Trail Making Test B performance (Figure 1)[324].



dCA and PI are associated with White matter injury. In 42 elderly participants with vascular disease (not from the MBS cohort) we also found that less effective dCA (both phase and gain) was cross-sectionally associated with lower white matter microstructural integrity (Fractional Anisotropy[FA] and Mean Diffusivity [MD] using DTI) (Purkayastha and Sorond, 2013; *Cerebral Blood Flow and Metabolism*, under review) (Figure 2 shows the relationship for FA. Similar relationship was observed for MD, MD data not shown). In the same cohort, we also found that lower cerebrovascular compliance as measured by the pulsatility index (PI) was cross-sectionally associated with WMH (Figure 3). While our preliminary findings are intriguing, they are limited by the small sample size and the cross-sectional nature of the analyses.





INCLUSION AND EXCLUSION CRITERIA. We propose to study 250 participants in this protocol.

Participant Selection

The participants eligible for this study are all individuals between the ages of 18 and 89 years old with at least one of the following diagnoses:

- Hypertension
- Hyperlipidemia

- Obesity
- Smoker
- Sickle cell anemia
- Diabetes
- Cognitive Impairment without dementia (must score a 20 or more on the mini mental status exam [MMSE] at screening)
- Extrapyramidal disease including Parkinson's disease
- Cerebrovascular disease (ischemic, hemorrhagic, or small vessel disease)
- Gait disorder
- Falls

These participants will be recruited through two strategies. As previously proposed, we will continue to recruit through advertisement in the hospital and outpatient clinics across the hospital. In addition, we will also specifically recruit study participants from the CARDIA cohort. CARDIA is an NHLBI-sponsored 4-center collaborative longitudinal investigation of factors related to the evolution of coronary heart disease risk factors in 5115 young black and white men and women initially ages 18-30 at baseline in 1985-86. There have been seven follow-up examinations to date, at Y2, Y5, Y7, Y10, Y15, Y20, and Y25. They are currently undergoing Y30 assessments in this cohort. Northwestern University is one the four CARDIA sites with Dr. Donald Lloyd-Jones as the site PI where 1000 participants remain enrolled. The availability of 30 years of longitudinal detailed vascular and other medical data makes this cohort a unique population to examine vascular contributions the brain and cerebrovascular outcomes.

Inclusion Criteria:

- Carry at least one of the above diagnoses
- Be able to provide informed consent
- MMSE score greater than or equal to 20

Exclusion Criteria:

- Absence of TCD temporal acoustic windows
- Scores below a 20 on the MMSE at screening
- Significant cardiac disease (such as active angina or symptomatic heart failure)
- COPD
- Anxiety Disorder
- Those who will not pass the standard MRI screening will be excluded from the MRI portion of the study, but will be able to undergo the TCD and clinical assessments.

MRI Exclusion Criteria:

- Claustrophobia
- Any metal implants
- Not able to lie flat for 60 minutes

Inclusion will be limited to adults between 18 and 89 years of age who are capable of providing informed consent. Pregnant women and prisoners will not be included.

STUDY-WIDE NUMBER OF PARTICIPANTS. We plan to enroll 250 participants in this research study at Northwestern Medicine.

STUDY TIMELINES. We anticipate to enroll the study participants over 3 years and to complete data analysis over a 6 month period once study accrual has been met. The total study time will be 1.5-2 hours for the TCD/cognitive measures and 1 hour for the MRI. The MRI may be done on the same day, or may be scheduled for a second visit. This study will require no additional visits or follow-up.

PROCEDURES INVOLVED. This study will involve up to 2 visits and a total participation time of 3 hours. Total study time will be 1.5-2 hours for the TCD/cognitive measures and 1 hour for the MRI. All participants will undergo a detailed medical history as well as a screening MMSE (those scoring below 20 will be excluded from further participation). Enrolled participants will undergo a TCD battery, cognitive assessment, and gait assessment. The MRI may be done on the same day, or may be scheduled for a second visit. This study will require no additional visits or follow-up.

Devices:

Arterial Pressure. Beat-to-beat blood pressure will be obtained non-invasively from the arterial waveform in a finger of the hand by the Finapres Blood Pressure System (Finapres Medical Systems).

Transcranial Doppler. Cerebral blood flow velocities will be continuously recorded in bilateral cerebral hemispheres. A 2 MHz pulsed flat transcranial Doppler probe (MultiDop X, DWL) will be placed over each temporal bone to insure insonation of the M1 segment of each MCA and the probes will be fixed in place for the duration of the test using a Velcro headband.

End-tidal CO_2 will be monitored using a nasal cannula connected to a CO_2 monitor. (Vacuumed Medical #17515 CO2 Analyzer)

Measurements:

TCD Measures. We have previously published the detailed methodology for all these procedures [307,308,324,325]. Once the participants have been instrumented we will record 10 minutes of resting data. Then they will be asked to perform the Nback task (a cognitive task) on the computer and finally they will be asked to perform the cerebral vasoreactivity test. Vasoreactivity will be assessed using the CO_2 breathing and hyperventilation method. Participants will be asked to breathe room air for 3 minutes, then inspire an air mixture of 8% CO_2, 21% O_2, and 71% nitrogen for 3 minutes and then mildly hyperventilate for 3minutes. This is a standard air mixture that is used routinely in cerebrovascular labs for vasoreactivity testing and the oxygen content is the same as in normal room air.

The resting data will be used to calculate cerebral autoregulation and pulsatility index, the cognitive trial will be used to calculate neurovascular coupling and the breathing trial will be used to measure vasoreactivity.

Dynamic Cerebral Autoregulation (dCA). TCD assessment of dynamic CA is based on transient changes in cerebral blood flow velocity in response to sudden rapid changes in arterial blood pressure. Spontaneous oscillations in arterial blood pressure and cerebral blood flow velocity are used to calculate the auto and cross spectra between these two signals[326-331]. Dynamic CA is measured from the gain and the phase of the cross spectral analysis on these oscillating signals.

All data segments for MFV and ABP will be visually inspected and edited for artifact and ectopy, and only stationary data will be used for this analysis. Frequency domain analysis will be performed on beat-to-beat ABPs and MFVs. A power spectrum analysis technique based on the Welch algorithm of averaging periodograms will be used. The time series will be interpolated at 4 Hz to obtain equidistant time intervals and then divided into 5 equal overlapping segments. Each segment will be detrended, Hanning filtered, and fast-Fourier transformed to its frequency representation squared. The periodograms will be averaged to produce the spectrum estimate. Coherence between low-frequency mean arterial ABP

and MFV will be calculated from the cross-spectra and autospectra of stationary data segments in the sitting and standing positions. Coherence will be computed as (cross-spectra)2/(input signal autospectrum)x(output signal autospectrum). The signals are considered coherent over the frequencies at which coherence values exceed 0.5. Transfer magnitudes and phases will be calculated for each subject over the frequency range meeting this criterion, with MATLAB software. Transfer functions will be determined by dividing the cross-spectrum by the input autospectrum for each vessel insonated.

Cerebral Vasoreactivtiy (VR): Cerebral VR is calculated from the slope of the relationship between blood flow velocity and changes in end-tidal CO_2 during 2 minutes of breathing a normoxic air mixture containing 8% concentration of CO_2 which has replaced a fraction of the nitrogen content of the air. The oxygen content is not changed. This mixture is a standard gas that is being used in the standard way for testing cerebral vasoreactivity[40]. There is data to support that VR is a measure of endothelial function in the cerebral small vessels [332-334]. VR and dCA test different properties of the cerebrovascular system and provide powerful tools to assess cerebral small vessel function in humans[335-337].

Neurovascular Coupling (NVC): Neurovascular coupling or functional hyperemia is measured as the percent increase in cerebral blood flow velocity during the performance of the Nback cognitive task [307]. To evaluate the cerebral blood flow response to the N-Back task, the percent change in blood flow velocity during the task will be calculated as follows. To allow BFV measures to stabilize after changing tasks, mean values are extracted from the middle 100 second time window for each 120-second task block. The mean percent change for each MCA is calculated as a ratio of the percent difference between the BFV during the N-back (BFVNB) and its corresponding "Identify the letter X" control period (BFVIDX) divided by BFV during Identify X (BFVIDX) and multiplied by 100 ([(BFVNB -BFVIDX)/ (BFVIDX)] *100). We report the percent change in BFV during the N-back task for each MCA individually (right or left MCA blood flow changes) as well as combine the values for the right and left MCA to report a total change in BFV during the N-Back task in bilateral MCA territories (total blood flow changes). Performance will be calculated as percent correct during each task.

Pulsatility Index (PI): The PI is calculated from the cerebral blood flow velocity waveform using the systolic, diastolic and mean measures of flow velocity (peak systolic-end diastolic velocities/mean flow velocity).

Cognitive Measures. Once the above measures are collected the TCD and BP devices will be removed and we will collect 7 cognitive measures. These tests will measure executive function, verbal learning and memory, working memory, processing speed, and inhibition most sensitive to the vascular contribution to cognitive impairment. The final measure of episodic memory is more likely related to the changes associated with Alzheimer's-type dementia and is included as a comparison when considering non-vascular causes of cognitive decline.

- *Trails Making Test.* This is a neuropsychological test of visual attention and task switching, and can provide information about executive function. It is sensitive to detecting several cognitive impairments such as Alzheimer 's disease and dementia. It consists of two parts in which the subject is instructed to connect a set of 25 *dots* as fast as possible while still maintaining accuracy.
- *Hopkins Verbal Learning Test – Revised.* This test is a simple measure of verbal learning and memory (recognition and recall). It requires recall of a series of 12 words over 3 learning trials, free recall after delay, and a recognition trial. The assessment takes 5-10 minutes with a 25 minute delay before completing the final recall and recognition trials.
- *Wechsler Adult Intelligence Scale Digit Span.* This test is a measure of short-term and working memory. There are two parts: Digits Forward and Digits Backward. Digits Forward primarily taps short-term auditory memory while Digits Backward measures the ability to manipulate verbal

information while in temporary storage. First, the subject listens to and repeats a sequence of numbers said by the interviewer. In the second part, the subject listens to a sequence of numbers and repeats them in reverse order. In both parts, the length of each sequence of numbers increases as the subject correctly responds.

- *Pattern Comparison Processing Speed Task.* This task is included because reductions in processing speed may be an expected consequence of microvascular disease. This tasks measures speed of processing with a simple picture same different discrimination procedure that takes approximately 3 minutes.
- *List Sorting Working Memory Test*. This task requires the immediate recall and sequence of visually and orally presented stimuli. Participants are asked to repeat the items displayed in the order of size (smallest to largest). In one set of trials the stimuli are within a single category (animals v. food), in the more complex trials the items can be classified on two dimensions (size and category). The test takes approximately 7 minutes.
- *Flanker Inhibitory Control and Attention Test.* The test requires the participant to focus on a centrally presented arrow stimulus while "inhibiting" attention to arrows that flank it on either the left or right. Performance on trials where the flankers and stimulus are congruent are compared to trials where the stimuli and flankers are incongruent. This test takes approximately 3 minutes.
- *Picture Sequence Memory Test.* In this test of episodic memory participants are asked to recall sequences of pictures presented over two learning trials.

Gait Measures. All participants will be asked to walk across a 25 feet mat (ZenoMat, Protokinetics, PA) and their walking speed will be measured during 2 trials.

Magnetic Resonance Imaging. Prior to the imaging studies, each subject will be familiarized with the scanner environment including the scanner noise. Imaging will be performed on a 3 Tesla MRI. The MRI protocol will include T1, T2, FLAIR, SWI, DTI and ASL sequences. We will obtain the number, distribution and total volume of lacunar strokes, number and distribution of cerebral microbleeds, total and regional volumetric measurements of the gray and white matter, cortical thickness, and white matter hyperintensities in the supratentorial structures of the brain. Diffusion tensor imaging data (DTI) will be used to calculate fractional anisotropy (FA) and mean diffusivity (MD) in normal appearing white matter and white matter hyperintensities. Arterial spin labeling (ASL) data will be used to measure cerebral blood flow. This work utilizes several complimentary procedures including surface and voxel-based image analysis procedures utilizing the FreeSurfer image analysis tools developed by our colleagues at Massachusetts General Hospital, as well as the FSL tools developed by our collaborators at Oxford. These tools have been tested and validated across several studies and are publically distributed, documented and utilized by a large number of investigators (more information can be found online- Freesurfer: surfer.nmr.mgh.harvard.edu; FSL: http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/

Small vessel disease: The whole brain will be searched for lacunar infarcts, defined as focal 10 mm diameter hyperintensities on T2-weighted images. Infarcts located in white matter have to be hypointense on T1-weighted and fluid attenuating inverse recovery images to distinguish from leukoaraiosis or white matter hyperintensities. Microbleeds will be identified using susceptibility weighted imaging (SWI), a novel high-resolution 3-D T2* sequence uniquely attuned to detection of hemorrhage [338,339]. White matter hyperintensities will be measured using the methods we have described previously. [307,324]

Volumetric measures: These measures will be gathered using the procedures we have detailed in our prior publications [307,324,340-344]. The volumes of interest are the following: Intracranial cavity, white matter hyperintensities in supratentorial brain, total and regional white matter, total gray matter, total

cerebrospinal fluid, brain parenchyma fraction, caudate volume, putamen volume, thalamus volume, globus pallidus volume, cerebellum volume, total cortical gray matter, regional cortical gray matter (primary motor and somatosensory areas, prefrontal, frontal, parietal, temporal and occipital lobes, anterior cingulated cortex, insula and hippocampus).

Diffusion Tensor Imaging (DTI) measures: These measures will be gathered using the procedures we have detailed previously [345-349]. In brief DTI data will be motion and eddy-current corrected, and subsequently used in computing fractional anisotropy (FA) and diffusivity (radial and axial) with the FSL Diffusion Toolbox. The multiple image modalities are registered to the T1 with Boundary-based Registration (BBR) [350] and the segmentation of WMH from healthy WM will be accomplished with a multi-spectral Gaussian classifier for each subject based on the atlas values.

Arterial Spin Labeling (ASL): These measures will be gathered using the procedures we have detailed in our prior publications[351,352]. Quantitative cerebral blood flow maps as well as mean cerebral blood flow values across volume and ROI will be calculated for the analyses in this proposal.

RECRUITMENT METHODS. Study participants will be recruited through two strategies. We will recruit hospital-wide advertisement using the attached flyers. There will be a contact number on the flyer. In addition we will also recruit from the CARDIA Cohort. The local CARDIA staff will send an invitation letter for the participants to contact our study team for additional information and participation. Interested participants will call us and after a brief telephone screen we will invite them to the Cerebrovascular Laboratory to formally explain the protocol, obtain informed consent and enroll in the study. The entire visit including the MRI will take about 2.5-3 hours. They will be paid $125 for completing the full Study. If they fail to enroll because of lack of TCD acoustic windows, they will be paid $25.00 for their short visit to the lab. If they opt out of the MRI or are not eligible for the MRI, but complete the TCD and clinical assessment, they will be paid $75. Typically, they will receive a check 4-6 weeks after participation (1st visit for those who opt out of MRI and second visit for those who complete the MRI). All participants will also be provided with a parking voucher if they use the parking facilities at the hospital.

5.11. Medical History, Hospitalizations, Medications, and Healthcare Access and Utilization

Medical history information is extremely important to assess in CARDIA for several reasons. CARDIA began as a study of cardiovascular risk development, and as such assessment of conditions such as hypertension, defined as self-report of use of medications for its treatment and/or BP measurements, are important for achieving this objective, and serving in analyses as endpoints[33,83]. In addition, use of certain medications or presence of specific medical conditions could affect or be affected by lifestyle, as well as affect cardiovascular risk levels, or increase risk for clinical outcomes. For example, longer duration of abdominal obesity was associated with greater risk of incident DM, independent of the degree of abdominal obesity, family history of DM, energy intake, physical activity, smoking, alcohol and other variables[172]. More recently, CARDIA has included in its objectives the study of the healthy lifestyle and the maintenance of CVH[353], the development and extent of subclinical disease[173,354], and the development of early clinical disease[17,22,24].

During the Y30 Exam, participants are asked to complete questionnaires and interviews in order for the study to thoroughly review self-reported health conditions, medication use, and healthcare access and utilization. Data are collected on all hospital admissions, reasons for admission, and for specific outpatient procedures and treatments for conditions of interest. Participants also undergo standardized interviews to assess these conditions of interest during annual contacts.

Tobacco Use - Smoking, particularly cigarette smoking, has long been known to be a major risk factor for cardiovascular events, including events due to atherosclerosis and to coronary spasm[355,356], as well as for subclinical disease[357-359]. Smoking is also known to increase inflammation, a process known to be intimately involved in all stages of atherosclerosis[360] and may link the pathogenesis of atherosclerosis, COPD, and other chronic conditions[361]. In addition, the relationship between genetic variants and cardiovascular risk differs in the context of age, gender and environmental exposures such as smoking.

Not only is smoking important to CVD endpoints, it also is a significant determinant of other endpoints and disease measures important to CARDIA, including asthma, COPD, and glucose intolerance[361,362]. Further, cigarette smoking was related to all-cause mortality in analyses of early mortality in CARDIA[363]. Even during the early years of CARDIA, we observed an excess of both major and minor ailments in current smokers at baseline compared to baseline never smokers who were not exposed to passive smoke[364].

A particular strength of CARDIA is its ability to examine potential race/ethnicity, gender, and SES because of its diverse cohort, offering a unique opportunity to explore the interaction of biological and environmental factors in the development of CVD differences by race. These issues are extremely important in analyses of health behaviors and lifestyle factors as they pertain to outcomes of interest to CARDIA. For example, black participants in Health ABC and in CARDIA with a higher proportion of African ancestry are at greater risk for losing lung function while smoking[365]. Thus, detailed data on smoking exposure is collected in CARDIA.

At Y30, we are adding questions about electronic, or e-cigarette, use. Since their US debut in 2010, e-cigarette use has sharply increased across gender, age, and race/ethnicity groups[41]. E-cigarettes are purported to not expose the user to the non-nicotine toxicants that are released from burning tobacco in combustion cigarettes and thus be safer products. But, at present, we have limited data on how e-cigarette use influences combustion cigarette behaviors and further mystifying the situation, the impact of e-cigarette use on cardiovascular and lung health is unknown. Some theorize that if smokers use e-cigarettes in place of combustion cigarettes, this could represent a net health benefit. Alternately, the reality may be that e-cigarettes are more commonly used as a complement and without a reduction in combustion cigarettes. In this scenario, even if e-cigarettes were less risky than combustion cigarettes, we would not expect to see a reduction in risk. Further, if e-cigarettes reduced (but did not eliminate) combustion cigarette use, we might predict different effects on various tobacco-related diseases. For example, the risk of CVD increases sharply even at low levels of smoking (and exposure to secondhand smoke); other diseases caused by smoking are more dose-related. Given the well-recognized hazards of combustion cigarettes and the skyrocketing popularity of e-cigarettes, there is an urgent need to better understand the implications of e-cigarette uptake.

Alcohol use - Alcohol intake is known to affect several cardiovascular risk factors, and has complex relationships with CVD and other outcomes. The relationships between alcohol intake and CHD depend on drinking pattern, age, sex, and possibly type of alcohol (e.g., red wine[366]), with the latter particularly controversial and confounded by personal characteristics and other lifestyle factors[367].

Average volume of alcohol consumption is made up of quantity consumed, on average, on drinking days, and the frequency of drinking days per year[368]. Alcohol quantity and frequency have been differentially related to various diseases/outcomes, and risk factors, including MI[369], BMI[370], diet[371], and suicide[372]. Moderate drinking has been associated with reduced risk for all-cause and CVD mortality, and heavier drinking with increased all-cause, CVD, ischemic stroke, and certain cancer risks[373,374]. For example, a recent meta-analysis showed that an alcohol intake up to 46 g/d had a protective effect against cerebral infarction morbidity in women, with the greatest reduction in risk of cerebral infarction observed at a

consumption level of approximately one alcoholic drink/day, whereas there was a monotonically increasing risk for hemorrhagic stroke with increasing intake[374].

Alcohol affects a diverse array of vascular and biochemical functions, many of which are potentially beneficial for atherosclerosis. These include increased fibrinolysis, decreased platelet aggregation/function decreased myocardial ischemia-reperfusion injury, increased endothelial cell-dependent vasorelaxation, decrease myocardial ischemia-vasorelaxation, decreased plasma clotting factors (factor Vii and fibrinogen), and insulin sensitivity[375,376]. As much as 50% of the lower risk of CHD related to alcohol is estimated to be through its effects on HDL[377]. A recent randomized cross-over trial tested beer (30 g alcohol/d, 660 mL), the equivalent amount of polyphenols as non-alcoholic beer (990 mL), and gin (30 g alcohol/d, 100 mL) for four weeks[378]. The phenolic content of beer reduced leukocyte adhesion molecules and inflammatory biomarkers, whereas alcohol mainly improved the lipid profile and reduced some plasma inflammatory biomarkers related to atherosclerosis. Alcohol consumption is also related to reduced-risk of DM in some studies[379]. On the other hand, heavy drinking may cause myocyte and left ventricular dysfunction[380,381]. A recent meta-analysis found that heavier alcohol consumption >20 g⁄day was associated with the risk of development of hypertension in both women and men[382]. With regards to light to moderate alcohol consumption (<20 g⁄d), women had a potentially reduced risk of hypertension, while men had an increased risk of hypertension.

CARDIA has published several findings in relationship to alcohol intake. In data from Y15, moderate alcohol intake was not associated with lower risk of CAC, while binge drinking was related to presence of CAC, with the association being strongest in black men[383]. Through Y20, alcohol intake was not associated with hypertension in adjusted analyses except in white women where the incidence was lower in drinkers[384]. Compared with the participants who never drank, those who became/stayed drinkers had smaller increases, while those who quit drinking had the highest increase in fibrinogen over 13 years (Y7 to Y20) of follow-up[385]. There are also differences in the longitudinal patterns of alcohol use by race, gender, and psychological profiles in CARDIA[386], and negative associations of heavy drinking in young adulthood with employment in later years, particularly in blacks[387]. In examining associations between racial discrimination and unhealthy behaviors, we found significant positive associations of discrimination with smoking and alcohol consumption in African-Americans and with smoking in whites after adjustment for selected characteristics including individual and neighborhood-level socioeconomic conditions and segregation[76].

In CARDIA, alcohol intake is assessed for different types of beverages (wine, beer, and liquor) and then summed for total consumption. Questions on binge drinking are also asked.

Non-medical drug use - Illicit drug use is common among young adults, while only a minority meet criteria for abuse or dependence (http://www.oas.samhsa.gov/NSDUH/2k4nsduh/2k4tabs/toc.htm). Most available data, however, are from the latter group of users. In addition, nonmedical use of prescription pain medications has grown rapidly in the US, subsequent restrictions have led to increases in heroin use, and laws on marijuana use have loosened in several states in recent years. The long-term effect of these trends on the health of US population is unknown, but may be adverse. Recent research on cannabis use has shown that use increases accidents, can produce dependence, and there are consistent associations between regular use and poor psychosocial outcomes and mental health in adulthood[388].

In CARDIA, hard drug use in healthy young adults, even when hard drug use stops, is associated with a subsequent decrease in general self-rated health that may be partially explained by persistent tobacco use[389]. CARDIA has also found significantly greater odds of higher lifetime marijuana use in blacks reporting discrimination, compared to those who did not, perhaps indicating unhealthy coping responses[390]. On the other hand, occasional and low cumulative marijuana use, which was nearly as

common as tobacco exposure, was not associated with adverse effects on pulmonary function over 20 years[391]. Non-medical drug use is collected for recent and lifetime use for several substances.

Interim hospitalizations - CVD outcome ascertainment is important in a study of longitudinal change in cardiovascular risk. While CVD morbidity and mortality has been relatively low due to the young age of the cohort initially, there has been a steady increase in the number of possible endpoints in CARDIA. For selected analyses, the number of outcomes is becoming common, and interesting, enough for analysis. For example, using ECGs from Y0, Y7, and Y20, the natural history of early repolarization was examined using outcomes data collected over 23 years[392]. The presence of early repolarization was not independently associated with adverse outcomes, including cardiovascular death and MI, once adjusted for sex and race. In addition to analyses of antecedents of CVD outcomes, standardized assessment of other outcomes of interest, such as hospitalized asthma, gives specific information on diagnosis and therapy.

Standardized self-report of hospitalization and potential outpatient treatment and procedures of interest, as well as informant report in case of death, is collected at each exam and each annual contact of the cohort. At Y30, records will be collected for specific types of hospitalization, including hospitalization for acute MI and chest pain; hospitalization for stroke; outpatient cardiovascular procedures; hospitalization for asthma or COPD; in- or outpatient diagnosis and treatment for pulmonary thromboemboli; and potential cardiovascular death. Once collected, medical records are adjudicated by physicians using standardized criteria.

Women's reproductive health – CVD risk factors such as total and LDL cholesterol levels and particle density are significantly more adverse in post-menopausal than in pre- or peri-menopausal women[393-398]. Levels of many risk factors after the menopause approach those of men, yet rates of CHD events remain markedly higher in men before age 75, with women lagging behind by at least ten years. In addition, female specific factors including early menopause, premature ovarian insufficiency, polycystic ovary syndrome, and pre-eclampsia have been related to increased CVD risk and are considered female-specific risk factors[399-401]. Early menopause has also been related to non-atherosclerosis CVD, including HF, and estrogens have been postulated to protect the heart from ventricular remodeling[402,403]. Other hormones besides sex steroids, including anti-mullerian hormone, may also be relevant regulators of the cardiovascular system[404].

The influence of the transition from pre- to peri- to post-menopause on risk factor levels, on subclinical disease, and in narrowing the risk gap between men and women in various forms of CVD is relatively unexplored, particularly in longitudinal data including men and women. Whether these transition-related changes are independent of age, of age-related weight gain and declines in physical activity, and in aging women vs men requires further investigation. How these changes relate to sex steroid and other hormone levels in middle age also deserves further study.

CARDIA, with its long term risk factor data and timing through midlife, is well suited to address the question of risk factor changes within female participants as they move through the peri-menopausal transition. Other studies such as the Study of Women Across the Nation (SWAN) lack a male comparison group. CARDIA observations also occurred in the relative absence of hormone replacement (at Y25, only 8.1% of the women reported current use of hormones, excluding oral contraceptives), since CARDIA women were undergoing natural menopause at a time when hormone replacement therapy (HRT) was no longer a preferred long-term post-menopausal therapy due to data from the Women's Health Initiative (WHI)[405,406] and Heart and Estrogen/Progestin Replacement Study (HERS)[407]. While the menopausal transition has been explored in the context of subclinical atherosclerosis, examining the effects of perimenopause and menopause[399,408-411], there are few data on other forms of subclinical disease.

Independent of endogenous hormone decline, vasomotor and other symptoms surrounding the peri-menopausal and post-menopausal periods predicted increased risk of future clinical CVD[412,413]. Hot flashes may represent a different endocrine response to menopause. CARDIA has been collecting data on hot flashes since Y15 and will do so again at Y30. Continued tracking of hot flash prevalence and incidence and their association with subclinical disease may provide additional clues into racial and other differences in disease incidence and associated risk factors.

The women in CARDIA will be 48-60 years old during Y30. Given that the average age of menopause is 51 years, most if not all women will be post-menopausal or experiencing the peri-menopausal transition. This is an optimal cohort to examine the effects of menopause on CVD risk, including the opportunity to examine longitudinal associations with subclinical cardiac disease, using echocardiograms from Y5, Y25, and Y30 CAC. CARDIA also offers the opportunity to examine racial differences in biological aging in both men and women.

Women will again complete a detailed questionnaire on menstrual history, including intervals between periods, menstrual flow, instances of amenorrhea, symptoms (e.g., hot flashes, vaginal dryness), and use of oral contraceptives and hormone therapy.

5.12. Physical Activity History

In 2008, *Physical Activity (PA) Guidelines for Americans*, published by the US Department of Health and Human Services, recommended that all adults should strive to engage in a minimum of 150 minutes per week of moderate- to vigorous-intensity physical activity[414]. This recommendation was based on a large body of epidemiological and exercise training studies that showed that physical activity was associated with reduced risk of premature death, CHD, stroke, hypertension, hyperlipidemia, type 2 DM, metabolic syndrome, depression, cognitive function (older adults), and improved cardiorespiratory and muscular fitness. Since the publication of those recommendations, there has been increasing evidence that prolonged sedentary behavior is associated with increased risk of CVD mortality[415], obesity[416,417], cardiometabolic risk factors[418], and type 2 DM[416] independent of physical activity.

Physical activity is a complex behavior that can occur in several domains and contexts, including occupation, domestic or self-care, transportation, and discretionary or leisure-time. Physical activity can be further characterized in terms of the frequency and duration across a range of intensity levels, ranging from light to vigorous. The consequences of maintaining a physically active lifestyle include increased energy expenditure, improved physical fitness and the promotion of health[419,420]. Sedentary behavior is distinct from physical activity and not simply the low end of the PA spectrum; it may be best represented by periods of prolonged sitting[421].

In the CARDIA Study it is important to measure both types of behaviors since PA and SB have independent health consequences. It is also important to understand the prevalence of different activities, and how this activity spectrum changes as the CARDIA population ages. Further it is important to reliably rank people for overall level of and intensity physical activity, and to determine the impact of types and levels of physical activity and sedentary behavior on the risk of cardiovascular events.

Furthermore, it is important to determine the role of ethnicity on the associations between physical activity or sedentary behavior and CVD, particularly in the hypertension area. Some studies have shown that blacks who performed moderate to vigorous activity have less hypertension than their inactive peers[422-424].

The existing questionnaires which initially appeared to be best suited for use in CARDIA were the Minnesota Leisure Time Physical Activity Questionnaire[425] and the Seven Day Physical Activity Recall[426]. The former, which spans twelve months, has been validated against physiologic endpoints, but has not

been shown to be adequately reliable on reapplication, and takes too long to administer. The latter spans only seven days, has considerable accuracy, has shown adequate validity and is suitably brief[427]. However, it too suffers from low repeatability since one week's physical activity is often not representative of general patterns.

In devising a briefer new questionnaire, it was theorized that people could accurately recall or summarize for themselves what and how much activity they do within broad categories. Using an extensive database from the Minnesota Heart Health Program, tabulations were made of activity participation for men and women aged 25-29 years. Based on these tabulations questions were worded for thirteen sets of moderate or vigorous, mostly recreational activities (e.g., vigorous job activities) which might be logically classed together. In this way, details were avoided that are poorly recalled and often irrelevant. A recent evaluation of the CARDIA Physical Activity History (PAH) questionnaire showed that the estimates of physical activity were comparable to those obtained from a more detailed questionnaire that was similar in structure to the CARDIA PAH with the exception that frequency and duration were directly assessed[428]. This same questionnaire has been administered at all exams since baseline.

At the Y30 Exam, most of the original CARDIA PAH is asked, including the series of the thirteen various moderate and vigorous intensity activities. The Y30 Exam also includes a few items from the Baecke self-administered activity questionnaire, but questions excluded at Y25, specifically, comparison to others of the same age and sex and household and childcare activities, are again excluded.

Sedentary behavior has been measured by a proxy question about television watching since the Y5 Exam. This question is again asked at Y30, along with additional questions about time spent sitting in a variety of domains or contexts (e.g., sitting while using the computer for non-work activities or playing video games) that were asked for the first time at Y25 (Form 91).

5.13. Genetic Analyses

CARDIA *collected* buffy coat for DNA extraction at Y10, Y15, Y20, and Y25. At Y15, cryo-preserved cells were collected for future transformation and immortalization. DNA has been extracted from all Y10 buffy coats and constitutes the primary source of genetic material for the CARDIA studies. This resource has been supplemented with material from the later years' collection. Similarly, at Y30, DNA will be extracted to supplement existing material and complement DNA sets for participants for whom DNA was not previously available in order to obtain as complete a genetic dataset as possible.

5.14. Gut Microbiome (Gut Microbiome and CVD Risk Study)

The ancillary study *A Novel Gut Microbial-Dependent Nutrient Metabolite and Atherosclerosis* (Gut Microbiome and CVD Risk Study) (K01 HL127159; Katie Meyer, PI) adds a gut microbiome component to the Y30 Exam (Chicago Field Center).

Currently, we lack gut microbiome data from population-based and diverse human cohorts. We address this gap through collection of stool and microbiome analysis in a sample (n=300) of CARDIA participants balanced with respect to age and European-/African-American race (n=75 in each gender-race subgroup). In addition, much microbiome research has come from small-scale clinic-based studies, which lack long-term and in-depth phenotypic data on study participants. By collecting microbiome data from CARDIA, we will be in a unique position to quantify associations between gut microbiome and CVD risk measures accounting for covariates, such as long-term diet and physical activity.

We will use a stool collection kit that we tested in a small sample of adults in North Carolina. Participants were selected from an ongoing cohort study to roughly match the age, gender, and racial

composition of the CARDIA cohort. Of the 20 individuals invited to participants, 17 completed the study. Participants were asked to score the following statement: "The stool collection kit was convenient and easy to use" on a scale of 1 to 5, with 5 reflecting "very much" and 1 indicating "not at all." The average score was 4.7. Participants were also asked to provide any general feedback that might help us improve the kit. We received no negative comments and one person responded "I'd just like to say that the instructions were easy to understand and I wouldn't change a thing." Although study participants may over-report satisfaction, we note that participants reported overall lower satisfaction with a separate pilot activity to collect all urine over a 12 hour period. Of the 17 samples received, one indicated a need for additional emphasis in the instructions, which we have done, to limit the volume of stool collected.

Individuals will be determined ineligible for the CARDIA microbiome study if any of following apply: they have taken antibiotics in the past month; they are currently pregnant; they have had an acute gastrointestinal illness or infections in the past week; they have Inflammatory Bowel Disease, such as Crohn's Disease or Ulcerative Colitis. Only CARDIA baseline participants will be invited to participate in this study. The study will be complete once 300 participants have completed the study. There will be an equal distribution of African American Males and Females and Caucasian Males and Females.

Stool samples will be collected by study participants in their homes during the CARDIA exam period (June 2015-August 2016). Participants will be recruited into the study at the Year 30 exam by Northwestern Clinic staff. Samples will be analyzed Aug 2016-Jan 2017, bioinformatics performed Feb 2017-June 2017, and statistical analysis conducted July 2017-Jan 2018. We know of no significant risks associated with stool collection, but have given great thought to the development of our stool collection kit to ensure maximal participant safety and convenience. We note in particular that our kit does not require contact with stool beyond what is experienced in daily living or as a part of standard medical tests (e.g., colon cancer screening).

Our kit includes disposable gloves, stool collection tubes (with scoop attached to the lid), and a water soluble bag to line the collection commode; these features ensure that the participant has minimal exposure to their stool. Samples are doubly-enclosed in plastic tubes, sealed into a biohazard bag, and shipped in an insulated cardboard box; these features provide several layers of separation between the samples and participants (as well as other individuals who come in contact with the box). Aside from the stool collection, participants will complete a short survey about variables related to gut microbiome composition, such as antibiotic use and recent gastrointestinal illness.

Our analysis will focus on sequence data from a marker gene (16S rRNA) that has a conserved portion specific to microbiota, as well as variable regions to distinguish phylogenetic groups. We will derive gut composition data from the 16S rRNA sequences. Sequences will be clustered based on a 97% pair-wise sequence similarity, and clusters—operational taxonomic units (OTUs)—will be assigned to bacterial lineages or taxonomies (e.g., genus) through alignment against a reference database. Taxonomic assignments will be used to build a phylogenetic tree, specifying the hierarchical evolutionary relationships among OTUs (e.g., genera within phyla), and providing information about biologically meaningful differences among OTUs (greater evolutionary distance implies greater biologic difference). Due to variability in sampling and sequencing depth, we will normalize the counts by calculating proportions. For each participant, we anticipate that we will have counts and proportions for ~200 OTUs (~100 genera) and a phylogenetic tree.

From these data, we will derive ~15 diversity measures, including measures of alpha-diversity such as richness and evenness, and measures that combine features of diversity (e.g., richness and abundance), including the Shannon Index. We will also derive between-sample (beta-diversity) measures of the compositional similarity/difference between samples, such as Bray-Curtis to measure between-sample dissimilarity based on OTU abundance. Diversity measures will be used to derive summary metrics, such

as Principal Coordinates Analysis (PCoA), which provide information about how data cluster in the community. Summary metrics also reduce dimensional structure for regression analysis. We anticipate that our final set of participant-level gut microbial community variables will include ~200 taxonomic counts (~100 genera), ~5 diversity measures, and the first ~10 clusters from PCoA for a total of ~215 measures.

Several features of OTU data raise challenges for regression analysis, including their high-dimensionality (~100 genera), correlational structure, and distributional attributes (skewed, zero-inflated, and over-dispersed). Some of these challenges can be circumvented by using summary diversity measures or PCoA clusters, which can be included in standard regression models. There are also substantive reasons for modeling diversity measures, as they have been associated with many health outcomes in the literature. We will, therefore rely on diversity measures for our primary analysis. Secondary analysis will include individual regression analysis of distinct taxonomic entities, and penalized regression to include all taxonomic entities in the same model, accounting for their clustered nature. Power will be lowest for the 100 regressions analysis. To account for multiple comparisons (100 distinct regressions), we will use the Benjamini-Hochberg procedure to calculate false discovery rate (FDR). The FDR is less stringent than family-wise error rates (e.g., Bonferroni) at given nominal significance level (alpha), and is preferable for high-dimensional data (here 100 models) as it balances the number of true and false positives. Assuming 10% true positives and a 10% FDR, we will have 80% power to detect differences in continuous variables (e.g., lipids, insulin resistance, blood pressure) of roughly 1 standard deviation comparing participants in the highest and lowest quartiles of genera categories. Differences of this magnitude are clinically meaningful. We will have even greater power for the analysis of gut microbial diversity measures.

5.15. Gut Microbiome (CARDIA Brain MRI Microbiome Study)

The ancillary study CARDIA Brain MRI Microbiome Study (funded by Intramural Research Program funds, NIA; Lenore Launer, Katie Meyer, PIs) adds a gut microbiome component to the Y30 Exam (Birmingham, Minneapolis, and Oakland Field Centers).

Background. Exciting new data are emerging showing evidence of gut microbiota and brain interactions that modulate neuronal, hormonal and immunological pathways in the brain. Identifying specific gut microbiota, or clusters of microbiota that may reduce the risk for Alzheimer's disease (AD), would be a major advance in identifying prevention strategies, such as dietary guidance.

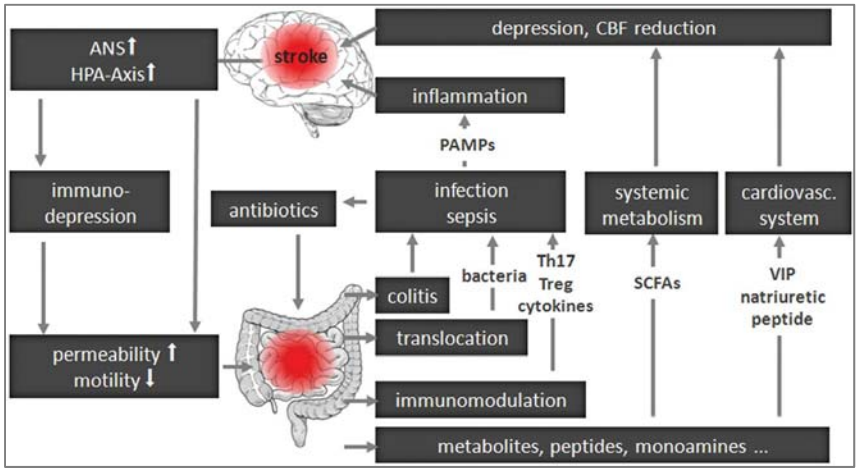
This hypothesis is still in the early stages of testing, particularly in human populations. It is an exciting development, as the current understanding of the gut-brain model brings together bi-directional pathways between the brain and inflammation, stress, systemic metabolism, and the autonomic nervous system. Animal models and clinical studies show possible gut microbe mediation of neuromodulators such as brain-derived neurotrophic factor (BDNF), serotonin, γ-Aminobutyric acid (GABA), and short chain fatty acids. These factors play a role in neurologic diseases, including depression, autism, Parkinson's disease and Alzheimer's disease[429-431]. Additionally, specific microbes have been shown to modulate the response to different drugs, such as metformin in T2 diabetes [432]. The link between diabetes, small vessel disease, cognitive impairment and the microbiota, is of particular interest[433].

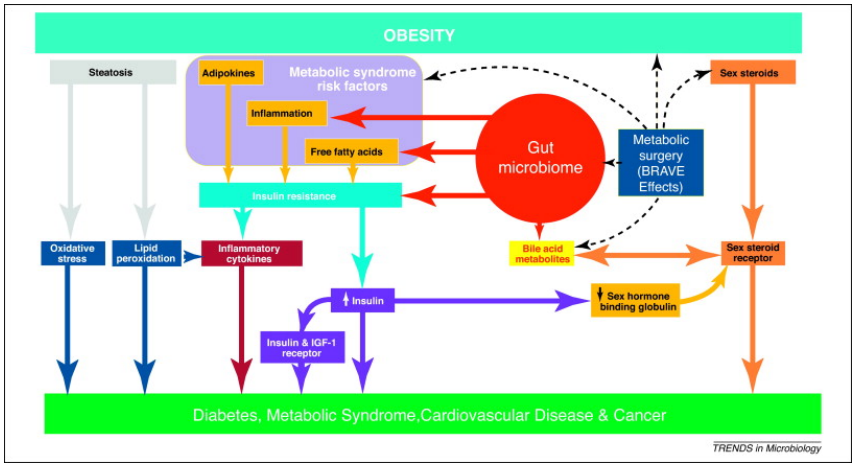
Study Objectives. We propose to test the hypothesis in the longitudinal sub-cohort of 300 participants who participate in the Y30 CARDIA Brain MRI Substudy. CARDIA is an ideal platform to study the gut-brain axis: the cohort is well described and two repeat state-of -the-art 3T MRI scans have been acquired, allowing the investigation of the association of microbiota to MRI indicators of tissue volume

and integrity, perfusion, vascular reactivity and fiber track connectivity in more than 40 regions of interest in the brain.

To ensure comparability with the *A Novel Gut Microbial-Dependent Nutrient Metabolite and Atherosclerosis* (Gut Microbiome and CVD Risk Study), the same protocol [see above] will be used from the kits, distribution of the kits to the participants, shipment of the sample to Dr. Meyers, quality control and analysis. In addition the same short survey about variables related to gut microbiome composition, such as antibiotic use and recent gastrointestinal illness.

Eligibility for the study includes: meets inclusion criteria for the MRI. From this sample, participants will be ineligible for the microbiome study if any of following apply: they have taken antibiotics in the past month; they are currently pregnant; they have had an acute gastrointestinal illness or infections in the past week; they have Inflammatory Bowel Disease, such as Crohn's Disease or Ulcerative Colitis.





5.16. Physical Activity Measurement (CARDIA Activity Study)

The ancillary study *10-Year Changes in Objectively-Measured Physical Activity and Sedentary Behavior in the CARDIA Cohort* (CARDIA Activity Study) (1R56HL 125423-01A1; Kelley Gabriel and Barbara Sternfeld, PIs) adds accelerometer-based assessment of physical activity and sedentary behavior to the Y30 Exam.

The primary goal of this study is to obtain a second collection of accelerometer data in the CARDIA cohort to evaluate the 10-year changes in objectively-measured PA and SB from early (ages 38-50 years)

to late (ages 48-60 years) midlife in relation to CVD risk while the Y30 core CARDIA exam is in progress. A secondary goal is to conduct a series of three methodological studies obtain preliminary data to support a forthcoming R01 to examine the impact of physical activity change on future disease outcomes and successful aging. Conducted in conjunction with the on-going Y30 CARDIA core exam (June, 2015 to May, 2016), this ancillary study will involve Y30 CARDIA core exam participants (estimated n=3,445) and will include the collection of accelerometer-derived PA and SB (first collected at Y20), providing, for the first time, objective measures of PA and SB change that span early to late midlife. We will also utilize core exam data from Y0 to Y30, including self-reported PA and SB, and measured cardiometabolic risk factors and disease outcomes.

CARDIA Activity Study Overview:

Ancillary study participants will be recruited from each of the four CARDIA clinic sites. Eligibility criteria include: (1) Active CARDIA Study participants and (2) ambulatory without assistance from another person (participants using assistive device are eligible).

For participants, willing to participate, that have not yet attended the Y30 Exam, s/he will be reintroduced to the accelerometer (also assessed using a similar model at the Y20 Exam) and receive instructions on how to properly wear the accelerometer, complete the tracking log, and return the materials using a pre-paid and addressed envelope, in-person, during the Y30 Exam visit. For participants, willing to participate, that have already attended the Y30 Exam, s/he will receive written instructions, the accelerometer, tracking log, and pre-paid and addressed envelope in the mail once the informed consent document has been signed and received at the clinic site. Comparisons of the in-person versus mail-based instruction and distribution of the accelerometer in terms of the differences in percentages of (1) ancillary study refusal and (2) compliance to valid wear time rules represents the first methodological sub-study. The second methodological sub-study will be conducted at the Oakland, CA site, only, and includes a comparison of the ActiGraph 7164 model (used at Y20) and ActiGraph GT3X model (used at Y30) in 100 (n=25 per race/sex group) participants who will simultaneously wear both monitors on their waist for the 7-day observation period. The third methodological sub-study will be conducted at the Birmingham, AL site, only, and includes examining the feasibility of continuous 24-hour sleep-waking time assessment in CARDIA participants. One hundred (n=25 per race/sex group) participants will wear two GT3X ActiGraph monitors, simultaneously, one on the waist for physical activity and sedentary behavior assessment and the other on the wrist for sleep duration and quality assessment. All CARDIA Activity Study participants will receive a telephone call 2-3 days after they started wearing the monitors. During this telephone call, CARDIA Activity Study staff will (1) determine if the participant is wearing the monitor, (2) ask for any remaining questions from the participant, and (3) remind participants to return the monitors after 7 days of data collection. If the accelerometer and related materials are not returned, the participant will receive reminder postcards and telephone calls.

Procedures to be used:

Accelerometer (Physical Activity) Measures: We will collect accelerometer data over seven consecutive days using the ActiGraph wGT3X-BT activity monitor (Pensacola, FL), a small, tri-axial piezoelectric accelerometer (4.6 cm x 3.3 cm x 1.5 cm; 19 gm). ActiGraph monitors have been extensively validated in laboratory and free-living conditions. Either in-person or on the telephone, participants will receive an accelerometer package that includes: (1) an initialized accelerometer, (2) detailed written wear instructions, (3) *Activity Monitor Tracking Log*, (4) contact information, and (5) pre-addressed and stamped envelope to return the monitor and record after seven days. In the tracking record, participants will enter the (1) time s/he put the monitor on in the morning, (2) time removed in the evening, (3) time that the monitor was removed and replaced for ≥30 minutes throughout the day (e.g., bathing or swimming activities), and (4) list of activities done for ≥ 10 minutes. Approximately 2-3 days

after the participant is scheduled to begin wearing the monitor, CARDIA Activity Study site staff will call to confirm monitor wear and inquire about any questions or problems.

Each CARDIA site will initialize the monitors to start at 12:00am on Day 1 of data collection using the study participant's unique study ID number. Raw tri-axial data will be sampled at 40 Hz for seven consecutive days. Once the accelerometer and tracking record are returned, data will be downloaded by CARDIA Activity Study site study staff and prepared for processing and analysis. Data will be processed using ActiLife6 software through a digitally matched filter and reintegrated up to 60 second epochs and screened for wear time using the Troiano algorithm (also used at the Y20 Exam). Weekly summary accelerometer estimates will be averaged (across days) for all participants with ≥ four valid days of ≥10 hours per day of wear time. The primary estimate, total accelerometer counts, will be calculated using summed counts detected over wear periods, while the secondary estimates, time (i.e., minutes) spent in different intensity levels will be derived using standardized cut-point threshold values. Data checks, including a descriptive analysis of the accelerometer-derived physical activity estimates, will be performed monthly.

5.17. AHASFRN-15SFRN23900002

AHASFRN-15SFRN23900002 is comprised of two ancillary studies: Project 1—Racial Differences and US Population Estimates of Nocturnal Hypertension and Non-Dipping (Paul Muntner, PI) and Project 2—Mechanisms of Nocturnal Hypertension and Non-Dipping Blood Pressure Pattern (David Calhoun, PI).

5.17.1 Ambulatory Blood Pressure Monitoring Measurement (Racial Differences in ABPM Study)

The ancillary study *Project 1—Racial Differences and US Population Estimates of Nocturnal Hypertension and Non-Dipping* (Racial Differences in ABPM Study) (AHASFRN-15SFRN23900002; Paul Muntner, PI) adds an ambulatory BP monitoring measurement to the Y30 Exam.

Rationale: The current study is designed with the mission of the American Heart Association "to reduce deaths from heart disease and stroke by 20 percent by the year 2020" in mind. The results of our study will help guide the development of diagnostic strategies and interventions which will reduce the burden of hypertension-related outcomes associated with nocturnal hypertension and non-dipping BP. Studies from Europe and Asia have shown nocturnal hypertension and non-dipping BP patterns to be associated with an increased CVD risk. However, few population-based studies in the US have conducted ambulatory blood pressure monitoring (ABPM). The preliminary data that are available suggest that these adverse phenotypes are substantially more common than hypertension based on clinic BP. Nocturnal hypertension and diurnal BP patterns assessed using ABPM are not currently part of routine clinical screening. If our hypotheses are confirmed, ABPM may become a valuable tool in clinical practice for diagnosing nocturnal hypertension and non-dipping BP and ultimately assessing the risks of CVD and CKD.

The primary goal of this study is to measure out-of-office blood pressure, including during sleep, in CARDIA to evaluate racial differences in nocturnal hypertension and non-dipping BP. Secondary goals are to determine the association between psychosocial and behavioral factors with the risk of nocturnal hypertension and a non-dipping BP pattern and determine associations of nocturnal hypertension and non-dipping BP patterns with target organ damage. We will also determine whether these associations differ by race. Finally, we will estimate the prevalence and number of US adults with nocturnal hypertension and non-dipping BP. We will also estimate the economic costs and benefits of screening for these phenotypes in US adults and the cost-effectiveness of treating nocturnal hypertension and non-dipping BP. This study will be conducted in conjunction with the on-going Y30 CARDIA core exam (June, 2015 to May, 2016) and will enroll Y30 CARDIA core exam participants from the University of Alabama at Birmingham (UAB) and Northwestern University (estimated n=800). Data collection will

include ABPM, actigraphy, and self-report data on sleep quality, risk for sleep apnea, stress and anxiety. We will also utilize core exam data from Y0 to Y30 and measured cardiometabolic risk factors and disease outcomes to achieve our study aims.

Racial Differences in ABPM Study Overview:

Ancillary study participants will be recruited from the UAB and Northwestern University CARDIA clinic sites during their Y30 study visit. Eligibility criteria include: (1) Active CARDIA Study participants and (2) clinic SBP/DBP < 180/110 mm Hg as measured by CARDIA clinic staff during their study visit or based on measurements by CARDIA staff conducted prior to initiation of ABPM for participants completing this ancillary study on a day other than when they complete their Y30 study visit. The only exclusion criterion for the proposed study is the inability to complete ABPM. For example, participants who cannot rest their arm every 30 minutes for 24 hours to allow for a BP measurement will be excluded (e.g., truck drivers) as will participants with an arm circumference too big for an extra large cuff (>50 cm). Additionally, we will exclude participants who do not have an arm available for placement of a BP cuff (e.g., double mastectomy, etc.). This will be a decision made by the study staff. Participants who work at night will be excluded if they cannot complete the ABPM on a non-work day.

Study visits:

The Racial Differences in ABPM Study will involve two study visits. The first study visit will entail fitting participants with the ABPM and actiwatch devices and measuring participants' neck circumference. When possible, this visit will occur immediately after the Y30 CARDIA study visit (i.e., the same day as the Y30 CARDIA study visit). If this is not feasible, participants will be called and asked to schedule a visit for this ancillary study. Participants will be asked to return to the CARDIA clinic 24 hours after initiating the ABPM period to return the study equipment and complete questionnaires. Some participants will not be able to return to the clinic for this second visit. For these participants, we will provide the option of returning the ABPM device and acitwatch via overnight mail (e.g., *UPS* or *FedEx*). Additionally, participants returning the ABPM device by mail will be asked to complete the study questionnaires upon completion of the ABPM period and to return the questionnaires by mail with the ABPM device.

Procedures to be used:

Twenty-four hour ambulatory monitor (ABPM): Prior to fitting participants with the ABPM device, the proper BP cuff size will be determined by measuring each participant's arm circumference at the mid-point between the acromion and olecranon. The participant will also be queried about typical sleep and awake times, which will be used to prepare the ABP monitor for silent mode during approximate sleep times. The ambulatory BP monitor will be initialized and the participant will be fitted with a *SpaceLabs* 90227 monitor (OnTrak). The monitor will be programmed to record BP readings every 30 minutes during the daytime and at night.

Neck Circumference Measurement: Participants will be asked to stand with their head held erect and eyes facing forward. They will be asked to breathe normally. A flexible tape measure (A Gulick II Plus 300 cm anthropometric tape - Model #67019) will be used to measure neck circumference at the level of the cricothyroid membrane (thyroid cartilage). Neck circumference should be recorded to the nearest 0.1 centimeter.

Actigraphy: Participants will also be fitted with an Actiwatch activity monitor (*Philips Respironics*, Andover, MA) on the wrist of his/her arm to wear during the same 24-hour period. Unless contra-indicated, the Actiwatch will be placed on the same arm that has the ABPM device (i.e., the non-dominant arm for most participants). We will use an Actiwatch as this will allow us to determine both sleep/awake times and physical activity from the same device. The actigraphy data will be merged with

the corresponding ABP measurements, based on the synchronized time stamps recorded by both devices.

Questionnaire administration: Questionnaires will be administered following the ABPM period.

Post-ABP Questionnaire: This form has three questions and will be used to document any discomfort participants experience while wearing the ABPM.

Perceived Stress Scale Questionnaire: Psychological stress will be assessed using the Perceived Stress Scale. This is a 10-item questionnaire that measures several facets of an individual's perception of their overall stress, has strong psychometric properties, and has been widely used in behavioral medicine research.

State-Trait Anxiety Inventory –Trait Scale: The State-Trait Anxiety Inventory –Trait Scale (TAI) will assess symptoms of anxiety. The TAI is a 20-item, self-report of trait-levels of anxiety. The TAI has shown good test-retest reliability and high internal consistency estimates in both whites and African Americans.

STOP-BANG Questionnaire: We will screen for sleep apnea using the 8-item STOP-BANG Questionnaire. Upon returning the ABPM, participants will answer the 4 items in the "STOP" section of the screener. The four items in the "BANG" portion of the STOP-BANG Questionnaire are assessed with data collected elsewhere from the study visit (body mass index, age, neck circumference, and sex). STOP-BANG is a validated questionnaire used to screen for obstructive sleep apnea, and is frequently used in studies of sleep disordered breathing and sleep quality.

Pittsburgh Sleep Quality Instrument (PSQI): Sleep quality will be assessed using the 19-item PSQI. This is a validated questionnaire that been used in several studies. The PSQI measures sleep quality during the previous month on a 21-point scale, with higher scores representing poorer sleep quality. Scores are also calculated for seven subscales: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction over the last month.

Repeat ABPM: We will conduct a second ABPM on 5% of study participants. The second ABPM will follow the same procedu re as the first ABPM.

5.17.2 High and Low Sodium Dietary Intervention (Sleep Apnea and Dietary Sodium Study)

The ancillary study *Project 2—Mechanisms of Nocturnal Hypertension and Non-Dipping Blood Pressure Pattern* (Sleep Apnea and Dietary Sodium Study) (AHASFRN-15SFRN23900002; David Calhoun, PI) adds a high and low sodium dietary intervention to the Y30 Exam.

The primary goal of this ancillary study is to test the hypothesis that high dietary sodium is an important cause of abnormal BP patterns, including nocturnal hypertension and non-dipping BP. Our second objective is to determine if high dietary sodium increases nocturnal BP by causing and/or worsening obstructive sleep apnea (OSA). We will enroll an equal number of African American and white participants, allowing us to determine if African Americans, being more salt-sensitive than whites, manifest greater improvement in nocturnal BP and OSA in response to dietary sodium restriction. We will measure changes in thoracic fluid content and B-type natriuretic peptide (BNP) as indices of intravascular fluid status to determine if sodium-induced fluid retention serves as an important mediator of OSA severity. Lastly, we will determine if aldosterone levels predict improvements in nocturnal BP and OSA in response to dietary sodium restriction.

Aim 1. To determine if high dietary sodium intake contributes importantly to the development of abnormal diurnal BP patterns, including nocturnal hypertension and a non-dipping BP pattern, we will compare the effect of high versus low dietary sodium intake on abnormal diurnal BP in individuals with known nocturnal hypertension. **Hypothesis 1:** Dietary sodium restriction will eliminate or substantially

reduce nocturnal hypertension and non-dipping BP compared to high sodium intake. **Hypothesis 2:** African Americans will manifest larger reductions in nocturnal BP and better restoration of BP dipping from dietary sodium restriction compared to whites.

Aim 2. To determine if high dietary sodium intake contributes importantly to nocturnal hypertension and non-dipping BP by causing and/or worsening OSA, we will compare the effect of high versus low dietary sodium intake on the development and severity of OSA in participants with nocturnal hypertension. **Hypothesis 1:** Dietary sodium restriction will eliminate or substantially reduce the severity of OSA compared to high sodium intake. **Hypothesis 2:** Reductions in thoracic fluid content and serum BNP levels induced by dietary sodium restriction will be proportional to improvements in OSA. **Hypothesis 3:** Reductions in nocturnal BP and OSA severity in response to dietary sodium restriction will be most pronounced in individuals with high aldosterone levels.

Sleep Apnea and Dietary Sodium Study Overview:

Sixty ancillary study participants (30 African American and 30 white) will be recruited from the Birmingham CARDIA clinic site. Eligibility criteria include: (1) Active CARDIA Study participants who have undergone 24-hr ambulatory blood pressure monitoring per Dr. Muntner's Population Science ancillary study; (2) nocturnal hypertension (>120/70 mmHg) identified by 24-hr ambulatory monitoring. Exclusion criteria include: (1) Stage 2 hypertension (clinic BP >159/99 mmHg); (2) use of antihypertensive agents; (3) congestive heart failure (ejection fraction of <40%); (4) chronic kidney disease (creatinine clearance <60 ml/min); (5) insulin-dependent diabetes; (6) history of cardiovascular event (stroke, TIA, myocardial infarction, or revascularization procedure); (7) current use of continuous positive airway pressure (CPAP) for OSA; (8) shift work; (9) known circadian sleep disorders.

Participants will enter into a randomized evaluation of high (6.0 g/day) and low (1.5 g/day) sodium diets. The protocol will consist of seven days of the first randomized diet; a 4-week washout period during which subjects ingest their normal ad-lib diets; and then 7 days of the second randomized diet. Standardized assessments (office BP, 24-hr ambulatory BP monitoring, home polysomnogram, 24-hr urine collection, blood collection, measurement of pulse wave velocity/pulse wave analysis, and thoracic bioimpedance cardiography) will be done at the beginning and end of the 7-day dietary intervention periods. In addition, each dietary intervention will be extended for three days in support of a translational substudy being done in collaboration with Dr. Jennifer Pollock. Participants will be provided labeled swabs to collect buccal cells every four hours for each of the three days of the dietary extension period to determine the effects of high vs. low dietary sodium intake on expression of circadian clock control genes (*CLOCK, Bmal1, per1, per2, cry1, and cry2*) that regulate diurnal BP patterns. Participants will be fitted with an Actical activity monitor (*Philips Respironics,* Andover, MA) on their wrist to wear during the 3-day periods to provide estimates of sleep onset and offset. In addition, melatonin and cortisol levels will be measured from the buccal swabs to confirm normal circadian profiles in all participants.

Procedures to be used:

Twenty-four hour ambulatory monitor (ABPM): Prior to fitting participants with the ABPM device, the proper BP cuff size will be determined by measuring each participant's arm circumference at the mid-point between the acromion and olecranon. The participant will also be queried about typical sleep and awake times, which will be used to prepare the ABP monitor for silent mode during approximate sleep times. Next, the ambulatory BP monitor will be initialized and the participant will be fitted with a *SpaceLabs 90207* monitor. The monitor will be programmed to record BP readings every 20 minutes during the daytime and every 30 minutes at night.

Home polysomnography: Home sleep monitoring will be done with use of the *Resmed ApneaLink* device. The *ApneaLink* is a 5-channel (nasal airflow, respiratory effort, oxygen saturation, snore, and heart rate) portable monitoring system, which is easy to put on and remove. The apnea/hypopnea (AHI) will be computed using standard American Academy of Sleep Medicine criteria (apnea is defined as a cessation of airflow for ≥ 10 seconds; hypopnea is defined as ≥ 30% reduction in airflow followed by a 4% or greater reduction in oxygen saturation). The AHI will be calculated as the total number of respiratory events calculated by total recording time.

Pulse wave analysis and pulse wave velocity: Applanation tonometry for central pulse wave analysis computed from the radial artery waveform using a transfer function and carotid-femoral pulse wave velocity (*SphygmoCor*, *AtCor Medical*, Sydney, Australia) according to guidelines. Both tests were performed with the patient in a supine position after resting for at least 10 minutes.

Thoracic bioimpedance cardiography: Hemodynamic measurements will be obtained by thoracic electrical bioimpedance cardiography (*BioZ*, *SonoSite*, Bothell, WA). This instrument uses surface electrocardiographic electrodes applied at the base of the neck and at the base of the thorax. A low-voltage high-amplitude alternating current is introduced through the outermost sensors and sensed through the innermost sensors. The difference between voltage introduced and that sensed indicates the level of impedance in the thorax and is inversely proportional to the amount of thoracic blood volume.

6. Exam Implementation

6.1. Recruitment

Retention of CARDIA participants across the four field centers is a primary focus of the Y30 Exam period. Participants should, in general, be scheduled sequentially in order of CARDIA participant ID (PID), i.e. the order in which they were examined at the baseline CARDIA exam.

All participants in the Y0 Exam are eligible for the Y30 Exam. Pregnancy or lactation will not eliminate a participant from the entire exam, but may preclude participation in specific exam components. Pregnancy and/or lactation may extend the window of eligibility for a participant. Similarly, participants who have moved more than 50 miles from their home clinic will be permitted an extended window of eligibility in order to maximize retention of the cohort and minimize the costs of retention. Reimbursements will be allowed within limits, at the discretion of the field center Principal Investigator, to bring back for examination participants who have moved more than 50 miles from their home clinic.

The Y30 Exam recruitment plan is designed to re-examine at least 72% of the surviving CARDIA cohort examined at the Y0 Exam. The plan also provides for extensive monitoring of the process. Each field center is provided scheduling lists designed to achieve the completion of the re-examination within twelve months. Participants who have moved will be monitored so that special recruitment and cost planning can be made in advance. Clinic staff will make every effort to contain the costs associated with bringing moved participants to the clinic during the exam. A description of the initial contact and recruitment process can be found in the Y30 MOO Chapter 2—Guidelines for the Y30 Exam.

Every effort is made to maximize the return rate of the CARDIA cohort. The efforts include performing a partial exam on participants who refuse or are unable to participate in a full exam; and examination at a clinic (field center) other than the participant's home clinic. The CARDIA Study is aiming at as high a rate as possible in order to maximize the long-term potential of the study to chart the natural history and/or evolution of CVD risk factors.

6.2. Exam Flow

A sample form for tracking clinic flow is provided to each field center via the CARDIA internal website. Each clinic can determine their own flow based on their set-up, staffing, and needs. The following general flow should typically be followed when planning the exam flow for each participant (detailed information can be found in the Y30 MOO).

- **Greeting & Informed Consent**
- **Blood Pressure** … before phlebotomy and any potentially stressful interview
- **Anthropometry** … before snack
- Albumin measurement (urine specimen) … when participant can provide
- **Phlebotomy—fasting blood draw** … before 10:30 AM, if possible, and before the snack
- **Cognitive Function** (CARDIA Cognition Study) … after the snack
- **Interview** (self- and interviewer-administered questionnaires)
- **Phlebotomy—2-hour glucose blood draw** (PRF Women Study) … two hours after the fasting blood draw
- **Echocardiography***
- **Pulmonary Function** (CARDIA Lung Study) … after BP, anthropometry, phlebotomy, and echo because of albuterol administration
- **Exit Interview** and Brain MRI Substudy scheduling, if applicable

*Please note that the echocardiographer should have approximately 30-minute break after every third or fourth participant.

6.3. Prioritization Schedule

If a participant refuses to stay for the entire exam (i.e. *I only have one and a half hours available)* then the performance of exam components are prioritized as follows:

Priority Level 1

1. Consent and Consent Summary Form
2. Exit Interview (plus Follow-up contact worksheet)
3. Blood Pressure (Form 2)
4. Fasting Phlebotomy (Form 5) **and** Urine (Form 51)
    - Lipid Profile
    - Glucose
    - Insulin
    - Serum creatinine
    - Urinary albumin/creatinine
    - DNA, PAXgene
5. Height, Weight, Waist Girth, Hip Girth (Form 20)
6. Medical History (Form 8)
7. Interim Health Care Contact (Form 31)
8. Sociodemographics (Form 3)
9. Tobacco Use (Form 10 and Form 9-TOB)

Priority Level 2

1. Echocardiogram (Form 40)

Priority Level 3

1. CARDIA Cognition Study: RAVLT (Form 87), DSST (Form 88), Stroop (Form 89), MoCA (Form 94), Category Fluency Test (Form 97), Letter Fluency Test (Form 96), REALM-SF (Form 95), CARDIA Cognition Form (Form 98)
2. PRF Women Study: HbA1c and OGTT (Form 5), (Form 5A)
3. CARDIA Lung Study: Spirometry Completion Form (Form 80), Hx of Lung Problems (Form 12)

Priority Level 4

1. Physical Activity Questionnaire (Form 18)
2. Alcohol Use Questionnaire (Form 7)
3. Women's Reproductive Health (Form 68)
4. CES-Depression (Form 36)
5. Quality of Life SF-12 (Form 65)
6. Sedentary Behavior Questionnaire (Form 91)

Priority Level 5

1. Follow-up forms to Form 8
    - Follow-up Questions for Medications (Form 9-MED)
    - Pregnancy Questionnaire (Form 9-PREG)
2. Non-Medical Drug Use (Form 17)
3. Discrimination (Form 44)
4. Chronic Burden (Form 64)
5. Beverages Questionnaire (Form 90)
6. Diet Practices (Form 48)
7. Dietary Habits (Form 79)
8. Neighborhood Environment (Form 81)
9. Neighborhood Cohesion (Form 56)
10. Weight History (Form 8A)
11. Weight Change (Form 46)
12. CARDIA Brain MRI Substudy: (Form 92)

6.4. Quality Control

To ensure high quality data, QC procedures developed during prior CARDIA exams are used, and include:

- Initial field center personnel were centrally trained and certified by coordinating center staff, and locally recertified during the exam.
- Anthropometric technicians are evaluated monthly using duplicate measures from a 5% random sample of participants to assess inter-technician variability (initial measurement and then re-measured by a different technician). Terminal digit distribution is used to estimate technician's performance monthly as well.
- Overall field center, laboratory, and reading center performance is evaluated by a site visit early in the exam cycle.
- Laboratories are evaluated using duplicate blind measurements done for all laboratory testing. The QC sample size is 5% per assay. Results of internal and external QC procedures and long-term monitoring for the NIST assays will be provided by the central blood & urine repository and lipids laboratory, and reported bi-monthly.

- Random review of 5% of cognitive test administration booklets and interview materials are conducted. All cognitive function assessments are recorded as a measure of QC. Recertification of cognitive function technicians will occur at six months following initial certification. Problem-based reviews are conducted, as needed.
- The field center Clinic Coordinator reviews one regular interview per interviewer and completes a rating form monthly. A coordinating center staff member reviews the tape and completes an evaluation form to provide field center Principal Investigator feedback.
- QC of brain MRI data consists of 5% random samples re-reads to assess intra-reader's variability monthly; machine reproducibility is done on twelve volunteers (three per each field center); regular phantom scanning for machine validation; checks for protocol violation and quality of the images performed by the Brain MRI Reading Center (MRIRC).
- Pulmonary function acquisitions and readings are reviewed by the Pulmonary Function Reading Center (PFRC) and compared to the data recorded on Form 80. All images are assigned the quality scores that are reviewed at the PFRC monthly to evaluate technician's performance. Re-readings of the images are done in 5% to assess intra- (2.5%) and inter- (2.5%) reader variability. The PFRC submits QC reports to the coordinating center on a monthly basis.
- ERC duplicates 10% and reads continuously throughout the entire exam to assess inter- (5%) and intra- (5%) reader variability. Re-measure 3% of participants at each field center to assess intra- (1.5%) and inter- (1.5%) technician variability are done throughout the exam period. ERC reviews all studies for protocol violations and makes an assessment of quality of images. QC feedback is provided to the technologists for all studies and additional protocol training sessions are held, as necessary.
- Utilizing the Electronic Data Collection (EDC) System, all data are subject to range and logic checks. Missing data and outliers are reviewed at the coordinating center continuously.
- For paper exam form data entry, quality is assessed by utilizing duplicate entry of 10% of data forms using dual monitor method and monthly review of error rates.
- Equipment maintenance and calibration is evaluated using QC logs reported to the coordinating center on a monthly basis.
- QC Subcommittee meets monthly (or twice a month if needed) to review QC reports and approve follow-up actions.

6.5. Referrals and Results Reporting

The study will utilize criteria for medical referral of abnormal BP, blood lipids and glucose levels, eGFR and urine albumin levels based on commonly-accepted guidelines where available, e.g., the JNC7 for BP, ACC/AHA 2013 cholesterol guidelines for lipids, and American Diabetes Association (ADA) criteria for diagnosis of DM. Table 4 below summarizes the referral criteria established for this exam. For Y25, we added a cutpoint for DM diagnosis using HbA1c, which is retained for Y30. The referral criteria will be implemented for those participants who report they do not have DM. Among diabetics, HbA1c is used to assess adequacy of control, with <7% considered the ADA goal for control. This information will be included in the results report. Echocardiograms and MRI scans will be reviewed for presence of significant findings which will be reported to participants, and referrals made to their usual source of care.

Table 4. Summary of Year 30 Referral Criteria, CARDIA Study	
Measure	**Referral Value**
Systolic blood pressure	≥ 140 mmHg
Diastolic blood pressure	≥ 90 mmHg
Triglycerides	≥ 500 mg/dL
LDL cholesterol	≥ 190 mg/dL
Fasting blood glucose	< 60 mg/dL or ≥ 126 mg/dL
HbA1c*	≥ 6.5%
eGFR	< 60 mL/min/1.73 m^2
Urinary albumin excretion	≥ 300 mg/g [estimated from (albumin mg/dL)/(creatinine mg/dL)]
Echocardiogram	
LVIDd	>6.0 cm
IVSd	>1.4 cm
LVPWd	>1.4 cm
EF	≤45% (or LV systolic dysfunction graded moderate or severe)
Aortic Root Dimension	>4.5 cm
Left Atrial Dimension	>5.0 cm
RVSP	>40 mmHg
Mitral or aortic regurgitation	Moderate or Severe
Stenosis of any valve	Mild, Moderate, or Severe
Mitral Valve Prolapse	Moderate or Severe
Clinical Findings	Significant (i.e., Aortic dissection, vegetation, tumor, flail leaflet, thrombus, suspected pericardial tamponade, significant arrhythmia, severe left ventricular dysfunction, etc.)
Pulmonary function	<70% of predicted (based on height, age, gender, and ethnicity)
MRI	**Urgent referral (Alert) -** Finding possibly having clinical significance (tumor without significant mass effect, sub-acute infarct, AVM, aneurysm, obstructive hydrocephalus). **Emergent referral (Alert) -** Finding possibly having immediate impact on patient care (acute subdural or epidural hematoma, sub-arachnoid hemorrhage, acute intra-parenchymal hematoma, acute infarct, abscess and suspected tumor with significant mass effect).

***applied to participants reporting they do not have diabetes**

Participants will receive printed report results of clinically useful tests as noted in Table 5 below. Copies of the results reports/letters can be found in the Y30 MOO in Chapter 13—Referrals and Results.

Table 5. Summary of Year 30 Results Reported to Participant, CARDIA Study

Measure	How Reported to Participant	Results
Blood pressure	In-clinic results form	Systolic and Diastolic blood pressure
Anthropometry	In-clinic results form	Height, Weight, BMI, Waist circumference, Hip circumference
Lipids	Mailed results form	Total Cholesterol, HDL, LDL, Triglycerides
Chemistries	Mailed results form	Fasting glucose, eGFR
Urine	Mailed results form	Urinary creatinine, albumin, albumin/creatinine ratio
Echocardiogram	Mailed results form	Left ventricular chamber size, left atrium chamber size, left ventricular ejection fraction, left ventricular internal dimension in diastole and in systole, interventricular septal thickness in systole and in diastole, left ventricular mass, aortic root dimension, left atrial dimension, mitral early peak flow velocity, mitral late peak flow velocity
Pulmonary function	Mailed results form	FVC, FEV1, FEV1/FVC ratio
MRI	Mailed results form	Presence or absence of unexpected findings (alerts)

7. Data Management

The Data Management System for the Y30 Exam includes the following physical components:

1. A Virtual Private Network (VPN) for encrypted firewalled connections connecting coordinating center desktop machines, field center desktop and mobile machines with the primary server site located at the coordinating center in the University of Alabama at Birmingham, School of Medicine, Department of Medicine (DOM), Division of Preventive Medicine Primary Server Room, and a disaster-recovery (DR) site housed in the DOM Server Room.
2. A Virtual Machine Host server at the coordinating center primary site delivers Microsoft 2012 R2 virtual servers and Windows 8.1 virtual machines providing development, testing, and production instances of the following services:
   - Microsoft SQL (MSSQL) databases for electronic data entry, reading center results, and software development
   - Microsoft Team Foundation development environment
   - MSSQL Reporting Server
   - Statistical Analysis System (SAS) software
   - Internet Information Server, and
   - Remote Desktop Session Hosts (RDSH) services (formerly known as Terminal Server services)

3. The DR site system uses Dell AppAssure snapshots written to a disc-based repository. Throughout the period, the snapshots will archive onto magnetic media.
4. Coordinating center and field center personnel endpoint devices will connect to coordinating center services from Windows 8.1 BitLocker encrypted systems.
5. A consolidated Recruitment System for participant scheduling which is maintained on the coordinating center secure servers.
6. A reporting system that provides the retention and tracking information to the field centers
7. The data entry system for use at the field centers.

All data entry will be performed by field center staff. The data entry system automates much of the range checking and Level 1 data checking formerly done with SAS programs.

7.1. Flow and Management of Data Forms

For Y0, Y2, Y5, Y7, and Y10 Exams, the CARDIA Study used distributed data entry systems with data entry performed locally at each of the field centers. For the Y15, Y20, and Y25 Exams, we utilized a different approach with paper questionnaires and forms generated at each field center from software provided by the coordinating center. The Y30 Exam will utilize an EDC System.

The CARDIA Recruitment System once existed at each field center, but is now accessed over VPN connections to an RDSH server hosting the application at the coordinating center. Each field center will have access to the CARDIA Recruitment System. The Recruitment System allows on-screen access to scheduling information, as well as a variety of reports used in the recruitment process.

The CARDIA Recruitment System includes a configurable reporting feature. The reporting feature will allow the user to filter the master list of participants by Field Center, Status, Category, and Exam Type. Each participant is assigned a status based on the last action performed on that participant. Categories are manually assigned to each participant. Each field center will be provided with a set of pre-determined category codes for the master participant list. Utilizing the filters, field centers will be able to produce several reports, such as the *≥50 Mile List* which includes participants who have moved 50 miles or more from their "home" field center (the site at which they participated in the Y0 Exam).

To ensure the integrity of data collected, the process must be monitored and data tracked carefully. The information collected in this exam will be analyzed and published to advance understanding about the development of CVD. The value of this information is a reflection of the care and attention paid to the completeness and accuracy of the data during its collection.

Both push and pull strategies of moving data from one point to another are utilized in creating a system to track and verify the status of data at each site, at any point in time. These sites are collectively referred to as "centers" and include:

- Field centers: Birmingham, Chicago, Minneapolis, Oakland
- Coordinating center
- Assay laboratories: blood & urine chemistries laboratory, lipids laboratory
- Storage laboratories: central blood & urine repository, DNA laboratory
- Reading centers: Brain MRI Reading Center, Pulmonary Function Reading Center, Echocardiography Reading Center

The purpose of the Reporting System is to employ effective methods of data tracking for Y30 by utilizing technologically-advanced systems for maintaining data availability and sustaining accurate data status throughout the exam period. The aims of the Reporting System are to:

1. assure the availability of complete and accurate data
2. assure timely transfer of complete and accurate data
3. store and archive data in a readily-retrievable manner

Computer-based information systems for all aspects of data collection, transmission, and entry have been developed by the coordinating center's Data, Information, and Statistical Computing staff. All systems have been developed in the C#.net programming language and make use of the standard Windows application programming interface.

7.2. Electronic Storage and Management of Data

The electronic storage and management of the data begins at the field centers.

7.3. Field Center System

A field center system is now simply a BitLocker-encrypted Windows 8.1 desktop or mobile device. The system is a CARDIA Active Directory computer and must use the Dell SonicWALL NetExtender VPN client to access systems and processes of the Y30 EDC, which is a Windows application utilizing the standard Windows graphical user interface and security. The current hardware configuration scheme greatly expands the field center's flexibility in that all study software will be installed on the server to allow multiple users in separate locations. The system provides support for the following activities:

Participant Scheduling - The Recruitment System provides a one week window for the expected date based on each participant's baseline examination date. It has a calendar format so that participants scheduled for each day is clear for the clinic personnel. The System allows certain days to be blocked out so that no one is scheduled at a time or date during which the clinic is not open. It also graphically shows the week's schedule against the "ideal schedule" to assist staff in tracking daily progress for retention goals.

Participant Event Tracking - The System keeps track of each participant's scheduled clinic visits and the outcome of each visit. Thus, if a participant does not keep his/her appointment, the system can record this and return the participant to the pool of individuals who need to be scheduled. The System maintains a database of this information and can produce tracking and retention reports throughout the study period.

Data Sharing Between Field Centers - The Recruitment System maintains study-wide demographic databases of non-sensitive site-specific information. The Scheduling System works in conjunction with the data transmission system to provide synchronized information for the clinics. Data in the files associated with this synchronization are generated at the coordinating center and automatically uploaded to the field centers in the form of read-only files. All PIDs, but not demographic information, are available to all field centers in the event a participant is being scheduled at a clinic other than their home clinic (clinic where the participant was examined for the Y0 Exam).

Reports of Recruitment/Scheduling Progress – An extensive report generation capability is offered by the Recruitment System. Among the standard reports which are produced are the tracking and retention reports. All field centers have the ability to produce their tracking and retention reports for a real-time update.

Availability of Blank Forms – Blank forms will be available on the CARDIA internal website. Field centers will have the ability to print the blank forms in those cases where the participant is not willing or able to complete the electronic data forms, or there is a loss of connectivity.

Quality Control ID Generation - The master list of QC PIDs is generated by the coordinating center and the weekly assignments are done at field center level. During the early part of the examination period (first three months) over-sampling is used to be sure that all QC measures are working. After this initial phase, the sampling plan is implemented in such a way as to produce about a 10% QC sample for the entire examination period. The Scheduling System allows for the field center to record which participant is involved in each QC sample. All data relating to QC is transmitted along with the normal recruitment activities to the coordinating center for review and monitoring

7.4. Data Entry

Like the field center system, the data entry system is a server-based application. In structure, the system is flexible so that forms can be added and removed for the system without making extensive changes to the basic program. Details of this flexibility are included where appropriate in the description which follows.

The data entry program performs an extensive set of tasks which include:

Data Entry from the Clinic - The data entry system utilizes the EDC. The system performs a series of edits including range checks, checks for completeness of answers, and checks of skip patterns.

Data Export – Upon entry and verification of data, the information is written to a SQL database. Statistical Computing Unit staff has read-only rights to the databases. Using custom designed views, the Statistical Computing Unit is able to directly import the data into the SAS programs used for analysis. All variable names and data types have been chosen by the Data Analysis and Verification Group so that no translation of name or type is required after input to SAS for analysis purposes.

Problem Data Resolution – With range checks, completeness checks, and skip patterns, the majority of any data problems can be resolved by the field center staff before the data entry process is finalized and data is transferred to the coordinating center.

Quality Control Administration - This section of the program provides tracking for the QC forms.

7.5. Data Transfer System

Field Centers – Data entry performed at the field centers for the EDC, Recruitment, and Endpoints Systems will travel through the encrypted VPN tunnel directly into the coordinating center's MSSQL databases.

Reading Centers – The Brain MRI Reading Center (MRIRC) will enter form data into the EDC, and as with the field centers, the data will travel through the encrypted VPN tunnel directly into the coordinating center's MSSQL databases. The MRIRC, along with the Pulmonary Function Reading Center and Echocardiography Reading Center will transmit data files to the coordinating center through the Dropbox function. These files will be uploaded to the coordinating center's MSSQL databases.

Assay Laboratories – The assay laboratories will transmit data files to the coordinating center through the Dropbox function. These files will be uploaded to the coordinating center's MSSQL databases.

Storage Laboratories - The storage laboratories will transmit data files to the coordinating center through the Dropbox function. These files will be uploaded to the coordinating center's MSSQL databases.

8. Study Organization

The organization of the CARDIA Study and the CARDIA Study centers is illustrated in Figure 2 and Figure 3, respectively, at the end of this section. Below is detailed the role of the CARDIA Study entities.

8.1. National Heart, Lung, and Blood Institute

The CARDIA Study operates under contracts between the institutions involved and NHLBI. The NHLBI has assigned a Project Officer to serve as the main scientific link between NHLBI and the Program.

Functions and Responsibilities

- Provide necessary scientific and administrative communication, liaison, and direction to the Principal Investigators of the field centers and the coordinating center to assure fulfillment of the scientific objectives and administrative aspects of this program for NHLBI and National Institutes of Health review--including protocol, program progress, forms, and publications.
- Serve as a direct link from the organization of CARDIA to the Director of the NHLBI, to channel inquiries, recommendations, and policy directives. Since this is a contract, NHLBI will be the final authority for determining program policy and how problems should be handled. A CARDIA Observational Study Monitoring Board (OSMB), composed of outside scientists, will provide advice to NHLBI on the conduct of the study.

8.2. Observational Study Monitoring Board

An external OSMB, whose membership is assigned by NHLBI, meets annually to monitor and evaluate the study's progress in all areas. The OSMB advises and makes recommendations to the Steering Committee and NHLBI concerning any scientific or administrative issues which may be of concern.

CARDIA OSMB Members	
Investigator	**Affiliation**
Larissa Avilés-Santa, MD, MPH, Executive Secretary	National Institutes of Health—NHLBI
Emelia J. Benjamin, MD, ScM	Boston University, School of Medicine, Department of Medicine
Ingrid Borecki, PhD, Chair	Washington University, School of Medicine, Division of Statistical Genomics
Patricia Elmer, PhD, RD	Kaiser Permanente Center for Health Research
Amit Khera, MD	University of Texas Southwestern Medical Center, Medical School, Department of Internal Medicine
Thomas H. Mosley, PhD	University of Mississippi Medical Center
Steven Shea, MD, MS	Columbia University, College of Physicians and Surgeons, Department of Medicine, Division of General Medicine

CARDIA OSMB Members	
Investigator	**Affiliation**
David Strogatz, PhD	State University of New York—Albany, School of Public Health, Department of Epidemiology
Lisa Wruck, PhD	University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Biostatistics

8.3. Coordinating Center

The CARDIA coordinating center is a facilitator of the study's design, monitoring, analysis, and manuscript production. The coordinating center takes the lead role in the generation of the study protocol and corresponding MOO. It also takes the organizing role for the centralized training and staff certifications. It has the responsibility for developing and implementing systems necessary for data collection, editing, management, and analysis.

Functions and Responsibilities

- Prepare the protocol and exam MOO with the cooperation of the Steering Committee.
- Prepare and implement a system for data collection, transmittal, logging, editing, management, extraction, and analysis.
- Work with the investigators in the development, pretesting, construction of forms, and associated procedures.
- Obtain inter- and intra-observer reliability measurements.
- Obtain reliability for all questionnaires and procedures used in the study so that such information might be incorporated into the analyses.
    - Manage subcontracts for central laboratories as requested by NHLBI.
    - Monitor QC of external laboratories.
    - Interact with the Clinic Coordinators and Principal Investigators to orchestrate the training sessions and staff certification for the Y30 Exam.
    - Produce minutes of the Steering Committee meetings and conference calls.
    - Assist in the organization and conduct of site visits to each center to ensure compliance with the provisions of the exam MOO.
    - Monitor the completeness and accuracy of submitted data forms. Notify field centers and the relevant study units of error rates and/or deficiencies in forms submitted.
    - Design and implement a distributed data analysis system.
    - Notify the Principal Investigator if any specific local problems arise in the field center's performance. Notify Project Officer if timely resolution is not possible.
    - Issue periodic reports to NHLBI, the Steering Committee, and the OSMB. Specific reports will include predefined QC monitoring reports.
    - Develop new or modify existing statistical methods for data analyses.
    - Facilitate the preparation of study publications in cooperation with other investigators and according to the publications policies.
    - Take the lead on specific manuscripts depending on investigator interest and in accordance with the publications policies.
    - Lead efforts to periodically review and revise the publications policies.

8.4. Field Centers

The field centers are clinical research units supervised by the Principal Investigators and supported by individual agreement research contracts from the NHLBI. Each field center is responsible for following the provisions of the protocol, exam MOO, and QC MOO. The Principal Investigator of each field center is a voting member of the Steering Committee.

Functions and Responsibilities

- Recruit and train staff to perform the procedures of the study effectively.
- Retain participants according to the exam MOO and examine/evaluate them.
- Establish and maintain good relations with the participants, their families, other attending physicians (if any), and the public.
- Collect and transmit all required data, specimens, etc. in accordance with established procedures and schedules.
- Evaluate the progress of the study and alert the coordinating center and Steering Committee to major problems.
- Work with the coordinating center to maintain the quality of data collected.

8.5. Reading Centers

For the Y30 Exam, there are three reading centers, each functioning to review data from the field centers and report results to the coordinating center. As well, each reading center is responsible for training CARDIA field center staff in their area and participating in the subcommittee which oversees their exam component.

The Echocardiography Reading Center (ERC), João Lima, MD, MBA, Principal Investigator, is located at JHU. The ERC is responsible for performing echocardiogram readings, as well as developing, implementing, and supporting QC activities for these exams and readings. Additionally, the ERC is responsible for developing the protocol for echocardiographic imaging, creating training materials for sonographers, and setting up plans for image transfer.

The Pulmonary Function Reading Center (PFRC), David Mannino, MD, Principal Investigator, is located at the University of Kentucky. The PFRC is responsible for reviewing the pulmonary function data and providing quality scores for the tests (conducted by the ancillary study, *CARDIA Lung Study*, Ravi Kalhan, PI).

The Brain MRI Reading Center (MRIRC), R. Nick Bryan, MD, PhD, Principal Investigator, is located at the University of Pennsylvania. The MRIRC is responsible for quality and clinical review of all participant scans as well as quantitative image analysis of the studies (conducted the substudy, *CARDIA Brain MRI Substudy*, Lenore Launer, PI). The MRIRC is also responsible for training reading center personnel in the performance of the phantom and participant scans, data transfer mechanisms, and QC analysis.

8.6. Laboratories

There are two assay laboratories and two storage laboratories for the Y30 Exam.

Lipids Laboratory – Northwest Lipids Research Laboratories, Santica Marcovina, PhD, ScD

Blood & Urine Chemistries Laboratory – Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota, Myron Gross, PhD

Central Blood & Urine Repository – Solomon Park Research Laboratories, Patric Clapshaw, PhD

DNA Laboratory – Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Myriam Fornage, PhD

8.7. Role and Composition of Steering Committee

The Steering Committee is the governing body of the CARDIA Study. It is composed of the Steering Committee chair; the NHLBI Project Officer and Contracting Officers; Principal Investigators and Co-Principal Investigators of the four field centers and the coordinating center; Subcommittee Chairs; recognized CARDIA scientists; and essential coordinating center staff. The Steering Committee provides overall scientific direction for the study and serves as the focal point of the organizational structure.

The Steering Committee meets regularly to review study progress, currently twice each month on the second and fourth Tuesdays for one hour at 10:30 AM Central Time. Each center is required to be represented by an investigator or proxy at each meeting of this committee. If a vote is needed, it will require a simple majority of the members. Minutes are taken by a member of the coordinating center.

The Steering Committee performs the following functions:

- Guide and oversee the development of the study protocol and MOO.
- Review and approve major changes in the MOO and data collection forms.
- Provide advice and assistance to the coordinating center, field centers, or NHLBI on operational matters.
- Resolve problems brought to the Steering Committee by investigators, the coordinating center, and any other of the central units (central laboratories, NHLBI, etc.).
- Monitor the performance of all participating field centers through site visits according to information provided by the coordinating center in order to insure that an adequate number of participants are retained; that the field centers adhere to the protocol; and that high quality data are collected.

CARDIA Steering Committee Members	
Investigator	**Affiliation**
Norrina B. Allen, PhD	Northwestern University, Feinberg School of Medicine, Department of Preventive Medicine
Alexander Arynchyn, MD, PhD	University of Alabama at Birmingham, School of Medicine, Department of Medicine, Division of Preventive Medicine
Sejong Bae, PhD	University of Alabama at Birmingham, School of Medicine, Department of Medicine, Division of Preventive Medicine
Claude Bouchard, PhD, MSc	Louisiana State University, Pennington Biomedical Research Center
R. Nick Bryan, MD, PhD	University of Pennsylvania, Perelman School of Medicine, Department of Radiology
Jeffrey Carr, MD, MS	Vanderbilt University, School of Medicine, Department of Radiology & Radiological Sciences
Mercedes Carnethon, PhD	Northwestern University, Feinberg School of Medicine, Department of Preventive Medicine
Myriam Fornage, PhD	University of Texas-Houston, Health Science Center, Institute of Molecular Medicine
Samuel Gidding, MD	Nemours
David C. Goff, Jr., MD, PhD, Chair	University of Colorado, School of Public Health

CARDIA Steering Committee Members	
Investigator	**Affiliation**
Myron Gross, PhD, MS	University of Minnesota, Molecular Epidemiology and Biomarker Research Laboratory
David R. Jacobs, Jr., PhD	University of Minnesota, School of Public Health, Division of Epidemiology & Community Health
Cheryl Jennings	National Institutes of Health—NHLBI
Ravi Kalhan, MD, MS	Northwestern University, Feinberg School of Medicine, Department of Preventive Medicine
Catarina Kiefe, MD, PhD	University of Massachusetts Medical School, Department of Quantitative Health Sciences
Lenore Launer, PhD	National Institutes of Health—NIA
Cora E. Lewis, MD, MSPH	University of Alabama at Birmingham, School of Medicine, Department of Medicine, Division of Preventive Medicine
João Lima, MD, MBA	Johns Hopkins University
Kiang Liu, PhD	Northwestern University, Feinberg School of Medicine, Department of Preventive Medicine
Donald Lloyd-Jones, MD	Northwestern University, Feinberg School of Medicine, Department of Preventive Medicine
Santica Marcovina, PhD, ScD	University of Washington
Ilya Nasrallah, MD, PhD	University of Pennsylvania, Perelman School of Medicine, Department of Radiology
Jamal S. Rana, MD, PhD	Kaiser Permanente
Jared Reis, PhD	National Institutes of Health—NHLBI
Pamela J. Schreiner, MS, MS, PhD	University of Minnesota, School of Public Health, Division of Epidemiology & Community Health
James Shikany, DrPH	University of Alabama at Birmingham, School of Medicine, Department of Medicine, Division of Preventive Medicine
Stephen Sidney, MD, MPH	Kaiser Permanente, Division of Research
David Siscovick, MD, MPH	New York Academy of Medicine
Lewis Smith, MD	Northwestern University, Feinberg School of Medicine, Department of Preventive Medicine
Lyn Steffen, PhD	University of Minnesota, School of Public Health, Division of Epidemiology & Community Health
Jennifer L. Wammack, MPH	University of Alabama at Birmingham, School of Medicine, Department of Medicine, Division of Preventive Medicine
Colin Wu, PhD	National Institutes of Health—NHLBI
Kristine Yaffe, MD	University of California, San Francisco, School of Medicine, Department of Psychiatry
Elizabeth Zoller	National Institutes of Health—NHLBI

8.8. Role and Composition of Steering Committee Subcommittees

Clinic Coordination and Retention Subcommittee

The Clinic Coordination and Retention Subcommittee's purpose is to:

1. Develop a communications support network for the Clinic Coordinators, the coordinating center and the Project office.
2. Establish a mechanism for providing input and feedback to the Steering Committee.
3. Convene annual meetings to discuss clinic operational issues, and summarize these for the Steering Committee.
4. Provide encouragement to Clinic Coordinators to participate in other study activities.
5. Plan and coordinate a retention program which will maximize the number of baseline participants to be re-examined during the Y30 Exam.
6. Collaborate with the coordinating center in designing and updating a monitoring system to evaluate and track the retention efforts.
7. The Clinic Coordination and Retention Subcommittee will meet periodically to review the effectiveness of the recruitment/retention plan and create new strategies, as needed.

Design and Analysis Subcommittee

The Design and Analysis Subcommittee's purpose is to:

1. Develop analytic strategies to deal with longitudinal data.
2. Identify appropriate statistical models from specific epidemiologic problems.
3. Plan analyses and publications which encompass the hypothesized epidemiologic models.

eCARDIA Subcommittee

The eCARDIA Subcommittee's purpose is to:

1. Provide oversight for the development of electronic capability for annual participant questionnaire completion.

Provide oversight for the transition from paper to EDC for the Y30 Exam.

Endpoints Surveillance and Adjudication Subcommittee

The Endpoints Surveillance and Adjudication Subcommittee's purpose is to:

1. Develop strategies for obtaining clinical information on all deaths, and on hospitalizations deemed relevant to CARDIA.
2. Enhance the completeness with which this information is obtained.
3. Adjudicate the causes of these disease events.

Genetics Subcommittee

The Genetics Subcommittee is responsible for designing and coordinating genetic activities in CARDIA, including research questions, participant consent, analyses, substudies, publications and presentations. The Genetics Subcommittee's purpose is to:

1. Track ongoing genetic studies using CARDIA DNA.
2. Recommend to the Steering Committee approximately 50 polymorphisms on identified genes for genotyping.
3. Review proposed substudies and ancillary studies, from investigators both within and external to the CARDIA Study.
4. Establish an ancillary study agreement for investigators using CARDIA DNA that will address a formal policy on data distribution and submitting progress reports, what to do with a non-cooperative ancillary study investigators, and a formal policy on either destroying or returning excess DNA to its source.

Imaging Subcommittee

The Imaging Subcommittee is responsible for helping to plan and address issues concerning the measurement of coronary artery calcium and carotid intimal media thickness, QC of these data, and analysis and interpretation of results.

The Imaging Subcommittee's purpose is to:

1. Develop and oversee the implementation of the protocol, QC procedures, and analysis of carotid ultrasound, coronary calcium, and other relevant imaging data.
2. Advise the Steering Committee on issues related to CAC and Carotid imaging.
3. Communicate with the two CARDIA imaging reading centers.

Laboratory Subcommittee

The Laboratory Subcommittee is responsible for planning and overseeing laboratory measurements on blood and urine specimens form Y25 Exam, oversee use of stored specimens from prior exams, and guide analyses and interpretation of results.

The Laboratory Subcommittee's purpose is to:

1. Plan and oversee the implementation of the laboratory procedures for the Y30 Exam.
2. Review and recommend laboratory measures (for blood and urine) to be included in the Y30 Exam.
3. Review results of internal and external quality results during the Y30 Exam.
4. Meet periodically to discuss measures to be conducted in case-control design following the Y30 Exam.

New Investigators Subcommittee

The New Investigators Committee has the following objectives:

1. Orient new investigators to CARDIA so that they understand the study structure.
2. Welcome new investigators as members of the CARDIA family.
3. Integrate new investigators into CARDIA scientific activities.
4. Encourage new investigators in paper-writing and other subcommittee membership.
5. Promote new investigators participating in, and leading, ancillary studies.

Publications and Presentations Subcommittee

The Publications and Presentations Subcommittee is responsible for overseeing all CARDIA publications and presentations activities, with final adjudication of decisions by the Steering Committee, and stimulating and enhancing the timely production of an optimal set of publications and presentations. Further, they are to plan and implement a policy to oversee all study publications and presentations.

The Publications and Presentations Subcommittee's purpose is to:

1. Provide a system for tracking progress on each proposed manuscript through its completion.
2. Provide scientific leadership and long-range planning for CARDIA publications activities.
3. Determine areas and topics of high scientific priority for CARDIA manuscripts and periodically re-examine these priorities.
4. Periodically examine CARDIA publications (completed and in-progress) and determine whether scientific priorities are being met. Propose strategies for progress.
5. Recruit new investigators, including junior investigators, to produce manuscripts.
6. Identify areas of potential collaboration with other studies.

Quality Control Subcommittee

The QC Subcommittee is responsible for designing and overseeing QC approaches for all aspects of CARDIA not specifically addressed by another subcommittee, and will recommend strategies to improve the quality of CARDIA data.

The QC Subcommittee's purpose is to:

1. Design appropriate measures and techniques that assess data quality.
2. Design appropriate measures and techniques that assess quality of the laboratories and reading centers.
3. Review the QC reports on the data.
4. Make recommendations to improve the quality of the data, as needed.
5. Recommend remedial protocols to improve the performance of a laboratory or reading center.
6. Prepare a document summarizing and assessing the quality of the collected data.

Steering Committee Subcommittee Memberships		
Subcommittee	**Member**	**Affiliation**
Clinic Coordination & Retention Subcommittee	Phillip Johnson	Birmingham Field Center
	Christie Oden	Birmingham Field Center
	Julia Wilkoff	Birmingham Field Center
	Kathleen Beck	Chicago Field Center
	Shirlee Mohiuddin	Chicago Field Center
	Sue Giovanazzi	Chicago Field Center
	Melanie Jaeb	Minneapolis Field Center
	Pamela Schreiner, Chair	Minneapolis Field Center
	Martin Stolzman	Minneapolis Field Center
	Jenny Zahn	Minneapolis Field Center
	Paula Partee	Oakland Field Center
	Melissa Nelson	Oakland Field Center
	Stephen Sidney	Oakland Field Center
	Jennifer Wammack	Coordinating Center
	Alexander Arynchyn	Coordinating Center
	Jacqueline Causey	Coordinating Center
	Ellen Funkhouser	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
Design & Analysis Subcommittee	Young-il Kim	Birmingham Field Center
	Kiang Liu	Chicago Field Center
	Lu Tian	Chicago Field Center
	Lihui Zhao	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Charles Quesenberry	Oakland Field Center
	Sejong Bae, Co-Chair	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
	Colin Wu, Co-Chair	National Institutes of Health—NHLBI
	Myriam Fornage	University of Texas—Houston
	João Lima	Johns Hopkins University
	Michael Steffes	University of Minnesota
eCARDIA Subcommittee	Phillip Johnson	Birmingham Field Center
	Julia Wilkoff	Birmingham Field Center
	Kathleen Beck	Chicago Field Center
	Shirlee Mohiuddin	Chicago Field Center
	Sue Giovanazzi	Chicago Field Center

Steering Committee Subcommittee Memberships		
Subcommittee	**Member**	**Affiliation**
	Pamela Schreiner	Minneapolis Field Center
	Martin Stolzman	Minneapolis Field Center
	Paula Partee	Oakland Field Center
	Melissa Nelson	Oakland Field Center
	Stephen Sidney, Chair	Oakland Field Center
	Byron Chancellor	Coordinating Center
	Jacqueline Causey	Coordinating Center
	Ellen Funkhouser	Coordinating Center
	James Shikany	Coordinating Center
	Jennifer Wammack	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
Endpoints Surveillance & Adjudication Subcommittee	Beth Lewis, Chair	Birmingham Field Center
	Stephen Glasser	Birmingham Field Center
	Holly Kramer	Chicago Field Center
	Sanjiv Shah	Chicago Field Center
	Daniel Duprez	Minneapolis Field Center
	Stephen Sidney	Oakland Field Center
	Alexander Arynchyn	Coordinating Center
	Raegan Durant	Coordinating Center
	Gina Wei	National Institutes of Health—NHLBI
	Kirsten Bibbins-Domingo	University of California, San Francisco
	Deborah Levine	University of Michigan
Genetics Subcommittee	Edmond Kabagambe	Birmingham Field Center
	Spencer Huang	Chicago Field Center
	Rajesh Kumar	Chicago Field Center
	Sanjiv Shah	Chicago Field Center
	Pamela Schreiner	Minneapolis Field Center
	Dongquan Chen	Coordinating Center
	George Papanicolaou	National Institutes of Health—NHLBI
	Myriam Fornage, Chair	University of Texas—Houston
	Claude Bouchard	Louisiana State University
	Myron Gross	University of Minnesota
	Laura Rasmussen-Torvik	Northwestern University
	David Siscovick	New York Academy of Medicine
	Alexander Reiner	University of Washington
Imaging Subcommittee	Stephen Glasser	Birmingham Field Center
	Kiang Liu	Chicago Field Center
	Sanjiv Shah	Chicago Field Center
	James Carr	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Kelvin Lim	Minneapolis Field Center
	Stephen Sidney, Chair	Oakland Field Center
	Mark D'Esposito	Oakland Field Center
	Jamal Rana	Oakland Field Center

Steering Committee Subcommittee Memberships		
Subcommittee	**Member**	**Affiliation**
	Alexander Arynchyn	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
	Nakela Cook	National Institutes of Health—NHLBI
	Lenore Launer	National Institutes of Health—NIA
	Jeffrey Carr	Vanderbilt University
	Greg Evans	Wake Forest University
	Greg Terry	Vanderbilt University
	João Lima	Johns Hopkins University
	Christopher Cox	Johns Hopkins University
	Erin Ricketts	Johns Hopkins University
	Nick Bryan	University of Pennsylvania
	Lisa Desiderio	University of Pennsylvania
	Ilya Nasrallah	University of Pennsylvania
	Samuel Gidding	Nemours
	George Washko	Brigham and Women's Hospital
Laboratory Subcommittee	April Carson	Birmingham Field Center
	Carlos Iribarren	Oakland Field Center
	Jennifer Wammack	Coordinating Center
	Alexander Arynchyn	Coordinating Center
	James Shikany, Chair	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
	William Funk	Northwestern University
	David Siscovick	New York Academy of Medicine
	Santica Marcovina	University of Washington
	Michael Steffes	University of Minnesota
	Myron Gross	University of Minnesota
New Investigators Subcommittee	Nefertiti Durant	Birmingham Field Center
	Olivia Affuso	Birmingham Field Center
	Brita Roy	Birmingham Field Center
	April Carson	Birmingham Field Center
	Gareth Dutton	Birmingham Field Center
	Emily Levitan	Birmingham Field Center
	Norrina Allen, Co-Chair	Chicago Field Center
	Michael Cuttica	Chicago Field Center
	Kiarri Kershaw	Chicago Field Center
	Ju-Mi Lee	Chicago Field Center
	John Wilkins	Chicago Field Center
	Lihui Zhao	Chicago Field Center
	Suma Konety	Minneapolis Field Center
	Monique Hedderson	Oakland Field Center
	Kelly Young-Wolff	Oakland Field Center
	Sejong Bae	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
	David Goff, Co-Chair	University of Colorado

Steering Committee Subcommittee Memberships		
Subcommittee	**Member**	**Affiliation**
	Ravi Sharma	Johns Hopkins University
	Bharath Ambale Venkatesh	Johns Hopkins University
	Henrique Turin	Johns Hopkins University
	Dhananjay Vaidya	Johns Hopkins University
	Akshay Sood	University of Minnesota
	Deborah Levine	University of Michigan
	Emily Wang	Yale University
	Nicole Redmond	University of Alabama at Birmingham
	John Booth	University of Alabama at Birmingham
	Isobelle Galeon	University of Colorado, Denver
	Mark Huffman	Northwestern University
	Katherine Ingram	Northwestern University
	Laura Rasmussen-Torvik	Kennesaw State University
	Yacob Tedla	University of Wisconsin-Madison
	Catherine Vladutiu	University of North Carolina
	Anne Peery	University of North Carolina
	Christina Shay	University of North Carolina, Chapel Hill
	Christopher Whitlow	Wake Forest University
	Joseph Yeboah	Wake Forest University
	Prachi Dubey	University of Massachusetts
Publications & Presentations Subcommittee	Beth Lewis	Birmingham Field Center
	Norrina Allen	Chicago Field Center
	Donald Lloyd-Jones	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Pamela Schreiner	Minneapolis Field Center
	Jamal Rana	Oakland Field Center
	Stephen Sidney	Oakland Field Center
	James Shikany	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
	Catarina Kiefe, Chair	University of Massachusetts
	David Goff	University of Colorado
Quality Control Subcommittee	Phillip Johnson	Birmingham Field Center
	Kiang Liu	Chicago Field Center
	Mercedes Carnethon	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Lyn Steffen	Minneapolis Field Center
	Alexander Arynchyn, Chair	Coordinating Center
	Sejong Bae	Coordinating Center
	Ellen Funkhouser	Coordinating Center
	James Shikany	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
	Colin Wu	National Institutes of Health—NHLBI
	Myriam Fornage	University of Texas—Houston
	Erin Ricketts	Johns Hopkins University

Steering Committee Subcommittee Memberships		
Subcommittee	**Member**	**Affiliation**
	João Lima	Johns Hopkins University
	Christopher Cox	Johns Hopkins University
	Santica Marcovina	University of Washington
	Myron Gross	University of Minnesota
	Michael Steffes	University of Minnesota
	Ilya Nasrallah	University of Pennsylvania
	Greg Terry	Vanderbilt University

8.9. Role and Composition of Executive Committee

The Executive Committee is responsible for making major decisions regarding operational and policy matters of the study. In addition, the Committee is responsible for the review and approval of ancillary study proposals submitted to the study. The Executive Committee meets on a monthly basis. Its membership consists of the Principal Investigator from each of the four Field Centers, the Principal Investigator/Director and Deputy Director of the Coordinating Center, the Principal Investigator of the ERC, the Chair of the Steering Committee, and the NHLBI Project Officer.

Executive Committee Membership	
Member	**Affiliation**
Beth Lewis	Birmingham Field Center
Donald Lloyd-Jones	Chicago Field Center
Pamela Schreiner	Minneapolis Field Center
Stephen Sidney	Oakland Field Center
James Shikany	Coordinating Center
Sejong Bae	Coordinating Center
Jared Reis	National Institutes of Health—NHLBI
João Lima	Johns Hopkins University
David Goff, Chair	University of Colorado

8.10. Role and Composition of Working Groups

Brain MRI Working Group

The Brain MRI Working Group's purpose is to:

1. Initiate and support the completion of analyses and manuscripts using the Y25 and Y30 brain MRI data, including providing guidance on data availability, quality, and interpretation.
2. Foster collaboration among investigators from both within and outside of the CARDIA community interested in working with the brain MRI data.
3. Serve as a first point of review for all manuscript and abstract proposals utilizing the brain MRI data.
4. Address QC issues related to the brain MRI data.
5. Design and oversee the collection of brain MRI data during the Y25 and Y30 exam cycles, including the development of Form 92 and the Brain MRI Substudy MOO.

Cognitive Function Working Group

The Cognitive Function Working Group's purpose is to:

1. Develop the cognitive function assessment for the Y30 Exam and prepare the associated sections of the protocol and MOO.
2. Provide oversight of training and QC for cognitive function assessment.
3. Provide leadership for publications and presentations utilizing cognitive function data.

Diabetes & Obesity Working Group

The Diabetes & Obesity Working Group's purpose is to:

1. Encourage collaboration among group members to generate manuscripts on topics pertaining to DM and obesity
2. Promote timely completion of manuscripts on DM and obesity related topics through regular conference calls and status updates
3. Suggest measurement protocols and data definitions for DM and obesity to the Steering Committee

Diet Working Group

The Diet Working Group is responsible for designing and overseeing the approaches for colleting dietary data in CARDIA and to analyze and publish the findings.

The Diet Working Group's purpose is to:

1. Advise the Steering Committee on selection of instruments to collect information related to diet for inclusion in the protocol as well as aspects of measurement, interpretation and implementation in the protocol.
2. Provide suggestions for diet paper proposals.
3. Work as a liaison with ancillary projects that involve diet hypotheses.

Physical Activity & Fitness Working Group

The Physical Activity & Fitness Working Group's purpose is to:

1. Initiate and support analyses and manuscripts related to the physical activity and fitness data available in CARDIA, with particular emphasis on the data collected as part of the CARDIA Fitness Study, discuss issues that arise with the use of these data, and develop study-wide approaches to resolving these issues.
2. Provide general leadership for the CARDIA Study in the area of physical activity and fitness, including the identification of priority areas for analyses involving physical activity and fitness variables.
3. Make recommendations regarding the physical activity and fitness assessment plans for future CARDIA examinations.

Pregnancy-Related Outcomes and Exposures Working Group

The Pregnancy-Related Outcomes and Exposures Working Group's purpose is to:

1. Initiate, support, and encourage timely completion of analyses and manuscripts related to pregnancy-related outcomes and exposures.
2. Promote collaborative relationships among investigators interested in working with CARDIA data related to pregnancy-related outcomes and exposures, including providing advice on data availability, quality, and variable definitions.

3. Provide leadership for the CARDIA Study in the area of pregnancy-related outcomes and exposures, including identification of priority areas for analyses, data collection during examinations, and ancillary study proposals.

Psychosocial Working Group

The Psychosocial Working Group's purpose is to:

1. Advise the Steering Committee on selection of psychosocial aspects of CARDIA, including selection of constructs for inclusion in the protocol; measurement; interpretation; implementation in the protocol.
2. Provide suggestions for psychosocial paper proposals.
3. Work as a liaison with ancillary projects that involve psychosocial hypotheses.

Pulmonary Working Group

The Pulmonary Working Group's purpose is to:

1. Provide suggestions for pulmonary related paper proposals.
2. Advise the Steering Committee on issues related to pulmonary testing or data.

Renal Working Group

The Renal Working Group's purpose is to:

1. Foster research in CKD using the CARDIA cohort. CKD research includes predictors of incident CKD and CKD progression and the role of kidney disease measures as exposures for subclinical CVD progression and cardiovascular events.
2. Meets monthly via conference call to discuss manuscript proposals, abstracts, and manuscripts in progress using the CARDIA cohort. The working group also discusses controversies regarding definitions of kidney disease and kidney disease progression and statistical methods for analyzing kidney disease measures.
3. Foster early investigators with a research interest kidney disease. This fostering is accomplished by early investigators participating in conference calls and sharing their work with other experienced investigators in the field of kidney disease and its association with CVD.

Reproductive Aging Working Group

The Reproductive Aging Working Group's purpose is to:

1. Develop manuscript proposals and ancillary studies related to all stages of a woman's reproductive life from menarche through menopause, excluding pregnancy-related conditions.
2. Serve as a resource to the P&P Subcommittee for manuscripts related to reproductive aging.
3. Work synergistically with other working groups interested in exploring reproductive aging.

Tobacco Working Group

The Tobacco Working Group's purpose is to:

1. Advise the Steering Committee on tobacco-related questionnaire development and variable construction in Y30.
2. Provide guidance and expertise for developing proposals, analyzing data, interpreting results, and producing publications, presentations that feature tobacco use as a key area of exploration.
3. Cultivate the growth of tobacco-focused research within CARDIA and serve as a resource for tobacco issues across CARDIA research.

Working Group Memberships		
Working Group	**Member**	**Affiliation**
Brain MRI Working Group	Beth Lewis	Birmingham Field Center
	Kiang Liu	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Pamela Schreiner	Minneapolis Field Center
	Stephen Sidney	Oakland Field Center
	Alexander Arynchyn	Coordinating Center
	James Shikany	Coordinating Center
	Lenore Launer	National Institutes of Health—NIA
	Robert Kramer	National Institutes of Health—NIA
	Jared Reis	National Institutes of Health—NHLBI
	Harsha Battapady	University of Pennsylvania
	Nick Bryan	University of Pennsylvania
	Christos Davatzikos	University of Pennsylvania
	Lisa Desiderio	University of Pennsylvania
	Guray Erus	University of Pennsylvania
	Myriam Fornage	University of Texas—Houston
	Myron Gross	University of Minnesota
Cognitive Working Group	Virginia Wadley	Birmingham Field Center
	David Jacobs	Minneapolis Field Center
	Stephen Sidney, Chair	Oakland Field Center
	Rachel Whitmer	Oakland Field Center
	Ellen Funkhouser	Coordinating Center
	Lenore Launer	National Institutes of Health—NIA
	Jared Reis	National Institutes of Health—NHLBI
	Laura Coker	Wake Forest University
	Deborah Levine	University of Michigan
	Bonnie Qin	University of North Carolina
	Tina Hoang	University of California, San Francisco
	Kristine Yaffe	University of California, San Francisco

Working Group Memberships		
Working Group	**Member**	**Affiliation**
Diabetes & Obesity Working Group	April Carson	Birmingham Field Center
	Nefertiti Durant, Co-Chair	Birmingham Field Center
	Gareth Dutton	Birmingham Field Center
	Edmond Kabagambe	Birmingham Field Center
	Beth Lewis	Birmingham Field Center
	Mercedes Carnethon, Co-Chair	Chicago Field Center
	Arlene Hankinson	Chicago Field Center
	Ellen Demerath	Minneapolis Field Center
	David Jacobs	Minneapolis Field Center
	Erica Gunderson	Oakland Field Center
	Jared Reis	National Institutes of Health—NHLBI
	Amy Alman	University of South Florida
	Bethany Gibbs	University of Pittsburgh
	David Goff	University of Colorado
	Lisa Chow	University of Minnesota
	Michael Steffes	University of Minnesota
	Alethea Hill	University of South Alabama
	Katherine Ingram	Kennesaw State University
	Beth Lacy	Brown University
	Jia Pu	Palo Alto Medical Foundation Research Institute
	Penny Gordon-Larsen	University of North Carolina
	David Siscovick	New York Academy of Medicine
	Akshay Sood	University of New Mexico
	Claude Bouchard	Louisiana State University, PBRC
	Christina Shay	University of Oklahoma Health Sciences Center
	Joyce Lee	University of Michigan
	Greg Terry	Vanderbilt University
	Lisa VanWagner	Northwestern University
Diet Working Group	Edmond Kabagambe	Birmingham Field Center
	Emily Levitan	Birmingham Field Center
	Linda Van Horn, Co-Chair	Chicago Field Center
	Lyn Steffen, Chair	Minneapolis Field Center
	David Jacobs	Minneapolis Field Center
	James Shikany	Coordinating Center
	Jared Reis	National Institutes of Health—NHLBI
	Ka He	University of North Carolina
	Katie Meyer	University of North Carolina
	Barry Popkin	University of North Carolina
	Christina Shay	University of Oklahoma

Working Group Memberships		
Working Group	**Member**	**Affiliation**
Physical Activity & Fitness Working Group	Olivia Affuso	Birmingham Field Center
	Arlene Hankinson	Chicago Field Center
	Mercedes Carnethon	Chicago Field Center
	Juned Siddique	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Stephen Sidney, Co-Chair	Oakland Field Center
	Barbara Sternfeld, Co-Chair	Oakland Field Center
	Claude Bouchard	Louisiana State University, PBRC
	Tuomo Rankinen	Louisiana State University, PBRC
	Mark Sarzynski	Louisiana State University, PBRC
	Catrine Tudor-Locke	Louisiana State University, PBRC
	Kelley Gabriel	University of Texas—Houston
	Eli Puterman	University of California
	Daniel White	Boston University

Working Group Memberships		
Working Group	**Member**	**Affiliation**
Pregnancy-Related Outcomes & Exposures Working Group	Beth Lewis, Co-Chair	Birmingham Field Center
	Adina Goldberger	Chicago Field Center
	Melissa Simon	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Suma Konety	Minneapolis Field Center
	Lyndsay Avalos	Oakland Field Center
	Erica Gunderson, Co-Chair	Oakland Field Center
	Monique Hedderson	Oakland Field Center
	Wendy Bennett	Johns Hopkins University
	Janet Catov	University of Pittsburgh
	Imo Ebong	Wake Forest University
	Kirsten Bibbins-Domingo	University of California, San Francisco
	Meghana Gadgil	University of California, San Francisco
	Roberta Ness	University of Texas—Houston
	Elizabeth Dehmer	University of North Carolina
	Wanda Nicholson	University of North Carolina
	Catherine Vladutiu	University of North Carolina
	Sylvia Badon	University of Washington
	Daniel Enquobahrie	University of Washington
	David Siscovick	New York Academy of Medicine
	Ronit Calderon-Margalit	University of Washington
	Molly Waring	University of Massachusetts
	Rhonda Bentley-Lewis	Massachusetts General Hospital
	Janne Boone-Heinonen	Oregon Health & Science University
	Rebecca Cogswell	University of Minnesota
	Myron Gross	University of Minnesota
	Katherine Ingram	Kennesaw State University
	Catherine Kim	University of Michigan
	Helene Kirkegaard	Cornell University
	Nisha Parikh	Queen's Heart Physician Practice
	Joyce Tang	Northwestern University
	Lisa VanWagner	Northwestern University
	Emily Harville	Tulane University
	Maeve Wallace	National Institutes of Health—NICHD
	Melissa Wellons	Vanderbilt University

Working Group Memberships		
Working Group	**Member**	**Affiliation**
Psychosocial Working Group	Nefertiti Durant	Birmingham Field Center
	Mercedes Carnethon	Chicago Field Center
	Kiarri Kershaw, Chair	Chicago Field Center
	Veronica Womack	Chicago Field Center
	Benjamin Capistrant	Minneapolis Field Center
	Christie McCullum-Hill	Coordinating Center
	Sonia Arteaga	National Institutes of Health—NHLBI
	Tiffany Powell	National Institutes of Health—NHLBI
	Sandra Albrecht	University of North Carolina
	Luisa Borrell	Columbia University
	Julie Bower	Johns Hopkins University
	Ana Diez-Roux	Drexel University
	Catarina Kiefe	University of Massachusetts
	Eric Loucks	Brown University
	Karen Matthews	University of Pittsburgh
	Eli Puterman	University of California, San Francisco
	Nicole Redmond	University of Alabama at Birmingham
	David Williams	Harvard University
	Brita Roy	Yale University
Pulmonary Working Group	Lewis Smith, Chair	Chicago Field Center
	Ravi Kalhan	Chicago Field Center
	Rajesh Kumar	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Bharat Thyagarajan	Minneapolis Field Center
	Carlos Iribarren	Oakland Field Center
	Alexander Arynchyn	Coordinating Center
	William Beckett	University of Rochester
	David Mannino	University of Kentucky
	Mark Pletcher	University of California, San Francisco
	Akshay Sood	University of New Mexico
Renal Working Group	Beth Lewis	Birmingham Field Center
	Orlando Gutiérrez	Birmingham Field Center
	Paul Muntner	Birmingham Field Center
	Holly Kramer, Co-Chair	Chicago Field Center
	Melissa Simon	Chicago Field Center
	David Jacobs	Minneapolis Field Center
	Paul Kimmel	National Institutes of Health—NIDDK
	K. Bibbins-Domingo, Co-Chair	University of California, San Francisco
	Vanessa Grubbs	University of California, San Francisco
	Carmen Peralta	University of California, San Francisco
	Michael Shlipak	University of California, San Francisco
	David Siscovick	New York Academy of Medicine
	Michael Steffes	University of Minnesota
	Alexander Chang	Johns Hopkins University

Working Group Memberships		
Working Group	**Member**	**Affiliation**
Reproductive Aging Working Group	Beth Lewis	Birmingham Field Center
	Mamie McLean	Birmingham Field Center
	Pam Schreiner, Co-Chair	Minneapolis Field Center
	Erica Gunderson	Oakland Field Center
	Stephen Sidney	Oakland Field Center
	Barbara Sternfeld	Oakland Field Center
	James Shikany	Coordinating Center
	Melissa Wellons, Co-Chair	Vanderbilt University
	Duke Appiah	University of Minnesota
	Ronit Calderon-Margalit	University of Washington
	Emily Harville	Tulane University
	Catherine Kim	University of Michigan
	Sharon Merkin	University of California, Los Angeles
	Stephen Schwartz	Fred Hutchinson Cancer Research Center
	David Siscovick	New York Academy of Medicine
	Joyce Tang	Northwestern University
	Erica Wang	Cedars-Sinai Medical Center
	Dale Williams	Florida International University
Tobacco Working Group	David Jacobs	Minneapolis Field Center
	Kelly Young-Wolff	Oakland Field Center
	Alexander Arynchyn	Coordinating Center
	Christie McCullum-Hill	Coordinating Center
	Rachel Widome, Co-Chair	University of Minnesota
	Brian Hitsman, Co-Chair	Northwestern University
	Reto Auer	University of California, San Francisco
	Mark Pletcher	University of California, San Francisco
	Ralph Caraballo	Centers for Disease Control and Prevention
	Judy Kruger	Centers for Disease Control and Prevention
	Catarina Kiefe	University of Massachusetts

Figure 2. CARDIA Study Committee Structure

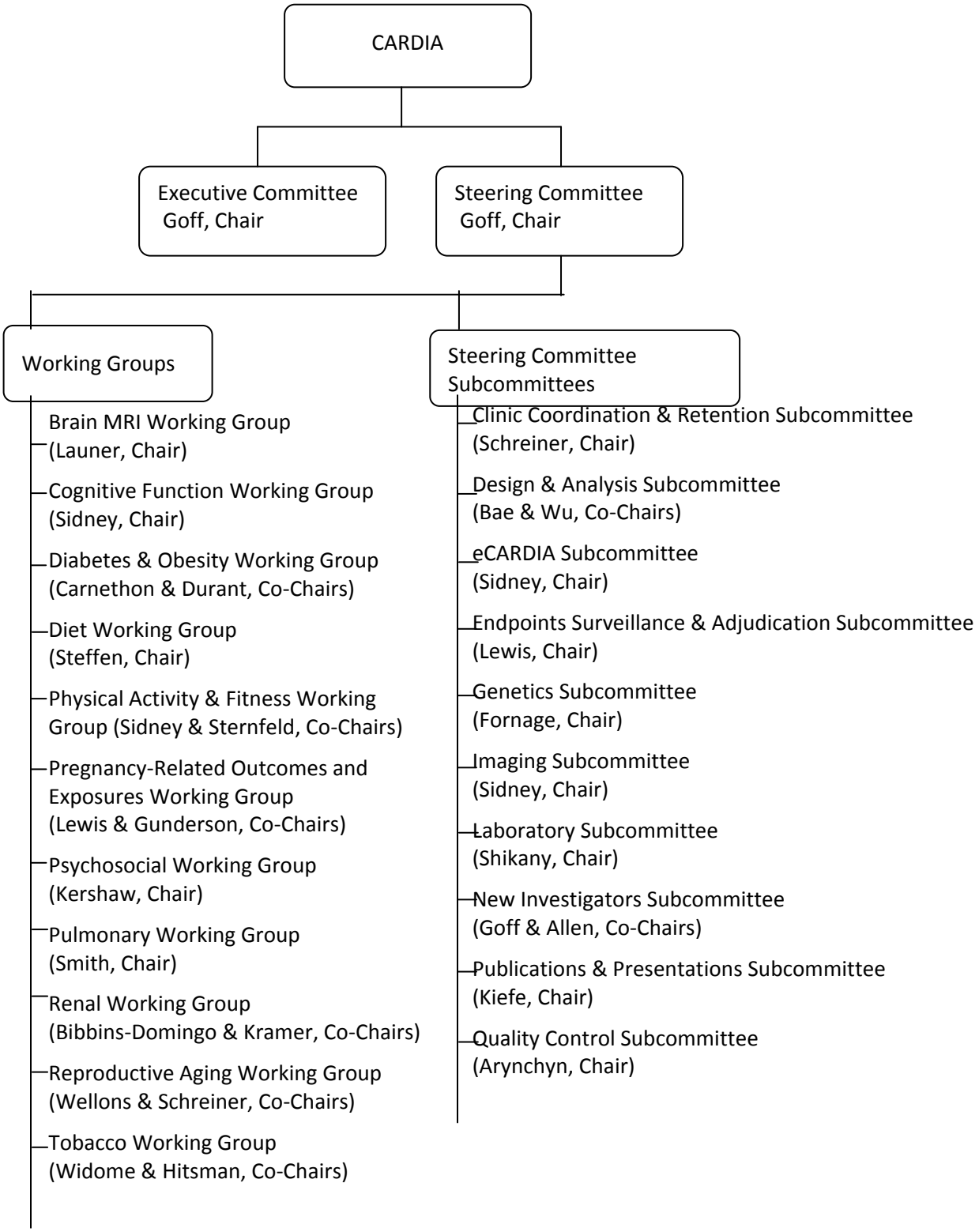
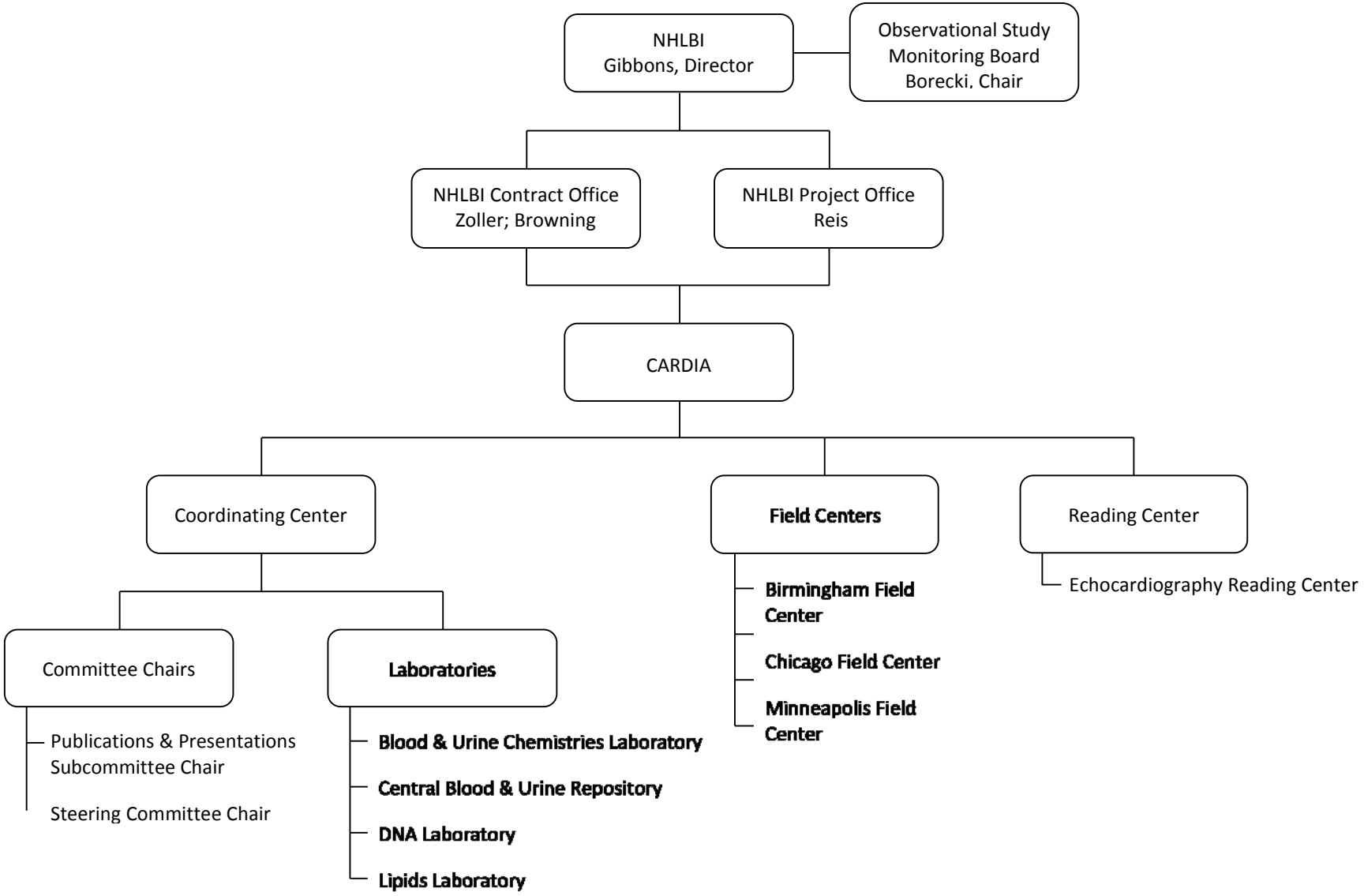


Figure 3. CARDIA Study Organization by Contract Status



9. Timeline

Base Period
July 1, 2013 – June 30, 2014

1. Protocol Development
2. Follow-up and Retention
3. Analysis and Publications

Option Period 1
July 1, 2014 – June 30, 2015

1. OMB Preparation
2. OMB Submission, clearance
3. Protocol Review NHLBI and OSMB
4. Staff Training and Certification
5. Pilot Testing
6. Year 30 Exam
7. Follow-up and Retention
8. **Analysis** and Publications

Option Period 2
July 1, 2015 – June 30, 2016

1. Year 30 Exam
2. Follow-up and Retention
3. Analysis and Publications

Option Period 3
July 1, 2016 – June 30, 2017

1. Follow-up and Retention
2. Analysis and Publications

Option Period 4
March 1, 2017 – June 30, 2018

1. Delivery of Final Datasets
2. Delivery of Biospecimens

Option Period 5
July 1, 2017 – June 30, 2018

1. Follow-up and Retention
2. Analysis and Publications

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4. The CARDIA Study Steering Committee. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 2 Exam Protocol.* The CARDIA Study Coordinating Center, University of Alabama at Birmingham;April 1987.
5. The CARDIA Coordinating Center. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 5 Exam Manual of Operations.* The CARDIA Study Coordinating Center, University of Alabama at BirminghamApril 1990.
6. The CARDIA Study Steering Committee. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 5 Exam Protocol.* The CARDIA Study Coordinating Center, University of Alabama at Birmingham;April 1990.
7. The CARDIA Coordinating Center. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 7 Exam Manual of Operations.* The CARDIA Study Coordinating Center, University of Alabama at BirminghamApril 1992.
8. The CARDIA Study Steering Committee. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 7 Exam Protocol.* The CARDIA Study Coordinating Center, University of Alabama at BirminghamApril 1992.
9. The CARDIA Study Steering Committee. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 10 Exam Manual of Operations.* The CARDIA Study Coordinating Center, University of Alabama at BirminghamApril 1995.
10. The CARDIA Study Steering Committee. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 10 Exam Protocol.* The CARDIA Study Coordinating Center, University of Alabama at BirminghamApril 1995.
11. The CARDIA Study Steering Committee. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 15 Exam Manual of Operations.* The CARDIA Study Coordinating Center, University of Alabama at BirminghamApril 2000.
12. Committee TCSS. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 15 Exam Protocol.* The CARDIA Study Coordinating Center, University of Alabama at Birmingham;April 2000.
13. The CARDIA Coordinating Center. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 15 Data Systems Documentation.* The CARDIA Study Coordinating Center, University of Alabama at BirminghamApril 2000.
14. Center TCC. *Coronary Artery Risk Development in (Young) Adults (CARDIA): Year 20 Exam Manual of Operations.* The CARDIA Study Coordinating Cetner, University of Alabama at Birmingham;Feburary 2005.

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