NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE ON AGING
INTRAMURAL RESEARCH PROGRAM

Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS)

Wave 5 Protocol

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Contractual Arrangements –Westat

1. Type of Contract/ Agreement:	**2. Sources of funding:**
Agreement Type: [x]Contract [ ]Subcontract [ ]Technology Transfer Agreement [x] Data Use Agreement (DUA) [ ] Material Transfer Agreement (MTA) [ ] Cooperative Research and Development Agreement (CRADA) [ ] Memorandum of Understanding (MOU) [ ] Memorandum of Agreement (MOA) [ ] Letter of Agreement [ ] Confidential Disclosure Agreement [X]If other; then specify: IRB Authorization Agreement Agreement Agreement Start Date: 2014 Agreement Expiration Date: upon completion of project Have funds been awarded? [x]Yes [ ]Pending [ ]No	[x]Institute/Department/Program Funds [ ] Another NIH Institute [ ] Another Federal Agency [ ] Foundation for the National Institutes of Health (FNIH) [ ]Industry [ ]Other Private Entity [ ]Other, specify: _____ **Name of Funder/s and/ or Sponsor/s:** _____ _____ _____ _____

Contractual Arrangements – University of Delaware

1. Type of Contract/ Agreement:	2. Sources of funding:
Agreement Type: [x]Contract [ ]Subcontract [ ]Technology Transfer Agreement [x] Data Use Agreement (DUA) [ ] Material Transfer Agreement (MTA) [ ] Cooperative Research and Development Agreement (CRADA) [ ] Memorandum of Understanding (MOU) [ ] Memorandum of Agreement (MOA) [ ] Letter of Agreement [ ] Confidential Disclosure Agreement [X]If other; then specify: IRB Authorization Agreement Agreement Agreement Start Date: 2009 Agreement Expiration Date: upon completion of project Have funds been awarded? [x]Yes [ ]Pending [ ]No	[x]Institute/Department/Program Funds [ ] Another NIH Institute [ ] Another Federal Agency [ ] Foundation for the National Institutes of Health (FNIH) [ ]Industry [ ]Other Private Entity [ ]Other, specify: _____ **Name of Funder/s and/ or Sponsor/s:** _____ _____ _____ _____

2.0.0 Statement of Compliance

The HANDLS study will be conducted in accordance with the design and specific provisions of this IRB-approved protocol, in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the requirements set forth in the US code of Federal Regulation applicable to clinical studies (45 CFR 46, parts A through D) concerning informed consent and IRB regulations; and in compliance with the International Conference on Harmonization's guidelines for Good Clinical Practices (ICH GCP). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the study participants. The Principal Investigator will promptly report to the IRB and the sponsor any changes in research activity and all unanticipated problems involving risk to human subjects, or others.

3.0.0 List of Abbreviations

HANDLS .....Healthy Aging in Neighborhoods of Diversity across the Life Span
MRVs.....Medical Research Vehicles
SES.....socioeconomic status
MRI.....magnetic resonance imaging
DXA.....Dual-energy X-ray absorptiometry
DNA.....deoxyribonucleic acid
AA.....African American
DTI.....diffusion tensor imaging
ADC .....apparent diffusion coefficient
SOP .....Standard Operating Procedures
SSB .....single strand breaks
DRC .....DNA repair capacity
SNP .....single nucleotide polymorphism
GWAS.....genome wide association study
COGENT .....Continental Origins and Genetic Epidemiology Network
CARe.....Candidate gene Association Resource consortium
NHANES .....The National Health and Nutrition Examination Survey
CKD .....chronic kidney disease
ESRD .....end stage renal disease
FA .....fractional anisotropy
GM .....gray matter
WM .....white matter
T2DM.....Type 2 Diabetes Mellitus
UMBC.....University of Maryland Baltimore County
MINI .....McGill Illness Narrative Interview
HIV .....human immunodeficiency virus
FTA.....fast technology for analysis
mRNA.....messenger ribonucleic acid
AMPM .....Automated Multiple Pass Method
REALM.....Rapid Estimate of Adult Literacy
WRAT.....Wide Range Achievement Test
IVA .....Instant Vertebral Assessment
mrem .....millirem
ATM.....automated teller machine
FDA .....Food and Drug Administration
NIA .....National Institute on Aging
NIH .....National Institutes of Health
OHRP.....Office of Human Research Protection

4.0.0 Protocol Summary

Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) – Wave 5

Short Title:	HANDLS
Conducted by:	National Institute on Aging, Intramural Research Program, Laboratory of Epidemiology and Population Sciences, Health Disparities Research Section
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Sample Size:	3720
Accrual Ceiling:	4000
Study Population:	The baseline HANDLS sample consists of 3720 community-dwelling African American and white adults aged 30-64. Participants were drawn from 13 neighborhoods (groups of contiguous census tracts) in Baltimore City, sampling representatively across a wide range of socioeconomic and income circumstances.
Accrual Period:	2004-2009
Study Design:	The heuristic study design is a factorial cross of four factors: age, sex, race, and SES with approximately equal numbers of subjects per "cell" (Figure 2 on page 26). HANDLS is planned as a 20-year longitudinal study of the 3720 individuals accrued (Figure 3 on page 26). Using our mobile medical research vehicles, we are revisiting each census tract for 2-3 months over the next 3 years.
Study Duration:	Start Date: 2004; End Date: 2024

Primary Objective:	The primary objective of HANDLS is to conduct a longitudinal study of minority health, aging, and health disparities focused on investigating the differential influences of race and socioeconomic status on health in an urban population.

5.0.0 Précis

The Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study is an interdisciplinary, community-based, prospective longitudinal epidemiologic study examining the influences of race and socioeconomic status (SES) on the development of age-related health disparities among socioeconomically diverse African Americans and whites in Baltimore. This study investigates whether health disparities develop or persist due to differences in SES, differences in race, or their interaction. Planned as a 20-year longitudinal study, HANDLS is unique because it assesses physical parameters as well as evaluating genetic, biologic, demographic, and psychosocial parameters of African American and white participants over a wide range of socioeconomic statuses. HANDLS also employs novel research tools, mobile medical research vehicles, in hopes of improving participation rates and retention among non-traditional research participants. The domains of the HANDLS study include: nutrition, cognition, biologic biomarkers, body composition and bone quality, physical function and performance, psychology, genomics, neighborhood environment and cardiovascular disease. Utilizing data from these study domains will facilitate an understanding of selected underlying factors of persistent black-white health disparities in overall longevity, cardiovascular disease, and cognitive decline.

HANDLS recruited a fixed cohort as an area probability sample of Baltimore City from August 2004 through November 2009 as Wave 1 (Figure 1). HANDLS Wave 2 entitled The Association of Personality and Socioeconomic status with Health Status – An Interim Follow-up Study began in June 2006 under a separate protocol. It was designed as a follow-up telephone interview approximately 18 months after the initial examination (Wave 1) was complete. Wave 2 provided interim contact with study participants, and important interim information regarding their health. Now completed, waves 3 and 4 were the first and second follow-up examinations and participants' second and third visit to our mobile Medical Research Vehicles (MRVs). The current protocol outlines Wave 5, the third follow-up examination and participants' fourth visit to our mobile Medical Research Vehicles (MRVs). Planned as a follow-up after 3-4 years, Wave 5 consists of health examinations, questionnaires, sensory assessments (visual and olfactory), health literacy assessment, renal function assessments, environmental assessments, and for a sub-set of participants; structural MRIs and a personality inventory.

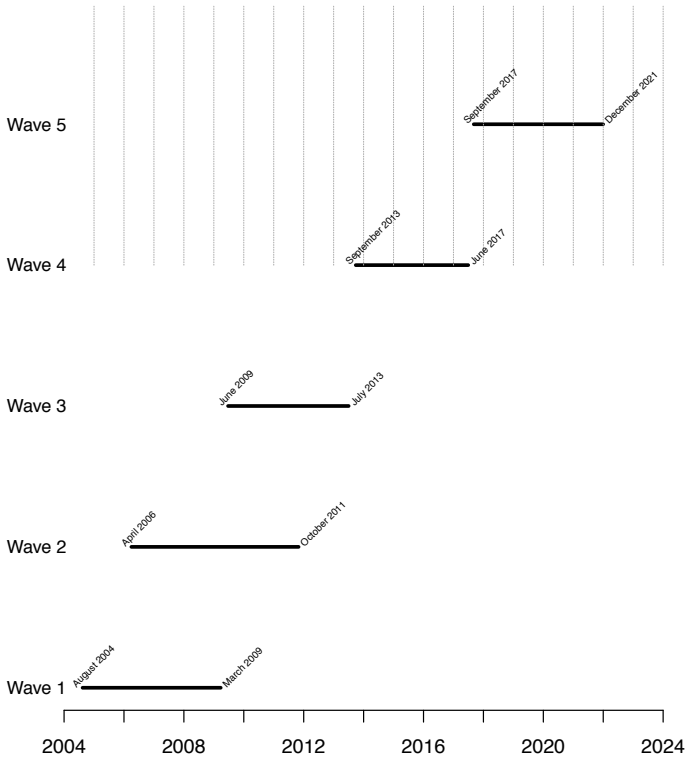

Figure 1. Present and projected HANDLS timeline

6.0.0 Background and Scientific Rationale

There are well-documented differences in health status among groups defined by age, race, ethnicity, and socioeconomic status (SES). Over the past decade or so, evidence from cross-sectional studies and nationally representative follow-ups suggests that there are persistent disparities among African Americans and other minority groups compared to Whites in morbidity[1-16] and mortality.[15,17-21] This is particularly evident in the steadily growing divide between well-educated white men and women and less educated African Americans.[22] Double jeopardy describes the constellation of health disparities conferred by old age and membership in a minority group.[23] Evidence suggests that there are unique disadvantages conferred by the combination of old age and minority status,[1-7,9,11-19,23-27] but the extent to which minority status is a direct cause of the disadvantage is unknown. Race, ethnicity, and SES are inextricably confounded in many studies. Membership in a minority group may be an indicator of the combinations of other effects such as low income, poor education, environmental exposure to toxic compounds, and lack of occupational opportunities. Recent data also suggests declining life expectancy and increased premature mortality among low-SES whites [28].

Independent of the effects of race and ethnicity, SES accounts for differences in the functional status associated with chronic disease, but has only a small role in predicting prevalence of chronic disease.[16] Further complicating this relationship, physicians' assessments and treatment differ by race and sex.[24,29] Addressing these disparities in health status requires data about the differences in risks for chronic disease associated with race, ethnicity, and SES in all groups regardless of their majority or minority standing.

The scientific objectives of HANDLS are to establish a single-site prospective longitudinal epidemiologic study of health disparities in socioeconomically diverse African Americans and whites residing in the city of Baltimore. Specifically, we designed HANDLS to disentangle the effects of race and SES on risk factors for morbidity and mortality, to examine the incidence and progression of pre-clinical disease, and to follow-up the development and persistence of health disparities, longitudinal health status, and health risks. The mechanisms or biologic and molecular pathways through which the health and longevity trajectories of individuals in American society are influenced are unknown at this time.

The present protocol focuses on predictors of change in cardiovascular function and fitness, risks for cerebrovascular conditions such as stroke, vascular dementia, and carotid stenosis, renal function, and pathological cognitive decline. We chose these specific areas as representing the health issues that are among the most prevalent, but least understood, in African Americans and low SES urban dwelling whites who have health burdens similar to African Americans. Specifically, we will measure heart function by EKG, muscle strength by grip strength, chair stand and single leg stand exercises, body composition by dual photon x-ray absorptiometry (DXA), cognitive performance with cognitive and neuropsychological tests, sensory functioning with the *Sniffin Sticks* olfactory assessment, and the Marco refractometer NIDEK for visual acuity and the TRC NW400 Non-Mydriatic fundus camera TOPCON for fundal images, skin microbiota using a skin swab, environmental exposure to toxins by collecting toenail clippings, and neuroimaging parameters by structural MRI.

We assess each of these areas by separate procedures for which we will investigate cross-sectional differences and longitudinal change within this sample and by comparison with other samples, particularly the National Health and Nutrition Examination Studies and other studies with which this study shares many procedures and tests. We will combine these measures in various ways to examine the risks for pathological outcomes such as stroke, dementia, and loss of functional independence.

7.0.0 Study Objectives

The primary objective of HANDLS is to conduct a longitudinal study of minority health and health disparities focused on investigating the differential influences of race and socioeconomic status on health in an urban population.

The scientific research questions for this interdisciplinary epidemiologic study of minority health and health disparities are:

(1) Do race and SES influence health disparities independently or do they interact with several factors (race, environmental or biologic factors, and cultural or lifestyle practices)?

(2) What is the influence of SES and race on age-related declines in function in an urban population?

(3) What is the influence of SES and race on the incidence and natural history of age-related disease?

(4) Are there early biomarkers of age-related health disparities that may enhance our ability to prevent or ameliorate the severity of these diseases?

For specific systems we will test the following hypotheses during Wave 5 of HANDLS:

Cardiovascular. (1) There will be significantly greater decline in cardiovascular health status as a function of SES and race independent of the effects of age in both men and women; for example, left ventricular mass, an important cardiac risk factor, is greater in African Americans than whites and is greater in African Americans of lower SES as compared to age-matched African Americans with higher SES, in both men and women; (2) Endothelial dysfunction is known to be more prevalent among African Americans. We hypothesize that it will not only be more prevalent in African Americans but also in low SES whites and those with evidence of oxidative stress markers because poverty and oxidative stress will be important modulating factors of endothelial function; (3) low SES will also correlate with lower Ankle-Brachial Index (ABI) values and increased longitudinal risk of cardiovascular morbidity and mortality.

Body composition and bone quality. Compared to white adults of comparable age, African Americans have: (1) A higher proportion of fat to lean mass of the total body, trunk and extremities, and greater odds of meeting DXA-defined criteria for sarcopenia and sarcopenic obesity; (2) Faster loss of lean mass, greater accumulation of fat mass and greater increase in the proportion of fat to lean mass of the total body, trunk and extremities, and greater risk of transition to sarcopenia and sarcopenic obesity; (3) Faster and earlier decline in bone density; and, (4) These associations are correlated with, and perhaps mediated by, differences in health habits such as nutrition, physical activity, and alcohol consumption.

Cognition. The rates of decline of various cognitive abilities will be the same in all groups regardless of race, ethnicity, or SES.

Muscle Strength. (1) African Americans have the same trajectory of muscle loss as other ethnic or racial groups after accounting for differences in occupational history, nutrition, and body mass and composition; (2) All ethnic and racial groups will show the same relationships among changes in muscle strength, physical activity, and cardiovascular fitness regardless of socioeconomic factors, nutrition, and comorbid conditions such as diabetes; and, (3) The greater strength reductions at older ages among lower SES individuals will be attributable to their greater severity of chronic diseases.

Covariates. Other variables such as nutrition, environment and neighborhood effects, genetic make-up, family history, activity level, access to health care, prevalent medical, dental, sensory, psychiatric conditions, caregiving status, renal function, oxidative stress, and inflammatory status may modulate the effects of SES and race on cardiovascular, musculoskeletal, and cognitive functioning. For example:
Skin and Health Disparities. Skin Microbiota. The microbiome of individual organisms conveys information about health status. In humans, alterations in the human microbiome are associated with diabetes, auto-immune disease, cancer as well as allergies.[30] The skin microbiome harbor billions of microorganisms, approximately 1 million bacteria reside per square cm of human skin.[31] The skin has a system of immune surveillance comprised of epithelial cells, lymphocytes, and antigen presenting cells. This system interdigitates with the innate and adaptive immune system.[32] For example, skin microbiota can affect expression of the complement system, an important part of host defense and inflammation targeted at invading microbes.[33] The interaction of the skin microbiome with the immune system appears to underlie many skin disorders as well slow wound healing.[34] Chronic slow wound healing is associated with diabetes, vascular disease, obesity as well as aging.[35] Dysfunctional interaction of bacteria, fungi, mites, and

viruses present on skin promote skin disease and chronic wounds. Clinically in the HANDLS population we have observed numerous examples of slow wound healing and would like to explore the role of the skin microbiome as a risk factor. We are particularly interested in exploring the effect of environment and host demographic factors on skin microbiota. This will allow us to help define normal healthy microbiota, how the skin microbiota interacts with the host immune system and most importantly whether there is any interaction between the skin microbiota and microbiota in other parts of the body.[36] We will sample by swabbing 4 areas of the upper extremities: inner aspect of the volar aspect and the antecubital fossae of both arms (~4 cm^2 area using a sterile cotton pledget soaked in sterile 0.15 NaCl with 0.1% Tween 20.[37] All samples will be stored -80^0C.

Oxidative stress and inflammatory state. As a translational research study, HANDLS permits investigation of health disparities in terms of socioeconomic, socio-cultural, and psychosocial parameters. HANDLS allows us to define a medical/biologic phenotype that may be amenable to dissection by bench scientists examining the molecular aspects of aging, disease and disability. The early appearance and increased severity of age-associated disease among African Americans and low SES individuals suggests that the factors contributing to the emergence of health disparities may also induce a phenotype of 'accelerated aging'. While others have attributed this to racism and other socio-cultural factors, we seek to understand the underlying biologic, genetic, and environmental factors that may result in this phenotype that ultimately contributes to the disparate life expectancies for low-SES and minority sub-populations. The health disparities induced phenotype of accelerated aging may be biologically similar to heritable 'progeroid' syndromes whose manifestations include increased susceptibility to oxidative stress, premature accumulation of oxidative DNA damage, defects in DNA repair and higher levels of biomarkers of oxidative stress and inflammation. While genetic background, environmental and behavioral factors influence health outcomes in all populations over the lifespan, health disparities may be the end product of an accelerated trajectory of dysfunctional interactions of these factors in populations at high risk or with high levels of risk exposure. Oxidative DNA damage includes single strand breaks (SSBs) and oxidative base damage. An increased baseline level of oxidative DNA damage is associated with several age-related diseases including: cardiovascular disease [42], diabetes mellitus,[43] cancer,[44] neurodegenerative disease,[45] and end-stage renal disease.[46] The level of oxidative DNA damage depends on a variety of factors. They may include age,[44] environmental exposure to genotoxic factors,[47] smoking,[48] ethanol intake,[47] and intracellular and extracellular metabolism.[49]

HANDLS examines this hypothesis by measuring biomarkers of oxidative stress and inflammation, assessing levels of the most widely studied oxidative DNA adducts. In addition, other important biomarkers of oxidative stress are being evaluated. Measures of inflammatory states include the pro-inflammatory cytokines, damage associated molecular pattern molecules like circulating mitochondrial DNA and C-reactive protein. Prospectively measuring biomarkers of oxidative stress in a longitudinal study may clarify whether oxidative stress plays a pivotal role in aging and in the development and or progression of age associated disease. It may also provide insights into the different trajectories of aging observed in individuals.

Genetics. Current technological advances in genotyping permit high throughput whole genome single nucleotide polymorphism (SNP) genotyping to proceed with the overall goal of examining the genetic contributions to the development of multi-gene complex clinical disorders. Of equal importance is the contribution this new knowledge will provide in furthering the examination of

the genetics behind the differences in medicinal drug responses frequently seen in individuals as well as to the discovery of new drug targets for a range of diseases with persistently high morbidity and mortality. Our primary aim is to identify the genetic factors that are associated with age-associated health disparities. We hypothesize that the prevalence and severity of age-associated disease in minority populations is related to in some cases genetic susceptibility factors. Genotyping will focus on identifying specific SNPs that may be related to disease susceptibility and or the severity of disease states and metabolic conditions that disproportionately affect this longitudinal cohort over the next 20 years. Examining the prevalence of these genetic polymorphisms is critical to understanding not only the association between the polymorphism and the disease but the molecular and biological functional outcome of these polymorphisms. Although race itself is not a definitive biologic factor but largely a proxy for social, cultural behavioral and environmental factors it is critically important for us to attempt to understand the role of genetic susceptibility to specific age-related heath disparities and clinical characteristics. The first step to gaining this understanding is to identify risk alleles for common diseases through genome wide association studies (GWAS). However, most of the early GWAS analyses failed to include diverse cohorts enriched for sub-populations at greatest risk. Therefore, inclusion of diverse population groups will hopefully enhance understanding of the effects of various genetic variants in different groups who may have different environmental exposures.

Whole genome SNP genotyping using the Illumina Infinium II platform for the first 1000 participants has been completed. Additional genotyping will take place using Illumina Neuro-X and EPIC chips. Planned work will proceed in conjunction with GWAS consortia including: the Continental Origins and Genetic Epidemiology Network (COGENT) and the Candidate–gene Association Resource consortium (CARe). Initial areas of research have focused on renal, metabolic, hematologic, and cardiovascular characteristics or conditions. Analysis of the data set is underway to determine genetic associations with hypertension, renal disease, cardiovascular disease, stroke and other age associated health disparities. In addition, other GWAS studies that have been completed focused on height, platelet count, water balance, and serum sodium concentration. Supplementary genotyping and sequencing will be performed dependent on the availability of funds.

Epigenetics. The disproportionate incidence and mortality from age-associated disease may also result from epigenetic mechanisms such a DNA methylation. One theory of aging focuses on the role of genes and the epigenome in the development of the aging phenotype. We will examine the hypothesis that human disease and disability may result from DNA modifications that are not the result of changes in the coding sequence of genes. The clinical relevance of DNA methylation states in the development of age-related disease has yet to be understood on a population basis. There is variation in methylation states from individual to individual. This may be related to age, gender, environmental exposure, and other genetic factors. Is it possible that our hypothesized phenotype of accelerated aging phenotype seen in low SES and minority communities is related to epigenetic factors such as methylation? Using the Illumina EPIC chip, we will examine methylation states within this longitudinal cohort to attempt to understand whether genetic methylation states are associated with the premature development of age-associated disease. We have chosen to employ DNA isolated from the lymphoid cells for this study. Our investigations will focus on identifying DNA methylation pattern factors that are associated with accelerated aging and the development of age-related health disparities conditions.

Environmental Exposure. To fully understand the transduction factors that lead to health disparities it is critical for us to understand the role that the environment may play for the interaction of the host with the environment is a fundamental source of disease. This is especially true in the context of health disparities where there are numerous reports that highlight disproportionate exposure for certain US sub-populations to environmental toxicants.[50] Poor, low resourced, economically disenfranchised minority communities are more frequently affected by higher that average levels of soil and air contamination by a variety of toxicants including lead, cadmium and arsenic. The role of environmental chemicals in health disparities is an area of active research because there is evidence that disproportionate exposure of selected toxins can be directly linked to specific health disparities. Renal disease is linked to environmental exposures of lead and cadmium at high levels. However, more recent data suggests that even chronic low levels of exposure may predispose to developing specific nephropathies, albuminuria and chronic kidney disease.[51] These low levels of exposure may not only be etiologic factors in renal disease but also promote disease progression of chronic kidney disease that may arise from other factors (i.e. diabetes mellitus, uncontrolled hypertension). Cumulative exposure to lead in adults leads to more rapid longitudinal changes in numerous measures of cognitive function suggesting that lead plays a role in accelerated cognitive aging.[52,53] There is also a longitudinal association between zinc levels and diabetes incidence in young adults and a relationship between zinc levels and redox status in diabetes that may portend more significant disease.[54,55] Selenium levels have also been linked with pre-diabetes, metabolic syndrome, and certain cancers.[56] Arsenic levels have been linked with numerous poor health outcomes including cancer (skin, lung, bladder), cardiovascular disease and possibly diabetes mellitus.[57,58] The links between the environment and health as well as the presence of environmental injustice among communities of color and among poor white communities provide a strong rationale for us to assess environmental exposures in our bi-racial socioeconomically diverse cohort.

Baltimore is home to many environmental hazards including polluting industries, a coal fired power plant, a coal disposal facility, and a trash incinerator among others. The trash incinerator alone emits lead, mercury, and dioxin into the air. Each of these industries in our urban setting results in higher levels of toxicants in the air and water creating environmental hazards that disproportionately occur in low income and minority communities in Baltimore many of which are the original 13 census segments from which HANDLS participants were recruited. Given the likelihood that our population cohort has been exposed to environmental toxicants, we would like to assess their exposure in the context of the observed age-related health disparities present in the cohort. We will examine possible long-term (1 year) cumulative exposures of several trace elements in HANDLS participants by collecting nail clippings from the distal edge of the nail plate on all 10 toes during their visit. Although chemical exposures can be quantified using blood and urine, these biofluids reflect short term exposures. We will assess chemical exposure using toenails because of their slow growth rate and lower rate of exposure to external contamination making them a better biomarker of chronic or longer-term exposure.[59-61] Toenail trace elements may be used as biomarkers that might be linked to various health disparities. Chronic exposure to lead, cadmium, arsenic, mercury among other agents have been implicated in cognitive decline, cancer, cardiovascular disease as well as mortality and may be non-invasively monitored using toenail clippings.[62-65] We are particularly interested in assessing arsenic levels because arsenic affects epigenetic silencing, promoter methylation and DNA repair capacity which we can assess as part of other domains of the HANDLS study. Using stainless steel, single use latex free nail clippers participants will be asked to provide toenail clippings

during their longitudinal visit at the MRV and during home visit for participants who do not come to the MRV for follow-up. We will also collect 10 milliliters of blood in trace metal free tubes. This is a minimal risk biomaterials collection. The major risks are incidental injury to the nail cuticle with a risk for bleeding or infection.

Renal function. The National Health and Nutrition Examination Survey (NHANES) reports that while chronic kidney disease (CKD) prevalence among Americans older than 20 years of age was 16.8%, rates for non-Hispanic Blacks and Mexican Americans were higher (19.9% and 18.7% respectively. This disparity is significantly highlighted when assessing the prevalence of stage 1 CKD. Prevalence of CKD 1 among non-Hispanics whites is 4.2% compared with 10.2% for Mexican Americans and 9.4% among non-Hispanic Blacks. The statistics for End-Stage Renal Disease (ESRD) mirror these disparities; African Americans have a 3.6 fold higher rate than whites and Hispanics have a 1.5 times higher prevalence rates than the U.S. non-Hispanic white population.[66]

The risk factors for CKD are multifaceted and difficult to dissect; they include: hypertension, diabetes mellitus, smoking, race, age, obesity and heart disease[67]. However, it is clear that other etiologic factors may also play a role including behavior, genetics, and the physical and sociologic environment as has been shown for ESRD [68-70]. Because of the complexity of the factors that influence the development of chronic kidney disease and the significant impact CKD and ESRD have on quality of life, disability and life expectancy [68-72], we set out to examine predictive factors for CKD, including poverty, genetics, food security, diet, and race. In hopes of providing early identification of participants with CKD, to improve outcomes and awareness of CKD among participants, serum Cystatin C levels will be measured in each participant. Cystatin C has been selected because the literature suggests that it may provide a more accurate estimate of GFR, especially when GFR is only mildly depressed.[73] Additionally, Cystatin C has been found to be a better predictor of cardiovascular mortality than creatinine among persons with mild CKD.

Caregiving. Health disparities may result from various forms of stress including psychological stress. Many studies have linked caregiving with significant levels of chronic stress for caregivers. This chronic stress is moderated by socioeconomic status, the condition and disabilities of the individual for whom care is provided, social support, and the age of the caregiver. Most of this research sampled primarily white caregivers. There remains a lack of research focused on middle and older aged, African-American women who are in multiple caregiving roles. We will examine the influence of multiple caregiving roles (i.e., occupancy of more than one caregiving role) on the physical and mental health outcomes of HANDLS participants. This aim is to gain greater understanding about the relation between multiple caregiving roles (i.e., occupancy of more than one caregiving role), and health status (physical and mental) among HANDLS participants. This proposed study could extend the caregiving literature in several ways. First, it will assess the influence of multiple caregiving roles on health status of caregivers, across race/ethnicity, class and gender. Previous studies lacked sample diversity and primarily focused on low-income African Americans.

Sensory Testing

Olfactory testing. Risk for dementia is associated with age[74], and there are significant and large disparities in the rates of dementia by race[75] and socioeconomic status[76]. According to the Alzheimer's Association, African Americans are about twice as likely than whites to develop Alzheimer's disease, the most common form of dementia[77]. African Americans are also less

likely than whites to be diagnosed with dementia. When African Americans are diagnosed, their diagnosis is likely later in the course of the disease[77]. Socioeconomic status, particularly the likelihood of adequate health insurance, complicates the identification of dementia because low educational attainment and low income are also risks for dementia[78]. As a consequence of these racial and socioeconomic disparities in dementia, the Department of Health and Human Services established the goal of addressing health disparities as a priority for research aimed at preventing and treating Alzheimer's disease[79].

As a study of racial and socioeconomic health disparities in African Americans and whites, the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study has the opportunity to study the early signs and symptoms of dementia. We will administer the odor identification test Sniffin' Sticks, a well-validated and inexpensive instrument to measure olfactory function. Odor identification with Sniffin' Sticks is performed by holding an uncapped canister that resembles a marker pen 2 cm from participants' noses while asking them to sniff the odor. Deficits in odor identification using Sniffin' Sticks are associated with risk for dementia[86,87].
There are no risks associated with administering Sniffin' Sticks. HANDLS participants who have decreased abilities to smell (hyposmia) or are unable to smell (anosmia) will be excluded.

Ocular Health (visual acuity and retinal pathological change). Given that there are notable health disparities in ocular diseases including glaucoma, diabetic retinopathy, and Age Related Macular Degeneration[88], we will begin to assess vision and refractive error in our participants as well as to assess retinal health and macular health by doing digital fundal photography using a non-mydriatic (non-dilation) camera that will allow us to assess markers of age and disease related retinal change. Although it is difficult to find data that captures the number of people affected by visual impairment in the United States, it has been estimated that perhaps as many as 142 million individuals over the age of 40 have visual impairment. The most common causes of the visual impairment include refractive error, age-related macular degeneration, cataract, diabetic retinopathy and glaucoma. Understanding and evaluating visual impairment is a critical part of health disparities research since poor eye health is associated with factors that contribute to already existing health inequities in low SES and minority populations. Poor vision is associated with increased risk for falls, social isolation as well as creating psychological stress that further enhances the adverse effects of already existing chronic medical condition[89]. This research is in keeping with recommendation 1 of the National Academy of Sciences September 2016 report entitled *Making Eye Health a Population Health Imperative: Vision for Tomorrow*.

We will assess visual acuity using the Marco refractometer NIDEK auto refract/keratometer ARK/760A and obtain fundal images using the TRC NW400 Non-Mydriatic fundus camera TOPCON in collaboration with Dr. Emily Y. Chew, M.D., Deputy Director, Division of Epidemiology and Clinical Applications and Deputy Clinical Director, National Eye Institute. There are no risks associated with these tests. Some participants may find the light used during the digital photography of the fundus bright. This is a transient effect with no long-lasting sequalae. Blind participants will be excluded.

Health literacy. Examination of the underlying factors of health disparities requires investigation of health literacy among populations at risk. Health literacy is defined as "the degree to which individuals can obtain, process, and understand the basic information and services they need to make appropriate health decisions…"[90] In 2004, the IOM estimated that almost 90 US adults

million adults had low levels of health literacy.[91] Work by multiple groups has linked health disparities to low levels of literacy and these disparities are not solely linked to income level, race or education levels.[92-94] Older adults are also more likely to have low levels of health literacy as well as those with multiple chronic illnesses or co-morbid conditions.[95-98] Reading and numerical skills are required to function effectively in health care environment. Inadequate health literacy affects several factors that may influence health disparities as well as severity of age-related conditions such as preventive care, medical compliance, and health care expenditures. Health literacy may also influence the recruitment and retention of low SES and minority individuals in clinical research. One of the gaps in our knowledge about reducing health disparities is how to modulate associated factors like health literacy to promote the reduction of health disparities. As many suggest, it is essential to integrate health literacy assessments in disparities research.[99]

We will assess health literacy in Wave 5 of HANDLS, using a short version of the REALM to examine its and to investigate the influence of race, sex, age, income, education and reading level on health literacy. We will also assess the associations of health literacy with chronic medical conditions, multiple co-morbidities, cognition, and symptoms of depression and other psychological factors. It provides an adequate evaluation of an individual's ability to read and understand health materials. Perhaps most significantly, we will use the health literacy data to develop appropriate HANDLS research study materials as well as health education messages tailored to our study population. Although we now assess all participant study materials for culturally competent and proficient communication as well as for readability using the Flesh-Kincaid Readability formula, it is likely that this additional information about health literacy levels will better inform our material preparation and review process. Given the very high smoking rates in our population, it is clear the standard health education messaging has not been effective. We hope that by evaluating health literacy in our population we can add to the literature information that will improve health education messages for vulnerable, at risk populations.

Mobile Health. HANDLS will test the feasibility of providing cellular phones or small internet ready devices to determine whether the device will help to improve compliance with HANDLS physician recommended healthcare follow-up stemming from their HANDLS medical examination. We will send electronic reminders to participants about physician's treatment recommendations explaining the risks for further complications should their healthcare needs go untreated. We will also send general health education information. For difficult to track participants, we will test whether providing the device will assist in maintaining contact between study visits and whether providing appointment reminders improves retention rates among the most difficult to track HANDLS participants.

7.1.0 Ancillary studies and sub-studies

7.1.1 Neuroimaging Sub-study Objectives (HANDLS Scan)

There are pronounced health disparities associated with race and socioeconomic status (SES) in various brain health endpoints including stroke, dementia, cognitive decline, and functional disability.[100,101] Particularly potent race disparities in stroke incidence are apparent at strikingly young ages, with a four-fold increased risk of stroke mortality among 45-59 year old African Americans (AA).[102] Efforts are needed at disentangling the respective influences of race and SES

in brain health, particularly early and subtle markers of brain pathology that predict future stroke, dementia, or cognitive and functional decline. Measures of subclinical or covert cerebrovascular disease assessed by magnetic resonance imaging (MRI), including gray matter and white matter volumes and white matter microstructure, offer such proven associations.[103,104] Identifying multi-level mediators of the relations of race and SES to subtle brain pathology is also crucial. Biomedical, behavioral, psychological, social, and environmental factors have been implicated as potential mediators of the relations of race and SES to a multitude of physical health outcomes,[105,106] but little is known about these pathways for brain health endpoints.[106,107] Recent quantitative MRI data in older adults revealed larger brain volumes, but greater white matter hyper-intensities in African Americans than whites.[108] The most pronounced relations of vascular disease to brain atrophy and white matter hyper-intensities were found in African Americans. MRI indices of subtle brain pathology have been associated with lower levels of cognitive and physical function and cognitive decline,[109,110] and may mediate relations of race and SES to these endpoints.

This protocol is a sub-study linked to the ongoing HANDLS study. In a subset of 500 HANDLS participants, we will assess total and regional gray matter and white matter volumes and white matter microstructure in 500 stroke- and dementia-free HANDLS participants (250 African American, 250 white; 50% women; ages 30-64 at baseline) over the full range of socioeconomic status using quantitative MRI data, including volumetrics and diffusion tensor imaging (DTI). The data for this ancillary study will be collected at the University of Maryland School of Medicine (UMD). UMD relies on their local IRB to provide oversite. The roles and responsibilities of UMD are limited to activities related to data collection and analysis (through a data transfer agreement) for the HANDLS SCAN sub-study, under the direction of Shari Waldstein and Les Katzel, Principal Investigators. Please see appendix – Protocol for HANDLS Neuroimaging Study for specific study procedures.

We will address the following aims and hypotheses:

Specific Aim 1. Examine race- and SES-related health disparities in MRI-assessed measures predictive of future stroke, dementia, or cognitive decline, and evaluate whether these relations differ by sex and age. The primary outcome measures will include total and regional gray matter and white matter volumes quantified by voxel-based morphometry, ischemic lesion volumes, and total and regional fractional anisotropy (FA) and the apparent diffusion coefficient (ADC) estimated by DTI.

Hypothesis 1. There will be significant interactive relations of race and SES with respect to MRI indexes of gray matter and white matter volumes, ischemic lesion volumes, and white matter microstructure such that lower SES African Americans will display the most extensive brain pathology, particularly in prefrontal regions. Moderated mediation by age and sex (i.e., that age and sex may moderate the meditational paths by which race and SES relate to brain outcomes) will be explored.

Specific Aim 2. Examine multi-level mediators of the relations of race and SES to brain MRI outcomes; potential mediators (i.e., vulnerability or resilience factors) include biomedical (e.g., cardiovascular risk factors, subclinical vascular disease, cardiovascular comorbidities), behavioral (e.g., diet, smoking, alcohol, physical activity), psychological (e.g., depression, vigilance, anger, stress, spirituality), social (e.g., social support and networks, racial discrimination), and environmental (e.g., neighborhood deprivation, access to health care) factors.

Hypothesis 2. The multi-level mediators of MRI-based measures of GM and WM will differ as a function of race and SES. For example, select psychological factors such as racial discrimination may be prominent influences in high SES African Americans (as per pilot data), whereas behavioral, social, and environmental factors may be the most prominent influences in low SES African Americans. Moderated mediation by age and sex will be explored.

Specific Aim 3. To examine whether MRI indexes of gray matter and white matter are proximal mediators of the relations of race and SES to cognitive and physical function.
Hypothesis 3. Lesser white matter integrity and lesser white matter and gray matter volumes, and higher ischemic lesion volumes will be associated with lower levels of cognitive (particularly executive) function and physical function. These associations will be most pronounced among lower SES African Americans. Moderated mediation by age and sex will be explored.

7.1.2 Predictors of Personality Sub-study Objectives

There is a robust association between personality traits and consequential health outcomes[111]. Conscientiousness and Neuroticism, in particular, are essential for healthy aging. Individuals higher in Conscientiousness – the tendency to be organized and disciplined – live longer [112,113], have better cardiovascular [114], metabolic [115,116], immunologic [117,118], respiratory [119], and mental [120] health, and are at lower risk of Alzheimer's disease [121,122]. Neuroticism – the tendency to experience negative emotions and vulnerability to stress – is likewise implicated in disease, including major depression [120,123] and coronary heart [124], lung [125], and Alzheimer's [126,127] disease. This robust evidence base has led to a great interest in harnessing the mechanisms and processes associated with these traits to develop more effective interventions to promote healthy aging [128]. This approach holds great promise to improve aging outcomes. Yet, little is known about how the individual's environment shapes these traits. As such, the purpose of this research is to identify how neighborhoods contribute to personality in adulthood. We also place personality in the context of a consequential health outcome – cognitive aging – to identify the dynamic interplay between personality and neighborhoods on cognitive aging.

This protocol is a sub-study linked to the main HANDLS study and is conducted out of Florida State University. Florida State University relies on their local IRB to provide oversite. The roles and responsibilities of FSU are limited to activities related to data collection and analysis (through a data transfer agreement) for the Predictors of Personality sub-study, under the direction of Dr. Angelina Sutin. Participants will be invited to complete a personality questionnaire on-line, over the phone or by mail.

This research has two aims:

(1) To identify neighborhood antecedents of trait psychological functioning and to test biological markers as a mechanism through which neighborhood factors contribute to personality traits. We will test the *hypothesis* that greater economic and social deprivation, exposure to neighborhood crime, and greater physical disorder will be associated with the *lower* Conscientiousness and *higher* Neuroticism and that these associations will be mediated by inflammatory markers.

(2) To test whether personality traits mediate and/or moderate the relation between neighborhood factors and cognitive functioning. We will test the *hypothesis* that personality mediates the

association between neighborhood factors and cognition such that neighborhood factors will inhibit self- and emotion-regulation traits, which contributes to greater cognitive decline in older adulthood. We will also test the *hypothesis* that higher Conscientiousness and lower Neuroticism buffer the harmful effect of neighborhood risk on cognitive aging.

We propose to recruit participants for the POP sub-study as they finish their standard assessment for HANDLS. At the conclusion of the MRV or home visit, participants will be informed about the study on personality and how to participate. Participants will be given three options: (1) they can complete the personality questionnaire online or on paper at the MRV, if time permits, (2) they can take a paper copy with them and return it by mail to Dr. Sutin at the Florida State University in a self-addressed stamped envelope, or (3) they can be contacted to complete the questionnaire by telephone with an interviewer. The procedure will be the same regardless of the medium of assessment. Informed consent will be obtained from willing participants at the MRVs; they will be given the consent form for the study with a full explanation of the procedures, risks and benefits and the contact information of study investigators. The questionnaire will consist of personality measures and questions about their neighborhood. Participants will be compensated with a retail gift card ($40), which will be provided or mailed to them after receipt of the completed questionnaire.

8.0.0 Expected Risks and Benefits

There is very little risk to participants in this observational study. The exposure to low dose radiation from the analysis of bone density and body composition by the densitometer and the risks associated with having blood drawn are the minimal risks.

The potential benefits to the participants include access to a full medical evaluation including screening for pathology in which early detection is advantageous. If the study doctor discovers any condition or problem, the information is provided to the participant immediately and their primary care doctor, with their permission. If the participant does not have a physician, efforts will be made to refer them for care. Participants will be reimbursed for time and inconvenience.

The potential benefits to society relate to improvement of overall health in a vulnerable population that currently bears a disproportionate burden of disease and disability in this country. Healthy People 2010, the nation's disease prevention agenda, have defined two national goals to reduce preventable threats to the nation's health.[129] The first is to increase the quality and years of healthy life and the second is to eliminate health disparities. However, in order to achieve this second goal it is critical to develop research initiatives that provide new insights into the relationship between psychosocial factors and health status by (1) incorporating biological measures into large scale epidemiologic health and survey research projects and (2) the development and inclusion of a diverse panel of biomarkers or biologic measures that evaluate biologic pathways that may be involved in the causal relationship between SES and health.[130] This is what HANDLS attempts to accomplish. If successful, HANDLS will provide unique information that will hopefully uncover findings that will provide a basis for the development of appropriate prevention and intervention strategies to reduce health disparities.

9.0.0 Eligibility

In this study, we are examining age related disorders in a target population of African Americans and whites in a representative sample of Baltimore City residents.

Inclusion criteria: (1) Verified HANDLS participants (age 30-64 at baseline recruitment); (2) able to give informed consent (or has a legal designee); and, (3) must have valid picture identification.

Exclusion criteria: (1) Pregnancy (for the MRV examination visit and the HANDLS Scan sub-study a urine pregnancy test is performed with women of child bearing potential during the medical screening prior to any testing or procedures. If positive, participant will not be eligible for the MRV examination visit until they are no longer pregnant. Participants with a positive pregnancy test will be invited to return for the MRV examination visit and/or the HANDLS Scan once pregnancy is resolved (pregnancy testing is repeated at each encounter, if indicated). The home visit protocol does not pose increased risk so pregnancy status is not required or obtained); and (2) Current cancer chemotherapy or radiation therapy.

10.0.0 Subject Enrollment

Plan to re-contact participants for Wave 5. The HANDLS study has recruited a representative sample of 3720 whites and African Americans between 30 and 64 years old from 13 neighborhoods in Baltimore city in both low and high socioeconomic strata as a fixed cohort following the overall design. We have used several methods to remain in contact with our participants since they initially enrolled in HANDLS. Specific examples include sending regular mailings such as newsletters, holiday and birthday cards to the addresses we have on file, participation in the wave 2 interim study, mailing study updates and reminders with change of address cards, and periodic reviews of the Baltimore city judicial system public records and the National Death Index database. While this does allow us to remain in contact with many of our participants, there still exists a sub-set of participants for whom traditional methods will not be successful.

For Wave 5 we employ a tracing and tracking specialist whose primary responsibility is to focus on conducting investigative fieldwork and extensive tracing & tracking procedures to locate missing participants. This requires (a) physically driving through all identified HANDLS study neighborhoods in Baltimore City to previously known addresses for missing participants, communicating with current residents (and or neighbors) of identified households to assist in locating participants; (b) contacting participant's family or friends identified by the participant as persons to be reached if participant cannot be located (c) using search engines on the internet, Baltimore City judicial system public records, National Death Index, Division of Vital Records, and similar methods to locate current residence or to verify status of missing participants; and, (d) other tracing and tracking methods developed over time and with experience.

Including this strategy will allow us to make every possible effort to locate as many of our participants as possible. It is particularly crucial in this first follow-up re-examination phase of the study.

11.0.0 Study Design and Procedures

The HANDLS study is an interdisciplinary, prospective epidemiologic longitudinal study examining the influences and interaction of race and SES on the development of cardiovascular and cerebrovascular health disparities among minority and lower SES subgroups.

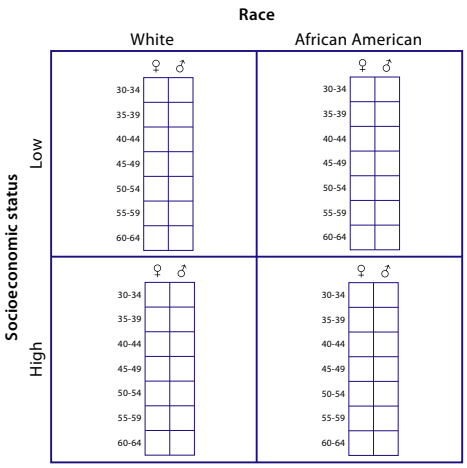

Figure 2. HANDLS sampling design

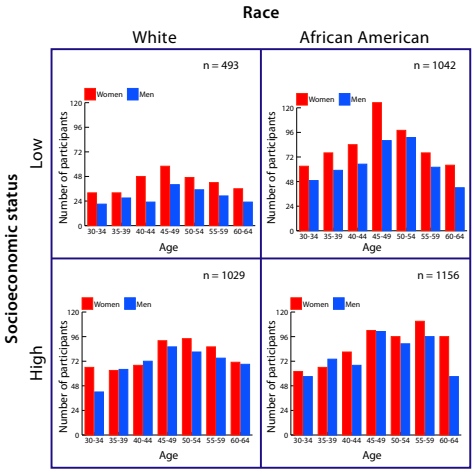

Figure 3. HANDLS baseline accrual

The baseline HANDLS sample consists of 3720 community-dwelling African American and white adults aged 30-64. Participants were drawn from 13 neighborhoods (groups of contiguous census tracts) in Baltimore City, sampling representatively across a wide range of socioeconomic and income circumstances. The heuristic study design is a factorial cross of four factors: age, sex, race, and SES with approximately equal numbers of subjects per "cell" (Figure 2). HANDLS is planned as a 20-year longitudinal study of the 3720 individuals accrued (Figure 3). Using our mobile medical research vehicles, we are revisiting each census tract for 2-3 months over the next 3 years.

The 13 neighborhoods identified were selected because they were likely to yield representative distributions of individuals between 30 and 64 years old who are African Americans and whites, men and women, and lower and higher SES.

Study sample. The study recruited an area probability sample of whites and African Americans between 30 and 64 years old from 13 neighborhoods in Baltimore City in both low and high socioeconomic strata as a fixed cohort following the overall design. By collecting a baseline assessment and 5 follow-up triennial assessments over approximately 20 years, there will be sufficient power (>.80) with 30 participants per group (race by SES by sex by age group) remaining after 20 years. There will also be sufficient power (>.80) to compare rates of change among groups after the baseline assessment.

Procedures. The primary study data for wave 5 is collected during the MRV examination visit or the home visit. These data include an interim medical history and physical examination since the

baseline examination; cognitive and sensory evaluation; assessment of health literacy, cardiovascular function: electrocardiogram, intimal medial thickness assessment by carotid Doppler, non-invasive baseline endothelial function assessment by peripheral arterial tonometry technology (Endo PAT II) (the IMT and endoPat will only be administered if not collected in a previous wave), assessments of muscle strength, lean body mass and bone density; laboratory measurements (blood and urine chemistries, hematology, biomaterials for genetic studies) and, an audio-administered questionnaire. For those participants who have difficulty ambulating independently, we recommend they complete the HANDLS home visit for wave 5 (see home visit table of procedures below). Westat staff will collect the home visit data. Westat relies on the IRB of record for the National Institute on Aging to provide IRB oversight through an IRB reliance agreement. The roles and responsibilities of Westat are limited exclusively to activities related to data collection for the HANDLS Home Visit Program (HHVP), under the direction of Catherine Torres, Principal Investigator.

A selected subset of participants is invited to participate in the optional studies, the neuroimaging sub-study and the predictors of personality sub-study. We conduct the neuroimaging study at University of Maryland School of Medicine and the predictors of personality study will be conducted out of Florida State University.

Results. Participants receive a copy of their clinical laboratory findings within 2 weeks of their examination visit (home visit or MRV). If a result is critical or needs further evaluation, the HANDLS clinician will contact the participant by telephone as soon as possible following the notification (within 24-48 hours), and will send a copy of the result to the participant the same day. If the participant agrees and provides consent to release information, a copy of the results are also sent to the participant's physician. If the participant does not have a physician the HANDLS nurse or social worker will facilitate a referral for follow-up care. The HANDLS clinician is available for further consultation with the participant and or their physician to provide additional information and or to facilitate follow-up care.

Approximately 8-12 weeks following their visit participants receive a Participant Report Packet with a copy of the results from their blood and urine tests, EKG, and DXA Scan. If the participant agrees and provides consent to release information, a copy of the results are sent to the participant's physician.

HANDLS Scan reports of incidental findings are received from the University of Maryland within one week of the scan. If a result is critical or needs further evaluation, the HANDLS clinician will contact the participant by telephone as soon as possible following the notification (within 24-48 hours), and will send a copy of the result to the participant via certified US mail the same day. If the participant agrees and provides consent to release information, a copy of the results is also sent to the participant's physician. If the participant does not have a physician the HANDLS nurse or social worker will facilitate a referral for follow-up care. The HANDLS clinician is available for further consultation with the participant and or the physician to provide additional information and or to facilitate follow-up care.

Home Visit

Measure or Procedure	Estimated Duration

Consent (completed by phone or in-person)	20 minutes
Specimen Collection, Vitals and EKG	45 minutes
Cognition & sensory test (smell)	60 minutes
Interim Medical History	30 minutes
Interim Physical Exam	45 minutes
Skin & toenail sample collection	10 minutes
Hand Grip	10 minutes
Questionnaires	15 minutes
Health Literacy	10 minutes
	10 minutes

Medical Research Vehicle Examination

Measure or Procedure	Estimated Duration	Location
Consent	20 minutes	MRV 2/3
Specimen collection (urine, blood, DNA)	20 minutes	MRV 3
Anthropometrics (height & weight)	5 minutes	MRV 1
Interim medical history	20 minutes	MRV 1
Interim Physical Exam	20 minutes	MRV 1
Skin & toenail sample collection	10 minutes	MRV 1
Cognition and sensory testing	60 minutes	MRV 2
Physical performance	15 minutes	MRV 1
EKG	10 minutes	MRV 1
Questionnaires	50 minutes	MRV 2
Health Literacy	10 minutes	MRV 1
Body composition & bone densitometry (DXA)	5 minutes	MRV 1
	10 minutes	MRV 2

Neuroimaging Sub-study

Measure or Procedure	Estimated Duration	Location
Neuroimaging Study	90 minutes	UMB

Predictors of Personality Sub-study

Measure or Procedure	Estimated Duration	Location
Personality Questionnaire	60 minutes	Self administer or Telephone

12.0.0 Procedure Description

Collection and Analysis of Biomaterials

Blood and Urine Fasting blood samples for clinical tests, banking plasma, serum, and DNA. As a part of the medical evaluation, blood tests are performed to look for anemia and other blood disorders, diabetes mellitus, thyroid disease, hepatitis, prostate disease, HIV disease and kidney disease. We are also using some blood samples to study genes that may play a role in age-related diseases like Alzheimer's disease, heart failure, high blood pressure, and cancer. The total amount of blood drawn from each participant is about 71.5 milliliters (~5 tablespoons). A random urine sample is collected for urinalysis, measurement of microalbuminuria, and storage.

Risks. There are some risks from having blood drawn. There is a risk of an infection from the needle puncture. There is also a risk of a black and blue mark, and the participant may feel faint. It is common to have a small black and blue mark, but it disappears after a day or so. Some people may begin to perspire or feel nauseated. These risks are very small. Our medical staff is well trained and has drawn blood many times. There is no risk for urine collection.

Buccal cell collection. As part of the medical evaluation buccal mucosa cells are collected from saliva samples using the Genotek Oragene DNA self-collection kit from each consenting participant. Participants are asked to spit into a DNA collection system (a small sample cup) to collect buccal mucosal cells. The extracted DNA will be used for epigenetic analysis as well as human mRNA expression profiling.

Risks. This is a completely non-invasive self-collection system. There are no known physical risks.

Alternative buccal cell collection method. The Whatman FTA collection system will be used as a back-up buccal cell collection method. This system collects buccal cells using a foam tipped applicator which is placed into the mouth and rubbed on the inside of both cheeks for 30 seconds

by the participant. The sample obtained is then transferred to the Indicating FTA cards. The extracted DNA will be used for epigenetic analysis.

Risks. Buccal mucosa smear risks include irritation of the inside of the cheek and/or gum line by the foam tipped swab used to collect cells and saliva.

Toenail clipping for environmental exposure assessment. The purpose of this test is to examine possible exposure to chemicals and/or toxins in the environment. We want to assess whether environmental exposure to certain chemicals and/or toxins contribute to the development of age-related diseases in our participants. We will clip a very small amount of nail from each of toes of both feet using a single use toenail clipper.

Risks. Cutting too close to the nail bed may result in blood loss (minimal) and risk of infection.

Skin swab. We want to study the interaction between microbiome and the immune system to examine if there is a relationship to poor wound healing and/or the development of age-related diseases. Skin bacteria will be collected using a sterile cotton swab brushed across the volar aspect of the dominant arm to examine skin microbiota.

Risks. None

Anthropometrics. We measure the height and weight and knee height of each participant.

Risks. None.

Medical history and physical examination. A physician or nurse practitioner performs an interim physical examination and medical history. The purpose of the physical examination and medical history is to document as unambiguously as possible any diagnosable conditions, to record medications and their frequencies and dosages, and to assess disabilities that might limit independent functional activities, that have developed or occurred since their last examination on the MRVs. In addition, we will examine subjects to ensure that they do not meet exclusionary criteria for any subsequent tests such as the DXA.

Risks. None.

Cognitive testing. We administer The Trail Making Test as a measure of executive function and the Mini-Mental State Examination[131] to screen for dementia. We assess symptoms of depression using the Center for Epidemiologic Studies Depression inventory (CES-D)[132]. These tests are given in a private, quiet room with an experienced psychometric technician.

Risks. None.

Sensory Testing

Sniffin' Sticks Test of Olfactory Function. The purpose of the Sniffin' Sticks test is to see if we can find problems with the ability to identify smells. Sometimes problems with smell can be related to medical, psychological or brain conditions.

Odor identification with *Sniffin' Sticks* is performed by holding an uncapped canister that resembles a marker pen 2 cm from participants' noses while asking them to sniff the odor.

Risks. There are no known risks associated with this test. Participants may find some odors unpleasant but the smell will go away quickly.

Visual Acuity and Fundus Photography. The purpose of this tests is to learn more about Age-related Macular Degeneration (AMD), a condition that can develop as we age. We will also test visual acuity.

Fundus Photography. Photographs will be taken of the back part of the eye (fundus) with a special camera. The participant will see some flashing lights.

Risks. The taking of photographs is not associated with any risk; however, the bright lights may cause temporary discomfort.

Visual Acuity Test. The study team will test participant's vision. Visual acuity (reading letters on an eye chart) will be measured using a machine called a refractometer.

Risks. These tests have no risk and will not cause discomfort. Participants will get a report of results.

Physical Performance Measures

The purpose of this performance battery is to estimate hand grip strength which has been associated with mortality and lower-extremity function and in this wave to carefully assess loss of functional capacity among participants. Use of these selected elements of the Short Physical Performance Battery and the hand grip will permit comparison to other nationally representative cohorts.[133]

Hand Grip Strength test. Handgrip strength in both hands, measured using an adjustable, hand-held, hydraulic grip strength dynamometer, is used as an overall assessment of physical strength and skeletal muscle function. Repeated measurement of grip strength over the follow-up visits will permit an estimate of strength loss over time. Grip strength is a commonly used indicator of health status and physical frailty and mid-life grip strength has been shown to be a strong predictor of early mortality.

The examination is done with the participant in the sitting position with the arm to be tested resting on the table and the elbow held at approximately a right angle. The dynamometer is held in the hand to be tested and is resting on a mouse pad. The participant is instructed to grip the two bars of the dynamometer in their hand, and to slowly squeeze the bars as hard as they can. The test is repeated on the other hand. This test is performed 3 times on each hand.

Exclusions. Participants who have had fusion, arthroplasty, tendon repair, synovectomy, or other related surgery of the upper extremity in the past 3 months will not be tested on the affected hand.

Sit-to-stand test. A commonly used performance-based test of physical function, the sit-to-stand test (also termed repeated chair stands), is used to assess functional status at study inception and to tract loss of functional capacity over time. Using a standard armless chair placed securely against a wall, the participant is first instructed to rise from the chair without using arms and return to a seated position. If this is done successfully, the participant is then asked to repeat that movement 10 times. Performance, both whether 10 stands are completed and time to perform 5 or 10 stands has been strongly associated with onset of functional limitation, physical disability, institutionalization, and mortality.

Exclusions. There are no formal exclusions from attempting the single chair stand; inability to rise from a chair without using arms excludes participants from doing repeated chair stands.

Tests of Standing Balance

Side-by-side stand. The side-by-side stand test should be performed with the participant standing a little less than an arm's length from a wall to provide an additional source of support if a loss of balance does occur. This test requires the participant to stand with feet side by side for 10 seconds.

Semi-Tandem Stand. The Semi-Tandem stand test should be performed with the participant standing a little less than an arm's length from a wall to provide an additional source of support if a loss of balance does occur. This test requires the participant to stand with the side of the heel of one foot touching the big toe of the other foot for about 30 seconds.

Tandem Stand. The Tandem stand test should be performed with the participant standing a little less than an arm's length from a wall to provide an additional source of support if a loss of balance does occur. This test required the participant to stand with the heel of one foot in front of and touching the toes of the other foot for 30 seconds.

Single leg stand. The single leg stand test should be performed with the participant standing a little less than an arm's length from a wall to provide an additional source of support if a loss of balance does occur. This test requires the participant to stand on one leg with the other leg flexed at the knee and held about two inches from the floor. The participant is asked to hold the position for as long as they can, up to 30 seconds. The single leg stand has been found to be a sensitive test of standing balance for middle age and older adults and has been used in numerous epidemiologic studies of well elderly without mishap.[133,134]

Risks. There are very minimal risks associated with the Physical Performance Measures. The only risks are that there is a slight risk of falling and the participant may feel tired after these tests.

Cardiovascular Function

Resting electrocardiogram (EKG). We place electrodes on the participant's skin to record their heartbeats. By looking at the electrical pulse of their heart we examine the heart rate and rhythm, and check if they have had a heart attack.

Risks. None.

Carotid Arterial Blood Flow and Arterial Stiffness. Carotid Doppler ultrasonography is the method of choice for noninvasive, in vivo examination of the structure and function of the carotid arteries. Intimal-medial thickness has emerged as a potent predictor of stroke,[135-137] myocardial infarction,[137] coronary artery disease[138] and cardiovascular disease[136] independent of other traditional cardiovascular risk factors. In this study, we will perform high resolution B-mode ultrasonography on the left carotid artery, for the evaluation of systolic and diastolic common carotid arterial diameters, carotid arterial flow, intimal-medial thickness, and plaques. We will also evaluate the right carotid artery for the presence of plaques.

Risks. None are known

Peripheral Arterial Tonometry via Endo PAT II System. Evidence suggests that the disproportionate cardiovascular disease risk among African Americans is not completely attributable to racial differences or to the standard cardiovascular disease risk parameters. Several sources of evidence suggest that the observed differences may be related to differences in the pathophysiology of the endothelium. The endothelium plays a central role in maintaining vascular tone and vascular homeostasis. Vasodilators including nitric oxide and prostacyclin and vasoconstrictors including endothelin-1, thromboxane A_2, and platelet activating factor are secreted by endothelial cells. Endothelial dysfunction results from decreased nitric oxide bioactivity. It is associated with vascular inflammation, vasoconstriction, and thrombosis and is one of the earliest manifestations of coronary atherosclerotic heart disease.[139] Numerous studies have demonstrated the increased prevalence of endothelial dysfunction among African Americans and is believed to play a critical role in the disparate incidence of hypertension and cardiovascular disease among this population.[140] What is the pathophysiological basis for the observed differences in arterial endothelial function? Endothelial function is modulated by race and sex. It is not completely understood how inflammatory factors, oxidative stress, and perhaps even the social determinates of health may modulate endothelial function.

We will measure endothelial function via Peripheral Arterial Tonometry (PAT) a non-invasive methodology to assess endothelium-mediated changes in peripheral vascular reactivity or vascular tone. The EndoPAT system measures nitric oxide mediated changes in vascular tone through the use of bio-sensors placed on the fingertips before, during, and after the occlusion of the blood flow in one arm by a sphygmomanometer. The release of the blood when the cuff is released leads to rapid return of blood flow which in turn causes a vascular dilatation (flow mediated dilatation and reactive hyperemia. The Endo-PAT II system provides an EndoScore or Reactive Hyperemia Index (RHI) which is a measure of endothelial function.

Risks. Occlusion of brachial artery may cause tingling in the arm and hand which will subside after the test. Patients s/p mastectomy should not have the cuff inflation performed on the side of the surgery. [141] [142] [139] [143]

Peripheral vascular assessment by ankle brachial index. The resting Ankle-Brachial Index (ABI) is calculated as the ratio of the systolic pressure in the ankle measured via hand held Doppler and sphygmomanometer at either the dorsalis pedis or posterior tibial artery and the systolic pressure measured at the brachial artery. The ABI is an indicator of peripheral artery disease as well as a prognostic marker of atherosclerosis and risk factor for future cardiovascular and cerebrovascular events.[144] [145] In this population at high risk for cardiovascular disease, measuring ABI will provide a benefit to participants at risk as well as an opportunity to evaluate covariates of abnormal ABI values in African American and low SES urban white cohorts. Participants will rest for 10 minutes; systolic pressure will be measured in both ankles and both with hand-held Doppler and properly sized sphygmomanometer.

Exclusions and Risks. Patients with open sores and ulcers at the ankle or antecubital fossa will be excluded as will those with AV shunts or grafts. Patients status post mastectomy or lower limb amputation will also be excluded.

The Carotid Arterial Blood Flow and Arterial Stiffness, Peripheral Arterial Tonometry via Endo PAT II System, and the Peripheral vascular assessment by ankle brachial index procedures may be performed on participants who did not receive them in a previous MRV visit.

Audio-administered questionnaires. We assess risk of poor mental health and questions about food security, smoking behaviors and income with an audio-administered (using a computer and headphones) questionnaire. Assistance is provided to the participants, if for example they have trouble seeing or reading the questions or are uncomfortable with using a computer.

Risks. None.

Health literacy. To assess health literacy in Wave 5, we will use a shortened version of the Rapid Estimate of Adult Literacy in medicine (REALM). The REALM assesses reading level through scoring pronunciation of a list of health care related terms by participants. It correlates with other measures of reading literacy and health literacy.

Risks. None.

***Bone density and body composition*.** We perform dual energy X-ray absorptiometry (DXA) on total body, lumbar spine, the hip and the Instant Vertebral Assessment (IVA) using a Discovery QDR series (Hologic, Bedford MA). DEXA delivers a small amount of radiation through an X-ray source while you lay on the scanner bed. Site-specific scans of the lumbar spine and right hip provide information on bone area (cm^2), and bone mineral density (g/cm^2). Total body scan measures both body composition and bone mineral density, including bone mineral content (g), bone area (cm^2), bone mineral density (g/cm^2), total body tissue (g), fat mass (g), lean mass (g), lean mass plus bone mineral content (g), and percent total fat (%). The IVA provides an assessment of vertebral fractures. Results of the total body scan are presented for the whole body as well as for the arms, legs, trunk, head, pelvis, and spine.

Exclusions. DXA studies are not administered to pregnant women or individuals weighing greater than 450 pounds due to the densitometer's limitations.

Risks. The NIH Radiation Safety Committee has reviewed the use of radiation in this research study and has approved this use as involving minimal risk and necessary to obtain the research

information desired. Although each organ receives a different dose, the amount of radiation exposure participants receive from these procedures is equal to a uniform whole-body exposure of less than 1 millirem. This calculated value is known as the "effective dose" and is used to relate the dose received by each organ to a single value. The amount of radiation received in this study is within the dose guideline established by the NIH Radiation Safety Committee for research subjects. The guideline is an effective dose of 5 rem (or 5,000 mrem) received per year.

The NIH Radiation Safety Branch monitors equipment and technique used in this study.

13.0.0 Collection and Storing of Human Sample Specimens and Data

Intended use of the samples, specimens, and data. Samples and data collected under this protocol may be used to study the differential influences of race and socioeconomic status on health in an urban population. Genetic testing will be performed.

Labeling of stored samples. Participants' stored samples will be labeled with HANDLS identification numbers that only the study team can link to participants. Any identifying information about participants will be kept confidential to the extent permitted by law.

How samples, specimens, and data will be tracked? Samples are tracked using the NIA Biological Sample Inventory system following NIH guidelines.

Storage and release of samples. Samples of the participant's blood are kept in a research laboratory at the National Institutes of Aging, NIH or one of our contract facilities. The subject's samples are tested immediately, or they may be frozen and used later. Informed consent allows subjects to determine future use and use for genomic projects. The subject's samples are stored with a confidential code. Samples may be kept until no cells remain or until the investigators decide to destroy them. If the participant gives us permission some samples are released to other doctors and scientists who are not associated with this institute. The Clinical Director and the Principal Investigators on this protocol will decide which researchers may receive samples. The subject's samples may be used in their research only if the research has been approved by an Institutional Review Board (IRB) and is related to the original research questions association with this protocol or for other research purposes as indicated below. Access to the samples will be limited by storing samples in a locked room.

What will happen to the samples, specimens, and data at the completion of the protocol? The stored material will be used only for research and will not be sold. At the completion of the protocol, samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol.

What circumstances would prompt the PI to report to the IRB loss or destruction of samples, specimens, or data? We will report any loss of samples (e.g., freezer malfunction to the IRB according to NIA protocol violation policy. In addition, we will report to the IRB any loss of unanticipated destruction of samples or data.

Participants may decide at any point not to have to have their samples stored. In this case, the Principal Investigator will destroy all known remaining samples and report what was done to the participant and the IRB. This decision may not affect participants' status in this protocol or any other protocols at NIH.

14.0.0 Data Collection and Management Procedures

HANDLS data are collected electronically or manually on the MRVs, over the telephone and in participant's homes. Data are kept in medical charts in locked file cabinets. Electronic data is kept on password-protected computers. All clinical research forms are filed in locked file cabinets. These materials are kept within a locked medical record room. Access to all study data is limited to HANDLS staff and investigators. Data are coded and entered by ID number only. Collaborators receive ID numbers only. No other identifying information is provided with the data unless there is a data use or materials transfer agreement in place, consent has been obtained from the HANDLS participant and the collaborators have obtained required IRB approval.

Data analysis. The study employs a standard statistic software package depending on the independent and dependent variables being analyzed. Data analyses include logistic regression and mixed effects modeling.

Data sharing agreement. Data generated by the HANDLS study is available through several mechanisms including publications, presentation of results at national scientific meetings, and via a proposal review mechanism routed through the HANDLS principal and co-investigators working group.

The HANDLS web site (http://handls.nih.gov) contains a data dictionary for each of the study domains outlining available data sets. This website also describes the proposal submission process for investigators who would like to use HANDLS data or biomaterials. Proposers are required to submit an electronic HANDLS concept sheet detailing the hypotheses and specific aims of the proposals as well as the required data sets and/or biomaterials. These proposals are reviewed by the HANDLS Working Group. Meritorious proposals are assigned a HANDLS Investigator to serve as liaison and collaborator working with the successful proposer facilitating the completion of the NIA and NIH data transfer or material transfer agreements required by federal regulations and to access and use the data set (s) or biomaterials required for the approved proposal. Proposals not completed and submitted for publication within the time frame stipulated in the proposal will be re-negotiated or terminated.

Data safety and monitoring. No data or safety monitoring board is required. The Principal Investigator will monitor and evaluate the progress of the study, including periodic assessment of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of contractors and other factors that can affect study outcome. This monitoring will also consider factors external to the study when interpreting the data, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study.

15.0.0 Quality Control

All data for the HANDLS study is collected by following detailed Standard Operating Procedures (SOPs) as outlined in the HANDLS Operations Manual. The majority of data is collected electronically, in real time, and is monitored at regular intervals for accuracy and adherence to the protocol by HANDLS computer programmers and information technology specialists. Manually collected data are stored in the research medical record and are reviewed for accuracy and completion daily by the HANDLS Medical Records Specialist. The HANDLS Clinical Study Manager selects medical records at random for monthly audits.

16.0.0 Statistical Considerations

Power analysis. Initial estimates based on the 2000 census data indicate that we needed to visit approximately 35% of the households in each census tract to collect the required 333 individuals. The initial sample of 3,500-4,000 participants is based on power analyses and assumptions about attrition over 20 years. For a power of 80% (the likelihood of finding an effect if it is really present), we can identify moderate effects (magnitude of the differences between groups) for various outcomes with as few as 30 participants per group at the end of the study. Working backwards by assuming 20% attrition after the baseline assessment and 15% attrition between subsequent assessments, we need approximately 3,500-4,000 participants at baseline to yield 1,680 after 20 years.

Data analyses. The study employs standard statistical software depending on the independent and dependent variables being analyzed. Data analyses include parametric and nonparametric statistics for cross-sectional comparisons applying logistic regression and mixed effects modeling as appropriate for the data. Longitudinal analyses will typically require either mixed-effects models, survival analyses, or proportional hazards depending on the data and specific outcome under study.

17.0.0 Regulatory Requirements

17.1.0 Informed Consent

Wave 5. There are two parts to the Wave 5 study. The first phase occurs in the field, at the medical research vehicles (MRVs) or in the participant's home, if they have limited mobility. If the participant has been identified as a home visit participant, consent will be obtained in the home. Among the preparations for their examinations on the medical research vehicles, participants are provided copies of the informed consent documents and are asked to read them. Participants are then instructed to view a consent film about the HANDLS study that explains the purpose of the study and all procedures they have previously reviewed in the informed consent documents. The HANDLS study consenter then reviews each document with participants a final time, page by page stopping to ask if they have any questions to ensure the participant has a clear understanding of the study, the degree of risk, potential benefits, and alternatives and then provides the participant with an opportunity to ask any further questions and to consider their decision to participate in this next wave of the HANDLS study. If participants agree to take part, signatures will be obtained using an IRB approved hard copy of the informed consent document or electronically using a PC tablet. HANDLS staff provides participants with printed copies for their records and a copy is placed in the research medical record. HANDLS staff sends participants copies of all signed informed consent documents with the results from their examinations.

Wave 5 – optional studies. Informed consent for the Neuroimaging Study will take place at the UMD follow guidelines set forth by their IRB. Procedures for informed consent for the Predictors of Personality sub-study will include the same procedures for the examination visit, except the participants will not see a video description of the assessment being conducted.

Durable Power of Attorney for Health Care Decision Making. During wave 4 participants were asked to designate a heath care agent by completing NIH form 200-(10-00) Durable Power of Attorney for Health Care Decision Making. Participants were informed that the designee will be

able to make decisions regarding their participation in HANDLS (and any clinical care related to their participation), in the event they are unable to make their own decisions due to diminished capacity. A copy of the form will be mailed to the person they name and participants will be encouraged to discuss their known desires and values with the designee, their personal physician and their family. During W5 if a participant no longer has decisional capacity, HANDLS staff will consider calling the participant's health care designee to discuss the feasibility and consent to participate in W5.

17.2.0 Compensation

The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, participants are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

Participants may be reimbursed up to a total of $250 for participating in the HANDLS - wave 5 study. They may be paid up to $160 for participating in the MRV visit or up to $100.00 for the Home Visit. If they participate in the Neuroimaging study at the University of Maryland they will be compensated an additional $50.00, and if they participate in the Predictors of Personality sub-study they will receive an additional $40.00 retail gift card.

If a participant is unable to complete all the tests they may receive a portion of that payment. They will receive payment in the form of an ATM debit card at the end of each phase. In most cases, the ATM card will be activated by the end of the study visit day. The participant will be instructed to take the card to an ATM machine of their choosing to withdraw payment. Written instructions regarding how to access payments will be provided. Occasionally participants are not able to complete all testing in one visit to the MRVs or some tests require repeating if there are questionable or abnormal results. We would like to be able to offer additional compensation for time and travel to return to the MRVs for return visits. The amount of compensation will vary between $20.00 and $80.00 depending on the length of time spent on the MRVs. We anticipate the return visits to be between 1-4 hours. This would include participants who never had a baseline evaluation.

17.3.0 Subject Confidentiality

HANDLS participants' confidentiality will be maintained by informing them of the following:

When results of an NIH research study are reported in medical journals or at scientific meetings, the participants will not be named and/or identified. In most cases, the NIH will not release any information about participant's research involvement without their written permission. However, if they sign a release of information form, for example for an insurance company, the HANDLS Medical Records Specialist will give the insurance company information from the medical records. Participants are informed this information might affect (either favorably or unfavorably) the willingness of the insurance company to sell them insurance.

The participants are informed that the Privacy Act protects the confidentiality of their medical record. However, the Act allows release of some information from the medical record without permission, for example, if the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations, require it.

To help us protect privacy, we have obtained a Department of Health and Human Services Certificate of Confidentiality issued by the National Institutes of Health. With this certificate the researchers cannot be forced to disclose information that may identify participants, even by court subpoena, in any federal, state, or local civil, criminal, administrative, legislative or other proceedings. The researchers will use the certificate to resist any demands for information that would identify them, except as explained below. The Certificate cannot be used to resist a demand for information from personnel of the U.S. Department of Health and Human Services that is used for auditing or program evaluation or for information that must be disclosed in order to meet federal regulations. A Certificate of Confidentiality does not prevent participants or a member of their family from voluntarily releasing information about themselves or their involvement in this research. If an insurer, employer, or other person obtains written consent to receive research information, then the researcher may not use the Certificate to withhold that information. The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without participant's consent, information that would identify them as a participant in the research project under the following conditions: It does not apply to state requirements to report certain communicable diseases. In addition, the study clinician may be required to report certain cases of abuse, neglect, or suicidal or homicidal intent to the appropriate authorities.

Information regarding who will have access to the data and use of personally identifiable data or private health information (PHI) are described in further detail in sections 14.0.0 (data collection and management procedures) of this protocol.

18.0.0 Participant Safety, Adverse Events & Problem Reporting

18.1.0 Participant Safety & Intent to Treat

As the HANDLS study cohort has aged, there are many participants who have developed new, more severe, or multiple chronic age-related medical conditions that require treatment. However, in this cohort participants are at times unable to regularly access needed care or are unable to be consistently compliant with medications and therapies. While participants are screened on the telephone and at times in person prior to setting their longitudinal visit appointments, participants usually do not fully relate their current medical status and level of compliance with prescribed medical regimens. Hence, we are at times confronted with participants in need of minor medical intervention. The frequency and severity of presenting symptomatology related to poorly controlled, chronic medical illness varies by participant and neighborhood demographics. Most often they report running low or out of medication because they cannot afford to fill the prescriptions to continue treatment. In those cases, once medically cleared, participants will be given a 2-4 week prescription and/or funds to fill the prescription. Once symptoms are controlled, they will return for their study visit. In these cases, with participants consent, treatment provided will be shared with the primary care physician of record. If the participant does not have a physician and is agreeable, they will be provided the available resources and assistance to obtain a primary care physician and or health insurance, if necessary.

We have determined that at times, it may be necessary to temporarily suspend the research visit for ethical and safety reasons to administer initial urgent care for treatment of significant symptoms and physical examination findings before the participant can be discharged home, to their medical provider or the local emergency department. Circumstances can become quite

complicated when participants exercise their right to refuse transport to an emergency department, HANDLS staff may not be able to contact the participant's primary care provider for an urgent appointment or the participant may not have a regular physician. There are five clinical presentations that we anticipate occurring episodically at the time of the HANDLS longitudinal visit and below we outline the proposed participant safety and well-being actions that will occur in keeping with standard medical practice and ethics:

18.1.1 Poorly controlled hypertension and related medical non-compliance

Some participants will have neglected to take their medications on the day of the exam despite being told the night before to take all anti-hypertensive medications and to bring all medications to the visit. This will result in an elevated screening blood pressure reading. Participants with values <200 systolic and <100 diastolic will be asked to take their medications immediately. Their blood pressure will be monitored for reduction upon which we will allow them to remain for the study visit. If there is no reduction in blood pressure, their visit will be terminated and they will be advised to take their mediation as directed and referred to their primary care physician for a blood pressure check for possible modification of their regimen. They will be re-contacted in 30 days to again assess their suitability for reappointment.

Some participants will be newly diagnosed or non-compliant and have no anti-hypertensive medication. These participants will not continue their visit; they will be referred to their primary care physician for evaluation and treatment if they have a primary care physician. If there is no primary care practitioner, we will provide information about appropriate medical clinics in the vicinity in the context of their insurance status and ability to pay. Social work assistance is available on staff to assist medical staff in navigating the financial assessments. They will be re-contacted in 30 days to again assess their suitability for reappointment.

Participants with SBP>200mmHg and/or a DBP >110mmHg will not continue with their visit and will be sent to the closest ER. If they decline, we will document their choice with an Against Medical Advice (AMA) note in the chart signed by the participant. However, in the interest of providing best available care, we will offer the alternate of administering Clonidine and providing a prescription for standard antihypertensive medications and an appointment for follow-up at the appropriate medical venue. They will be re-contacted in 30 days to again assess their suitability for reappointment.

18.1.2 Poorly controlled diabetes mellitus and related medical non-compliance

Some participants may present with symptoms of hyperglycemia due to medical or dietary non-compliance. If there are symptoms of hyperglycemia, a finger stick will be done. For participants with elevated glucose levels >400 but < 500 as determined by glucometer monitoring will be asked to take their medications if available. If they have been non-complaint with their medications or have no medications with them, they will be treated with sliding scale regular insulin and referred back to their primary care provider for evaluation after completing their longitudinal visit. If they have no medication at home, in the interest of providing the best care possible, we will provide a prescription for their previously prescribed hypoglycemic agent or an appropriate drug if they are unable to provide the name of the drug previously taken. They

will be referred to an appropriate provider for disease management. Participants with glucose >500 will be referred to the ER and their testing suspended. If they refuse ER transport, we will document their choice with an Against Medical Advice (AMA) note in the chart signed by the participant. We will treat with regular insulin and attempt to get the soonest appointment with their provider or a provider at an appropriate medical clinic. These participants will be contacted 30 days after the interrupted testing to assess their suitability for completion of their longitudinal visit.

Some participants may mistakenly take their hypoglycemic agents while they are fasting in preparation for their visit and become hypoglycemic despite being instructed the night before the visit via telephone to bring their meds to take at the MRV after they have had their blood drawn and are ready for breakfast. Depending on their glucose level using finger stick glucometer monitoring we will administer glucotabs, oral juices or injectable glucagon. Testing will resume when patient is no longer hypoglycemic by finger stick glucometer monitoring.

18.1.3 Poorly controlled asthma/chronic obstructive pulmonary disease (COPD).

Participants at times are non-compliant with their medications or have run out of their prescriptions and present with or develop symptoms of wheezing or dyspnea. Participants with a pulse oximeter of 85-90% will receive albuterol nebulizer and/or oxygen as directed by the medical staff. If the subject's saturation does not return to 90% or greater after treatment and remains stable, the subject will be referred to the emergency room. Participants with pulse oximeter values <85% will be sent to the local emergency department. They will be re-contacted in 30 days to reassess their suitability for reappointment.

18.1.4 Alcohol Withdrawal

Participants who exhibit early signs and symptoms of alcohol withdrawal during testing, will have their testing interrupted and a detailed history taken of their alcohol use. Participants with prior history of severe alcohol withdrawal will be sent to the emergency room. In some instances, it may become necessary to give the participant an initial dose of short acting benzodiazepine to prevent further deterioration prior to the participant being transported to the nearest emergency room by the Emergency Medical System (EMS). Participants with no prior history of severe alcohol withdrawal will be discharged home after receiving counseling and referral to an alcoholic detox program. Participants will be re-contacted in 30 days to reassess their suitability for reappointment.

18.1.5 Seizure Disorder

Participants with a known history of seizure disorder, who have a seizure while being testing, will have their testing for the day stopped. Depending upon the situation, EMS may be activated. Participants who have a singular seizure episode with full recovery will be discharged to their primary care provider. For participants who have repeated seizure episodes, EMS will be activated and the participant will be treated with short acting benzodiazepine prior to the EMS

arrival. Participants will be re-contacted in 30 days to reassess their suitability for reappointment.

For all clinical presentations in sections 18.1.1 to 18.1.5 above:

Participants will be asked to sign an Against Medical Advice (AMA) note if they decline EMS and or transport to an emergency room or their primary care physician's office per study clinician's advice.

If a participant's study visit is terminated due to a medical problem identified on the MRV, the study team will attempt to re-contact participant within four days to follow up and determine the outcome of treatment recommendations.

18.2.0 Adverse Events and Unanticipated Problem Reporting

Adverse events associated with HANDLS study procedures are expected to occur very infrequently. Most of the potential risks associated with study procedures (see Section 1.2) are limited to mild, transient discomforts of no clinical significance. Only clinically significant adverse events will be reported to the IRB. A clinically significant adverse event will be reported as a serious adverse event if it is life threatening, causes persistent or significant disability, leads to death, or requires medical or surgical intervention to prevent a life-threatening event, persistent or significant disability or death.

Anticipated minor protocol deviations and anticipated problems or adverse events, as described in sections 18.0.0 – 18.1.5 above, will be reported to the IRB annually, as part of the continuing review process.

HANDLS staff is trained to detect and respond to clinically significant adverse events. They are expected to report clinically significant adverse events to the Principal Investigator immediately or as soon as is practical. The Principal Investigators for the HANDLS Scan and the Predictors of Personality (POP) sub-studies are also expected to report clinically significant adverse events immediately to the NIA Principal Investigators and to follow the adverse event reporting policies of their institutions. The HANDLS principal investigator will be responsible for reporting all unanticipated clinically significant adverse events to the NIEHS IRB within 7 days of receiving notification that an event occurred.

Adverse Events, protocol deviations, unanticipated problems (UP), serious adverse events, sponsor and serious, are defined as described in NIH Human Research Protections Program (HRPP) Standard Operating Procedure (SOP) #16 entitled *Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations*. All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and clinical director as soon as possible but not more than 7 days after the principal investigator first learns of the event. Unanticipated problems defined as not serious will be reported to the IRB and clinical director as soon as possible but not more than 14 days after the PI first learns of the event. Unanticipated protocol deviations defined as not serious will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event. Serious adverse event deaths will be reported to the clinical director within 7 days after the PI learns of the event.

18.3.0 Reporting Waiver

Waiver of Reporting to the IRB of anticipated minor protocol deviations and adverse events unless determined to be an Unanticipated Problem

The following anticipated minor deviations in the conduct of the protocol will not be reported to the IRB unless a procedural or protocol change is required:

We anticipate that not all HANDLS testing will be completed at each participant visit due to scheduling conflicts or time constraints either anticipated or unanticipated. As per protocol, tests/procedures may be rescheduled for another time if it is within the current testing period (wave), without compromising study data.

The following anticipated non-UP adverse events will not be reported to the IRB unless associated with an Unanticipated Problem:

Syncope or near syncopal episodes, that occur before, during or after blood draws, hyperglycemic, hypoglycemic episodes, hypertensive urgency and hypotension that require minimal medical intervention, falls with minimal injury to the participant and do not require more than minimal medical intervention, Muscular strains or sprains which require minimal or no medical intervention, undiagnosed non-life threatening study results that require medical follow-up by primary care provider, such as critical laboratory values initiating a laboratory alert, EKG, DXA or MRI reports; and, any other non-life threatening or non-medically indicated treatment required event that occurs during the course of a participant's study visit.

19.0.0 Site and Clinical Safety Monitoring Plan

The NIA Clinical Research Protocol Office will perform routine visits to the HANDLS research site to ensure the safety and conduct of the study complies with 45 CFR 46 and NIA guidelines. Audits are performed to assure that clinical research is in compliance with FDA, DHHS domestic regulations, Clinical Practice Guidelines (GCP), and local and federal human subjects standards. An audit may be performed following an adverse event, protocol deviation or at the time of annual renewal. The Clinical Protocol Coordinator of the Clinical Research Protocol Office determines the frequency of monitoring visits. Participant records are randomly selected from the protocol to be audited. Targeted audits may also be carried out when there is specific concern regarding patient safety or data integrity. The principal investigator and clinical research coordinator of the study are notified at least three weeks in advance of the audit, and are asked to supply all research records and patient medical records for the audit.

The NIA Clinical Research Protocol Office (CRPO) staff and the Clinical Protocol Coordinator of the Clinical Research Protocol Office (CRPO) carry out the audits. Audit format follows the NCI guidelines for national cooperative group audits. Following intensive review of the research and medical records, a formal written report of the audit findings is sent to the principal investigator and the NIA Clinical Director. The site visits will be recorded in a visit log, by the monitor, and kept at the HANDLS research site.

The monitor will review various aspects of the study including, but not limited to:

(1) Compliance to the protocol;

(2) Review of written informed consent forms for participants enrolled;

(3) Comparison of clinic records (source documentation) to data recorded on case report forms to assure the completeness and accuracy of data collected;

(4) Continued acceptability of facilities and staff; and,

(5) Assessment of proper sample accountability, transfer and storage.

During the scheduled monitoring visits, source documentation will be made available to the monitor to substantiate proper informed consent procedures, adherence to protocol procedures, adequate reporting and follow-up of AEs. The Investigator (and as appropriate the research study staff) must be available to meet with the study monitor to discuss the findings from this review of Clinical Report Forms and source documents, make necessary corrections to case report form entries, respond to data clarification requests and respond to any other study-related inquiries of the monitor.

The principal investigator will be notified of any planned visit and a date will be set that is mutually agreeable. A report will be written to document all findings, solutions and discussions. The report or a follow-up letter summarizing the contents of the report will be sent to the principal investigator. Additional follow-up will be conducted by email and telephone as needed.

20.0.0 References

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Addendum to the Protocol