THE ASSOCIATION BETWEEN DIET QUALITY AND PREVALENCE OF ANEMIA IN AN URBAN POPULATION OF SOCIOECONOMICALLY DIVERSE AFRICAN AMERICAN AND WHITE ADULTS

by

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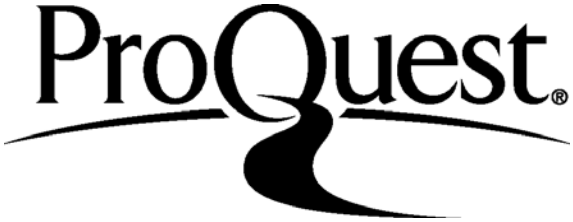

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ABSTRACT

Background- Anemia is a common health concern, especially for premenopausal women and the aging population. Previous research has shown that anemia can increase the risk of morbidity as well as mortality. Approximately one-third of anemia is attributed to nutritional deficiencies, one-third to anemia of inflammation (AI) or chronic disease, and one-third to unexplained etiologies. Diet can clearly play a role in nutritional anemia (NA), but less is known about the possible role of diet on AI or unexplained anemia (UA). Since the human diet is composed of a variety of foods with many nutrients, examining single nutrient intake may not be effective in evaluating the role diet may play in the occurrence of anemia. Overall diet quality may provide evidence to suggest that diet plays a role not only in NA, but also in AI and UA.

Objective- The primary aim of this study it to characterize the types of anemia present in an urban population of socio-economically diverse African American and White adults. The secondary aim is to determine if diet quality is associated with each type of anemia.

Subjects- The participants were 1,977 Whites and African Americans from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) baseline study conducted between 2004 and 2009

Methods- Two 24-hour dietary recalls were collected with the USDA Automated Multiple Pass Method by trained interviewers. Diet quality was assessed using the Healthy Eating Index (HEI)-2010. Anemia was defined by applying known criteria to fasting blood samples of hemoglobin, serum ferritin, transferrin saturation, and serum B_{12} and folate concentrations.

Stat analysis- Chi square and one-way analyses of variance were used to compare demographic characteristics of those with anemia and those without anemia. Logistic regression models were conducted to examine the association of HEI-2010 scores and anemia. Only participants with two dietary recalls and all blood biomarkers were included in the analyses.

Results- Of the 1,977 HANDLS study participants, 305 (15.4%) were identified as anemic. Of these 305 participants with anemia, 47.5% were diagnosed with anemia of inflammation, 29.8% with unexplained anemia, and 22.6% with nutritional anemia,. Based on unadjusted regression analysis; sex ($p<0.01$), race ($p<0.01$), poverty status ($p<0.01$), food assistance ($p<0.05$), education level ($p<0.01$) smoking status ($p<0.05$), hand grip strength ($p<0.01$), and BMI ($p<0.01$) were statistically significant predictors of general anemia. Diet quality as evaluated by HEI-2010 scores, were not significant predictors of general anemia, NA, AI, or UA.

Conclusion- Approximately 15% of HANDLS study participants had anemia, with the AI being more prevalent than either NA or UA. Literacy, BMI, and CRP (all associated with diet quality), and handgrip strength (a marker for nutritional status),

were all found to be predictors of general anemia. It is possible that no associations were found between anemia and diet quality because anemia reflects long-term dietary patterns and that diet quality as assessed by HEI-2010 only captures recent compliance to dietary guidelines.

Chapter 1

INTRODUCTION

Definition of Anemia

Anemia is characterized by a lower than normal number of red blood cells (RBCs) in the blood, or when the red RBCs do not function as they should; often times there is a decrease in hemoglobin concentration in the blood.[1,2] Hemoglobin, an iron-rich protein in the blood, is responsible for attaching to and transporting oxygen from the lungs to other tissues in the body. Thus, when there is a decrease in hemoglobin in the blood, or a lower amount of RBCs than normal, the body does not get enough oxygen from the blood.[1] Normal amounts of hemoglobin in the RBCs vary, but in general, in males, a normal amount of hemoglobin ranges from 13.8 g/dL to 17.2 g/dL; in women, this range is from 12.1-15.1 g/dL.[1] The most commonly accepted and used criteria for determining anemia in adults regardless of race comes from the World Health Organization (WHO)[3]. Anemia is defined as a hemoglobin concentration less than 13 g/dL in men and less than 12 g/dL in women.

Prevalence of Anemia

Anemia is one of the most prevalent disorders worldwide. Based on data from the World Health Organization (WHO) Vitamin and Mineral Nutrition Information System for 1993-2005, anemia affected over 1.6 billion people, accounting for nearly a quarter of the global population.[4] In 2010 Kassebaum and colleagues reported that

the global anemia prevalence was 32.9%. [2] In this study, it was found that high income regions, such as countries found in North America, tended to have the lowest amount of and lowest burden attributed to anemia compared to poorer countries and regions. Although North America may not be as affected by anemia as many other countries in the world, it is the area with the least amount of progress, and the only region that did not reduce anemia rates from 1990 to 2010.[2] For that reason it is crucial to express the importance of determining risk factors for anemia as well as possible programs to implement in order to decrease anemia prevalence.

The prevalence of anemia varies by age, gender, and socio-demographic background. The populations most affected by anemia are children and women, with prevalence among the aging population becoming more notable. [5] In the previously mentioned worldwide analysis by the WHO, it was found that 30.2% of non-pregnant women were affected by anemia; that is about 468 million women. [4] It has been shown that anemia incidence tends to increase with age[6] and is common in the elderly population; most often defined as those 65 years and older. The Third National Health and Nutrition Examination Survey (NHANES III) study found that in adults aged 65 years and older, anemia was present in 11% of men and in 10% of women.[7] The incidence of anemia rapidly increased after the age of 50 years and continued until reaching a rate of incidence over 20% in those 85 years and older. Approximately three million Americans over the age of 65 are anemic.[7]

Race may play a significant role in prevalence of anemia as well. Several studies have found that anemia is more prevalent in blacks than in whites.[7-9] In a

geographically representative sample of 19,836 participants, it was found that anemia was present in American blacks nearly three times as much as in American whites.[8] However, it is hypothesized that this increase in anemia is actually due to differences in hemoglobin concentration between races.

Research supports that blacks tend to have lower hematocrit, mean corpuscular volume, transferrin saturation, and hemoglobin than whites.[10] A study by Cresanta found that hemoglobin levels were 0.9g/dL lower in blacks than in whites,[11] with several other studies finding similar results.[9,12] Patel found that more blacks were classified as anemic using the WHO standards. However, the blacks classified as anemic were not at risk of the adverse events associated with anemia (increased morbidity and mortality).[13] Therefore, it may not be that anemia is actually more prevalent in blacks, but that the markers commonly used for assessing anemia may be inherently different between races. Thus, it has been suggested that the criteria for anemia classification may not be appropriate for all racial groups, specifically blacks.[10,11,13] Beutler and colleagues have proposed different values for lower limits of normal hemoglobin concentration based on race,[14] but little else has been done to validate these values or implement them in anemia diagnosis. Therefore the same criteria are still applied to all races.

Although less conclusive, there is some research indicating that socioeconomic status (SES) is associated with anemia as well. Iron deficiency, an important factor contributing to NA, is most common among low SES groups.[15] Several studies have found that low SES was an independent risk for increased risk of anemia.[16,17] Yet

unlike many diseases that tend to afflict low SES and poverty stricken groups, anemia is not limited only to these groups and can be found across all SES groups.

Chapter 2

REVIEW OF THE LITERATURE

Health risks of anemia

In the aging population anemia can have a varying degree of consequences, specifically related to morbidity and mortality. In one study of adults 65 years and older, Thein et al. found that anemia was associated with greater fatigue, lower handgrip strength, an increased number of disabilities, and more depressive symptoms.[18] Penninx et al. found that in a cohort of community-dwelling adults aged 65 years and older, those who presented with anemia performed worse in three timed function tests of standing balance, five repetitions of sitting and rising from a chair, and an 8-foot walk. These anemic individuals had more disabilities, lower muscle strength, and poorer physical performance than those who were non-anemic.[19] Several studies found that as concentration of hemoglobin increased, so did performance and ability;[18,20] another study found that treatment with epoetin alfa led to an increase in hemoglobin concentration, which in turn was associated with improvements in fatigue and the quality of life scores.[21] Anemia was also found to be associated with increased risk of recurrent falls, which is a critical risk factor affecting the quality of life of the elderly.[22] As well as all of these factors contributing to performance and ability, anemia was also associated with the increased risk for hospitalization.[23,24]

Penninx and colleagues found that patients who were anemic not only had an increased risk for hospitalization, but also had more frequent and longer hospital stays.[24]

Not only does anemia greatly affect the morbidity of individuals, but it has also been shown to increase the risk of mortality. After adjusting for age, sex, diabetes mellitus, and co morbidities, Culleton et al. found that anemia was associated with an increased risk of death in a large cohort of adults 66 years and older.[23] Chaves et al. found that the risk of mortality decreased with higher concentrations of hemoglobin until reaching the threshold of 13.9 g/dL.[25] A study of adults 71 years and older,[24] a study of adults 65 years and older,[9] and a study of adults 85 years and older, [26] all found similar results; anemia was associated with increased risk of mortality in adults.

Causes of anemia

Anemia has three main causes; blood loss, lack of RBC production, and high rates of RBC destruction.[1] The most common of these three is blood loss. Blood loss can be the result of menstruation, bleeding in digestive or urinary tract, surgery, trauma, or even cancer. RBC production can be influenced by diet, hormones, pregnancy, and some chronic diseases, as well as some inherited conditions such as aplastic anemia.[1] Some conditions such as sickle cell anemia, thalassemias, a diseased or enlarged spleen, or hemolytic anemia can result in the destruction of RBCs. These factors result in a wide variety of etiologies that may result in anemia.

According to data from the NHANES III survey, one-third of anemia was a result of a nutrient deficiency; one-third was attributed to chronic inflammation,

chronic kidney disease, or both; and one-third was unexplained anemia (UA).[7] Many other studies have found evidence to support this as well. In a study by Artz and colleagues, it was found that of 174 adults aged 65 years and older, that 25% of anemia was due to iron deficiency, 10% was anemia of chronic inflammation, 7.5% was anemia of hematologic malignancy, and 44% remained unexplained.[27] In another study of disabled women 65 years or older, anemia was once again associated with nutritional deficiencies, chronic inflammation, renal insufficiencies, and unexplained anemia.[28] When observing mild anemia in the elderly, another study found that chronic disease, thalassemia, and renal insufficiency were the most frequent types of mild anemia, but almost one third of the anemia were still unexplained.[29] Together, these studies reveal that many factors may play a role in the incidence of different types of anemia, and not all etiologies of anemia can readily be deciphered.

Nutritional anemia

According to the WHO technical report of 1968, nutritional anemia (NA) is defined as "a condition in which the hemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency."[3] NA can result from the inadequate intake of many vitamins and minerals. The nutrient deficiency most commonly associated with NA is iron. [7,30-32] Although iron is the most common contributing factor to nutritional anemia, other vitamins such as vitamins A, B_{12}, and C, folic acid, and riboflavin status have been shown to be associated as well. NHANES III found that of the anemia with nutrient deficiencies, 48.3% were deficient only of iron, 18.8% were deficient only of

folic acid, 17.2% only of vitamin B_{12}, 5.8% was deficient of folic acid and B_{12}, and 9.9% were deficient of all three nutrients.[7] Given these findings, this study will focus on anemia attributed to deficiencies of iron, folate, and vitamin B_{12}.

Iron deficiency anemia (IDA) is the most common cause of NA. IDA occurs when there are inadequate amounts of RBCs due to a lack of iron.[33] Iron is a nutritionally essential element, and can be found in a wide variety of food sources. While liver and other organ meats are particularly high in iron, the most common food sources of iron are red meats, oysters and clams, dark green leafy vegetables, dried fruits, and beans.[34] Iron plays a crucial role in oxygen transportation, cellular metabolism, and cell growth and differentiation.[35]

The development of iron deficiency occurs in stages; the first stage is the depletion of iron stores in the liver, spleen, and bone marrow, followed by a marked decrease in transport of iron to tissues, and finally the depletion of iron-containing proteins and enzymes, which results in IDA.[34] IDA is characterized by microcytic RBCs with a reduced amount of hemoglobin (hypochromic).[34] The impaired delivery of oxygen to the tissues can cause symptoms such as weakness, fatigue, poor concentration, and poor work productivity.[36-40] WHO estimates that over 800,000 deaths worldwide can be attributed to IDA and this disease remains among the 15 leading contributors to the global burden of disease.[41]

In addition to inadequate iron intake, excessive losses of iron, inadequate absorption, and increased iron requirements during growth can contribute to iron deficiency. Blood loss, specifically due to chronic gastrointestinal blood loss, is the

primary cause of iron deficiency in the adult population.[42,43] Several vitamins have been suggested to play a role in iron metabolism. It has been proposed that vitamin A enhances the mobilization of iron delivery and increases plasma iron and transferrin saturation, thereby increasing iron supply to hematopoietic tissue.[44] Deficiency of vitamin A may alter absorption, storage, release, or transport of iron to the marrow, contributing to a possible decrease in RBC production. [45] Vitamin C has been shown to enhance the absorption of non-heme iron, which is much more common in food sources but also less bioavailable.[46] Vitamin C may also counteract the inhibition of iron absorption imposed by dietary tannins or phytates[46] as well as protect against hemolysis caused by oxidative damage.[44] Several studies have shown that riboflavin can improve hematological status and enhance the effects of iron supplementation.[44] Deficiency of riboflavin may decrease mobilization and absorption of iron while increasing iron losses.[47]

Unlike IDA, folic acid and vitamin B_{12} deficiencies result in megaloblastic anemia. A deficiency of either of these vitamins is difficult to differentiate from each other and both result in the impairment of DNA synthesis.[48] The occurrence of anemia due to these deficiencies is far less common than IDA. The primary cause of folic acid deficiency is inadequate intake of folate rich foods.[49] Due to fortification in industrialized nations, deficiency of this vitamin is relatively rare. Another common cause of folate deficiency is excess alcohol intake. Excess alcohol intake can effect folate consumption, absorption, and storage and therefore contribute greatly to folate status.[49]

Vitamin B_{12} deficiency is common in elderly individuals and is prevalent even in wealthy, industrialized countries. The main causes of B_{12} deficiency are inadequate intake and malabsorption.[50] In the elderly, malabsorption is the primary cause of deficiency. With aging, there is often a deterioration of the gastric mucosa and diminished production of gastric acid, which is critical for releasing vitamins from food during metabolism.[50] Medications such as proton pump inhibitors and H2-receptor antagonists may also contribute to malabsorption. These medications are commonly taken for gastric ulcers or gastroesophageal reflux disease, and inhibit secretion of gastric acid, pepsin, and in some cases, intrinsic factor.[49] The decrease in these secretions may result in the reduction of vitamin release from foods and subsequently, vitamin absorption. A deficiency in either folic acid of vitamin B_{12} may lead to development of anemia, and dietary intake, alcohol consumption, and medication use should be considered when assessing the risk for anemia as well as the cause of anemia.

Anemia of Inflammation

AI is also known as anemia of chronic inflammation or anemia of chronic disease. AI is characterized by low serum iron and low iron binding capacity while serum ferritin remains at a normal or elevated concentration.[51] This anemia is most commonly associated with underlying conditions such as infection, chronic illness, malignancy, and rheumatologic disorders.[52] One factor that may be involved in the development of AI is hepcidin, a peptide responsible for regulating iron metabolism.[35,53] When inflammation is present, inflammatory cytokines, specifically

interleukin-6 (IL-6), up regulate hepcidin.[54,55] Hepcidin inhibits iron absorption, iron transportation, and iron release, which dramatically limits the delivery of iron to the bone marrow, therefore increasing the risk of anemia.[54,56]

Leptin may also play a role in the up-regulation of hepcidin. Leptin is an adipokine associated with inflammation, body fat mass, and energy metabolism. The evidence that suggests that leptin up regulates hepcidin provides a possible link (although not significant) between obesity, inflammation, and iron status.[52,57] Obesity causes chronic inflammation and is associated with the expression of proinflammatory cytokines (IL-6) and the increased production of leptin. These two factors both have been shown to lead to the up-regulation of hepcidin which in turn inhibits iron absorption; providing evidence to indicate that obesity may be an important factor contributing to AI.[58]

C - reactive protein (CRP), a general marker of systemic inflammation, may play a part in anemia as well. It is thought that increased CRP activity due to inflammation may inhibit erythropoiesis (RBC production) by reducing iron availability.[59] As with the blood markers of anemia indicated earlier, CRP levels may be influenced by race, gender and SES. Alley et al. found that African Americans as well as women were more likely to have high levels of CRP.[60] Another study found that CRP, as well as the important hepcidin regulator IL-6, is inversely associated with SES.[61] Importantly, it has been found that within the HANDLS population, CRP levels are inversely related to diet quality assessed by a micronutrient index score.[62] Thus, inflammation as indicated by CRP levels may be influenced by racial

differences, SES, and diet quality, and subsequently these influential factors may increase the risk for AI.

Other chronic diseases such as atherosclerosis and diabetes mellitus are also associated with AI, and evidence suggests that this may largely be due to elevated serum pro-inflammatory cytokines.[63-65] Although these chronic diseases can be the result of many differing etiologies, often times they can be attributed to modifiable behaviors, namely, the diet.[66-69]

Unexplained anemia

As previously stated, about one-third of anemia is the result of an unknown or unexplained etiology.[7] Many hypotheses about what is the underlying cause of UA exist, some of which include sex hormone deficiency,[70] myelodysplastic syndromes, and a deficiency of erythropoietin even in the absence of renal dysfunction.[71] More studies must be done to investigate the possible causes for these unexplained cases.

In summary, there are many possible causes of anemia. One well known cause is specifically related to nutritional deficiencies. NA is most often related to iron deficiency, as well as folate or B_{12} deficiencies, but as we have mentioned above, many other nutrients including vitamin A, vitamin C, and riboflavin have also been related to iron metabolism and thus, also to anemia. Many studies have focused specifically on the deficiency of one particular nutrient while neglecting to look at the overall quality of the diet. Considering humans do not consume single nutrients at a time, it may be more beneficial to investigate the nutrient-to-nutrient interactions that result from the combination of many nutrients within many food types.[72] Examining

diet quality would not only allow for a new perspective concerning NA, but perhaps also for AI and UA.

Chapter 3

PURPOSE OF THE STUDY

The objectives of this study are the following:

1. The primary aim of this study it to characterize the types of anemia present in an urban population of socioeconomically diverse African American and White adults.
2. The secondary aim is to determine if diet quality is associated with each type of anemia.

-We hypothesize that individuals with the lowest quality diets based of the HEI-2010 scores will have higher risk for nutritional anemias, AI and unexplained anemia.

Chapter 4

METHODS

HANDLS Study/ Population

Data for analyses came from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. The HANDLS study is a longitudinal population-based cohort study. This study was designed to assess the relationship that socioeconomic status (SES) and race may have with health disparities in minority and low SES groups.[73] The cohort consisted of African American and white adults 30-64 years of age living in the city of Baltimore, Maryland from 2004 to 2009. Participants were drawn from 13 neighborhoods in Baltimore and sampled by using a factorial cross of four factors; age, sex, race, and SES.[73] All participants were required to read and sign an informed consent form (Appendix A).

HANDLS baseline data collection included 2 parts.[74] Phase one was a household survey conducted in the participant's home. Participant background, demographic information, education experience, occupational status, household income, physical activity, and health status were ascertained during this interview. A 24-hour dietary recall was also conducted. In the second phase, participants came to Medical Research Vehicles (MRV) for an examination. This examination included a medical history survey, a physical exam, blood tests, cognitive testing, body

composition measurements, physical performance, and several other procedures. A second dietary recall was conducted during this phase.

This study included only those participants with two 24-hour dietary recalls and all blood markers of anemia from baseline of the HANDLS study. Participants with CKD were excluded due to the fact that the anemia would be because of the disease state and not due to diet quality. Those with sickle cell disease were also excluded. No other exclusions were made.

24-hour dietary recall

The USDA Automated Multiple Pass Method (AMPM) was used to conduct two 24-hour dietary recalls. The AMPM involves five steps designed to provide cues and prompt thorough recall for all foods and drinks consumed throughout the previous day.[75] Both interviews were conducted by a trained-interviewer. An illustrated Food Model Booklet as well as other visual measurement aids was used to increase the accuracy of food and drink quantity estimates. Each recall was then coded using USDA Survey Net data processing system to match the foods with codes in the Food and Nutrient Database for Dietary Studies version 3.0.[76]

Diet Quality Assessment

To assess diet quality the Healthy Eating Index (HEI)-2010 was calculated from both days of dietary recalls. The HEI was used to measure diet quality by assessing conformance to federal dietary guidelines.[77] HEI-2010 scores were calculated for each recall day (day 1 and day 2) and then averaged to obtain the mean HEI-2010 total and component scores. The HEI-2010 is composed of 12 categories

that determine adequacy and moderation of the diet (Appendix B). A score is given for each category and then these individual scores are summed for the total HEI score; the maximum score is 100, representing the ideal diet. For a detailed description of the procedure used for HEI score calculation, visit the HANDLS website (http://handls.nih.gov/06Coll-w01HEI.htm) (Appendix C). Only the total score was used in analysis.

Assessment of Anemia

Fasting blood samples were used for anemia diagnosis. General anemia was defined using the WHO standards; hemoglobin levels less than 13 g/dL in men and less than 12 g/dL in women. Using fasting measures of hemoglobin, serum ferritin, transferrin saturation, and serum B_{12} and folate concentrations, anemia was categorized into 3 groups: nutritional anemia (iron, folate, Vitamin B_{12}), anemia of inflammation or unexplained anemia. The values used for anemia diagnosis can be found in Appendix D.

Independent Variables

Variables such as age, sex, race, SES, handgrip strength, CRP, and other health related variables were collected during an audio questionnaire, the medical history exam, and the physical performance examination.

SES predictors include self-reported household income less or greater than 125% of the 2004 Health and Human Services poverty guidelines (Poverty Income Ratio [PIR]) and literacy level. Within the HANDLS population, literacy has been found to be significantly associated with diet quality, and thus is an important variable

to be used in this analysis of diet quality and anemia (Kuczmarski, Marie F.; in press). Literacy was assessed using the reading subtest of the Wide Range Achievement Test-3rd Edition (WRAT-3). WRAT-3 measures the ability to recognize and name letters and words. The total WRAT-3 Reading score is the sum of total correctly pronounced letters and total correctly pronounced words, and serves as the literacy measurement. The total WRAT-3 score has been converted to grade level equivalents.[78]

Handgrip strength, which is used as an indicator of total- body muscle strength and physical performance, serves as an indicator of overall nutritional status.[79] Handgrip strength was assessed using the Jamar Hydraulic Hand Dynamometer. The hand dynamometer registers the maximum kilograms of force per trial. Two trials were conducted with both the right and left hand. The mean of the two trials by the dominant hand will be used in this analysis.

During the medical history exam, information on diseases that could impact anemia status was obtained. The disease states were cardiovascular disease, diabetes, thyroid disorders, arthritis, Parkinson's disease, and cognitive impairment. Upon examination of the prevalence of these diseases among the study participants, it was found that there were only three participants with cognitive impairment and another three participants with Parkinson's disease, thus, these two disease states were dropped from analysis.

Statistical Analyses

Means and standard deviations for continuous variables and percentages for categorical variables were computed to describe the demographic characteristics and

determine the prevalence of anemia. Participants were divided into four groups; women with anemia, women without anemia, men with anemia, and men without anemia. In order to assess statistically significant differences across these four groups, one-way analysis of variance (ANOVA) tests for continuous variables and chi-square tests for categorical variables were conducted. Pearson correlation coefficients were computed for continuous variables and Spearman's correlation coefficients were computed for categorical variables to assess associations between anemia and demographic characteristics.

Logistic regression models were fit to examine the association of HEI-2010 scores and anemia. Anemia defined as either general, NA, AI or UA, was the dependent variable and diet quality as total HEI-2010 was one of the independent or predictor variables. Covariates included in the model were sex, race, <125%PIR, literacy [total WRAT score], body mass index (BMI), handgrip strength, and smoking status. These models were first run with HEI-2010 scores as a continuous variable. Tertiles of HEI-2010 scores were then generated and the logistic regression models were fit again with HEI-2010 as a categorical variable. The results of the logistic regression are reported as odds ratios (OR) and 95% confidence intervals (95% CI). Effect modification of the HEI-2010 score-anemia association by race was investigated by the inclusion of a race by HEI-2010 score interaction term within the logistic regression model.

All statistical analyses were conducted using SPSS Statistics Version 22. Statistical significance was set at $p<0.05$.

Chapter 5

RESULTS

Sample Population

Of the 1,977 HANDLS participants included in this sample, 56.3% were women (n=1109); and 44% were men (n=862)(Table 1). The mean (±SE) age of the sample population was 47.8 (±0.21) years. Over half (56.6%) of the population was black (n=1118). The average BMI was 29.8 kg/m^2, indicating that the population was overweight.[80] The mean (±SE) CRP of the population was 4.27 (±0.20). After assessing SES predictors, it was found that 42.1% of the population was below 125% PIR, and 26.7% of the population was receiving food assistance. Approximately 48% of the population described themselves as current smokers. The mean (±SE) total WRAT score for the population was 42.3 (±0.19), with 35.9% of the population having the equivalent of less than an 8th grade education literacy level (Table 1).

Characteristics of Participants with Anemia

As presented in Table 1, 305 (15.4%) of the 1,977 participants were identified as anemic based on the criteria presented in Appendix 1. Of the 305 anemic participants, 68.5% (n=209) were women while 31.5% (n=96) were men. The majority of those that were anemic were African American (81.6%) compared to Whites (18.4%). Based on chi square and one-way ANOVA analyses, participants

with anemia were significantly different from participants who did not have anemia for the following characteristics; sex, race, BMI, <125 PIR, smoking status, CRP, handgrip strength, total WRAT score, and grade literacy level. The only demographic variable not found to be significant was age (Table 1).

Table 2 provides descriptive information about participants categorized by sex. Of the 1,115 women, 209 had anemia. Women who were anemic were more likely to be African American, were significantly younger, and had significantly higher BMIs and CRP levels than non-anemic women. Additionally, a higher proportion of anemic women had incomes <125% PIR and received food assistance. Anemic women had significantly lower total WRAT scores and were more likely to have a literacy level equivalent to less than an 8th grade level. Smoking was more prevalent among women without anemia than those with anemia.

Of the 862 men, 96 had anemia. Similar to women, men with anemia were more likely to be African American, were more likely to have a literacy level equivalent to that of less than the 8th grade, and had significantly higher CRP levels than men without anemia. In contrast to women, men with anemia were older and had significantly lower hand grip strength than men without anemia.

Individuals diagnosed with anemia were classified into one of three categories; namely, nutritional anemia, anemia of inflammation, or unexplained anemia. Of the 305 anemic men and women, significantly more participants were diagnosed with anemia of inflammation (47.5%) than unexplained anemia (29.8%) or nutritional anemia (22.6%)(Table 3). Significantly more women were diagnosed with nutritional

anemia and unexplained anemia than men, while significantly more men were diagnosed with anemia of inflammation (Table 3).

Diet Quality and anemia status

As shown in Table 4, there were no significant differences between dietary quality as evaluated by mean HEI-2010 scores of the total sample who are anemic and those who are not anemic. The same results were found for both men and women.

When HEI-2010 scores were distributed over tertiles, there was a statistically significant increase in mean HEI-2010 score between each tertile for general anemia as well as each category of anemia. However, there was no significant difference in the prevalence within tertiles for anemia categories.

Characteristics found to be significantly associated with general anemia (Tables 1 and 2) were included in analysis of correlation coefficients (Table 6). Being African American, a woman, having a literacy level less than that of the 8^{th} grade, having a high BMI, having high CRP levels and being <125% PIR were all found to have significant positive correlations with the prevalence of anemia (Table 6). Handgrip strength and smoking were found to have significant negative correlations with anemia. To avoid multicollinearity, total WRAT scores and receiving food assistance were not included in subsequent regression analysis.

Of all six disease states examined, only diabetes mellitus was positively correlated with anemia (Appendix E) as well as being a significant predictor of general anemia (OR = 1.65; $p<0.01$) (Appendix F).

Based on unadjusted regression analysis; sex ($p<0.01$), race ($p<0.01$), poverty status ($p<0.01$), literacy ($p<0.01$), CRP ($p<0.01$), smoking status ($p<0.05$), hand grip strength ($p<0.01$), and BMI ($p<0.01$) were statistically significant predictors of general anemia (Table 7). After adjusting for sex, race, poverty status, literacy, BMI, hand grip strength, and smoking status [Model 2], only race and handgrip strength remained significant (Table 8). Diet quality as evaluated by HEI-2010 scores was not a predictor of general anemia when scores were examined as a continuous variable, or as categorical tertiles (Table 9). HEI-2010 scores remained insignificant when adjusted for sex, race, poverty status, literacy, BMI, handgrip strength, and smoking status.

Interaction effects were conducted on predictor variables, and race was found to have a statistically significant interaction effect with HEI-2010 scores (data not shown). Logistic regression models found a significant association between anemia and race in an unadjusted model (OR= 4.61; 95% CI=3.14, 6.78), as well as in the adjusted model (Table 10). Due to the interaction between anemia and race, logistic regression models were fit to African Americans and Whites separately to evaluate if race effected the association between anemia and diet quality. These logistic regressions revealed the association of diet quality measured by the HEI-2010 score and anemia was insignificant (Table 11 and 12).

Chapter 6

DISCUSSION

The our knowledge, this is the first study to investigate the association between diet quality as evaluated by HEI-2010 scores on overall anemia prevalence as well as the different categories of anemia in a socioeconomically diverse urban population. Approximately 15% of the HANDLS baseline study participants examined in this research were diagnosed with anemia; roughly half had AI, while UA was seen in 3 of 10 and NA in 2 in 10 participants. This finding is different from that reported in NHANES III, which had a relatively even distribution of about one-third for each category of anemia.[7]

The mean± SE total HEI-2010 score for the study population was 42.6±0.26. This is more than 10 points less than that of the average HEI-2010 scores found in the What We Eat in America, National Health and Nutrition Examination Survey (WWEIA-NHANES), 2007-2008- a nationally representative survey.[81] Total HEI-2010 scores were not found to be associated with prevalence of general anemia, either as a continuous variable or when distributed over tertiles. This result was also found for all categories of anemia. One factor that may contribute to the lack of findings may be the small variance in HEI-2010 scores among the HANDLS study population. The majority of the HANDLS population was not consuming a high-quality diet as suggested by the mean (±SE) HEI-2010 score of 42.6± 0.26; out of 100 maximum

points. The total HEI score is most useful when comparing very low diet quality score to very high diet quality scores.[82] Thus the lack of results may be due to the homogeneity of the diet quality scores.

Additionally, only the total HEI-2010 scores were used in analysis. The HEI-2010 is composed of 12 categories that determine adequacy and moderation of the diet.[77] A score is given for each category. It would be interesting to use each individual component in analysis to determine if any specific HEI component has an association with anemia. Components such as total-protein foods, seafood and plant proteins, and greens and beans contain iron-rich foods. Evaluating these components may reveal differences in iron consumption between those who are anemic or not anemic. Additionally, assessing the fruit and vegetable components may reveal differences in consumption that would allow for comparison of vitamin and mineral intake.

The HEI was developed as a way to assess diet quality at a specific point in time.[83] It is also possible, that no association was found because HEI-2010 only captures recent compliance to dietary guidelines while anemia is a health condition that reflects long-term dietary patterns. For this reason a nutrient based diet quality index-which can show the long term effect diet may have on nutrient status- may be more useful tool than the HEI-2010.

Among HANDLS participants, handgrip strength, literacy, BMI, and CRP were significantly associated with anemia. Handgrip strength is used as an indicator of total-body muscle strength, which can serve as an indicator of overall nutritional

status.[79] Thus, handgrip strength has been used as a tool in the analysis of diet quality.[84] In this study, participants diagnosed with general anemia had significantly lower handgrip strength than those without anemia; this remained true in male participants after review of sex specific analysis as well. Several other studies have found similar results, with anemia resulting in decreased handgrip strength.[18,19] However, these studies did not include any measure of diet quality in analysis. These studies clearly link handgrip strength to nutritional status and anemia, but no study was found documenting an association between specifically studying handgrip strength and an actual measure of diet quality, specifically HEI-2010 scores. The association between handgrip strength and anemia indicates that nutritional status which reflects diet quality, anthropometry, clinical and biochemical measures may be more important than diet alone.

Within the literature, there is evidence that literacy plays a role in diet quality. Several studies have found that low literacy levels were associated with poor food label comprehension, and lower food portion estimation accuracy.[85,86] Within the HANDLS population, literacy was associated with nutrient-based diet quality, [87] as well as significantly associated with diet quality of selected HEI-2010 component scores. (Kuczmarski, Marie F.; in press). This study found that lower literacy levels were associated with a higher prevalence of anemia. Since literacy appears to be associated with both diet and anemia in the same population, it seems likely that the link between diet and anemia may be nutrient or food group specific.

Within this study, a higher BMI was associated with a higher risk of general anemia. A possible link between obesity and anemia could be explained since being overweight or obese is accompanied by chronic inflammation. When inflammation is present, there is an increase in the production of leptin, an adipokine associated with inflammation, body fat mass, and energy metabolism.[57,58] Leptin in turn, up regulates hepcidin, which then inhibits iron absorption, transportation, and release, which limits the delivery of iron to the bone marrow.[54,56]

Despite the possible link between the inflammatory mechanisms and anemia; most studies have found there to be no association between overweight or obesity and anemia.[88-90] Ausk et al., found that those who were overweight or obese had the expected changes in serum iron, transferring saturation, and ferritin that accompany chronic inflammation, but they were not more likely to be anemic than normal weight individuals.[89] Although most studies have not found higher BMIs to be associated with increased anemia, the biological mechanisms associated with obesity support the idea that there could be a positive association. Since obesity is negatively associated with diet quality,[91,92] it is not unreasonable that there could be a link between anemia and diet quality.

CRP was significantly associated with general anemia and unexplained anemia in this study. It was interesting that CRP was not significantly associated with AI, which can explain why CRP is not used to diagnose AI. Within the HANDLS study, CRP levels were inversely related to diet quality assessed by a micronutrient index score.[62] These results could explain how diet may influence the prevalence of anemia.

The lack of association between HEI-2010 scores in this study could indicate that the HEI-2010 was not the most appropriate measure of diet quality.

Being African American, a woman, and having a lower income were found to be significantly associated with prevalence of anemia. These results are consistent with results of other investigators. Within this HANDLS sample, the ratio of African Americans to Whites with anemia was roughly 4:1. Both Dong and Zakai found anemia present in American blacks nearly three times as much as in American whites.[8,9] Although these results are consistent with other research, caution must be taken when interpreting the results.

Several studies have reported that hematocrit, mean corpuscular volume, transferrin saturation, and hemoglobin- all important biomarkers when diagnosing for anemia- are lower in blacks than in whites. [9-12] However, it was found that many of the blacks did not have the typical signs and symptoms or the increased morbidity and mortality that accompany anemia.[9,13] This could mean that although significantly more African Americans in the HANDLS population were diagnosed with anemia by the established study criteria, this classification may not be a true representation when applied clinically.

There are several strengths of this study. First, the study targets an understudied, large population of socioeconomically diverse African American and White individuals. Second, diet quality was based on two 24-hour dietary recalls which allowed for usual diet to be estimated.

There are some limitations of this study. Cross sectional data was used which limits the ability to make cause-and-effect conclusions concerning the observed associations. Another limitation is that only the total mean HEI-2010 scores were used in analysis; no analyses of HEI-2010 components were done. The individual component scores can provide valuable information about food intake, thus the total and component scores allow for a more in-depth evaluation of the diet.[82,93] Additionally, results of regression analysis were only conducted for general anemia, and not for NA, AI, or UA due to small sample sizes of persons with NA and UA. Finally, there is ample evidence to suggest a biological difference between white and black individuals which may affect anemia diagnosis as well as subsequent research results. Beutler and colleagues have proposed different values for lower limits of normal hemoglobin concentration based on race, but little else has been done to validate these values or implement them in anemia diagnosis.[14]

Conclusion

In conclusion, to the best of our knowledge, this is the first study to investigate the association between diet quality as measured by HEI-2010 scores and anemia. HEI-2010 scores were not associated with the prevalence of general anemia, nutritional anemia, anemia of inflammation, or unexplained anemia. Those who were diagnosed with general anemia were more likely to be African American, a woman, have lower income, have literacy levels less than that of the 8th grade, have elevated CRP levels, lower hand grip strength, and a higher BMI. Handgrip strength, literacy, BMI, and CRP- factors linked to diet quality or nutritional status, were associated with

prevalence of general anemia. Therefore, other diet quality indices may be more appropriate to explore the association between anemia and diet.

TABLES

Table 1: Characteristics of HANDLS study participants categorized by anemia status

Variables	**Total N=1977**	**Anemia N=305**	**No Anemia N=1672**	**P-value**
Sex; N(%) Women Men	 1115 (56.3%) 862 (44%)	 209 (68.5%) 96 (31.5%)	 906 (53.8%) 766 (46.2%)	**<0.001**
Age (years); $\bar{x}$±SE	47.8 ±0.21	47.58±0.57	47.79±0.23	0.713
Race; N(%) African American White	 1118 (56.6%) 859 (43.4%)	 249 (81.6%) 56 (18.4%)	 869 (52%) 803 (48%)	**<0.001**
BMI (kg/m^2); $\bar{x}$±SE	29.8 ±0.17	31.25±0.50	29.59±0.18	**0.001**
<125% PIR; N(%)	832 (42.1%)	150 (50.8%)	682 (40.8%)	**0.006**
Receiving Food Assistance; N(%)	443 (26.7%)	82 (31.7%)	361 (25.8%)	**0.049**
CRP (mg/L) ; $\bar{x}$±SE	4.99±0.23	8.87±1.03	4.27±0.20	**<0.001**
Current Smoker; N(%)	870 (48%)	114 (41.6%)	756 (49.1%)	**0.022**
Hand Grip Strength; $\bar{x}$±SE	35.1 ±0.43	32.11±0.62	35.58±0.49	**0.004**
Total WRAT scores (kg); $\bar{x}$±SE	42.3 ±0.19	40.69±0.46	42.53±0.20	**<0.001**
Grade Literacy, <8th grade; N(%)*	710 (35.9%)	139 (47.1%)	571 (35.1%)	**<0.001**

*determined by WRAT score
Abbreviations:
HANDLS= Healthy Aging in Neighborhoods of Diversity across the Life Span study
BMI= body mass index
PIR= poverty income ratio
CRP= C-reactive protein
WRAT= Wide Range Achievement Test

Table 2: Characteristics of HANDLS study participants by sex and anemia status.

	Total	**Women**			**Men**		
	N=1977	Anemia N=209	No Anemia N=906	p-value	Anemia N=96	No Anemia N=766	p-value
Age (years); $\bar{x}$±SE	47.8 ±0.21	46.19±0.7	48.03±0.31	**0.01**	50.61±0.91	47.5±0.33	**0.002**
Race; N(%) African American White	 1118 (56.6%) 859 (43.4%)	 169 (80.9%) 40 (19.1%)	 446 (49.6%) 454 (50.4%)	**<0.001**	 80 (83.3%) 16 (19.7%)	 432 (54.8%) 349 (45.2%)	**<0.001**
BMI (kg/m^2); $\bar{x}$±SE	29.8 ±0.17	33.2±0.62	30.89±0.28	**<0.001**	26.99±0.63	28.08±0.22	0.102
Poverty Index Ratio (PIR); N(%)* >125% PIR <125% PIR	 1145 (57.9%) 832 (42.1%)	 103 (49.3%) 106 (50.7%)	 510 (56.7%) 390 (43.3%)	0.053	 52 (54.2%) 44 (45.8%)	 480 (62.2%) 292 (37.8%)	0.129
Receiving Food Assistance, N(%)	443 (26.7%)	65 (36.5%)	204 (27%)	**0.012**	17 (21%)	157 (24.3%)	0.505
CRP (mg/L); $\bar{x}$±SE	4.99±0.23	8.39±0.81	5.05±0.29	**<0.001**	9.92±2.78	3.35±0.26	**<0.001**
Current Smoker, N(%)	870 (48%)	60 (32.4%)	376 (45.6%)	**0.001**	54 (60.7%)	380 (53.1%)	0.175
Hand grip Strength (kg) $\bar{x}$±SE	35.1 ±0.43	28.54±0.57	28.10±0.28	0.50	39.15±1.02	44.17±0.88	**0.048**
Total WRAT score; $\bar{x}$±SE	42.3 ±0.19	41.0±0.54	42.89±0.25	**0.001**	39.95±0.88	42.10±0.33	0.32
Grade Literacy; N(%)* <8th grade >8th grade	 710 (35.9%) 1212 (61.3%)	 94 (45.4%) 113 (54.6%)	 289 (33%) 588 (67%)	**0.001**	 45 (51.1%) 43 (48.9%)	 282 (37.6%) 468(62.4%)	**0.014**

Table 3: Prevalence of different types of anemia for HANDLS study participants by sex.

HANDLS participants diagnosed with anemia characterized by low hemoglobin	**Total N=305**	**Women N=209**	**Men N=96**
Nutritional Anemia, N(%)	69 (22.6)[§]	62 (29.7)[a]	7 (7.3)[a]
Anemia of Inflammation, N(%)	145 (47.5)[§‡]	78 (37.3)[a]	67 (69.8)[a]
Unexplained Anemia, N(%)	91 (29.8)[‡]	69 (33)[a]	22 (22.9)[a]

Within Columns: Same symbol within a column are significantly different at the level $p<0.05$.
Within Rows: Superscripts with the same letter within a row are significantly different at the level $p<0.05$.

Table 4: Mean (SE) HEI-2010 scores by anemia status and sex for HANDLS study participants

	Total Sample		**Anemia**		**No Anemia**	
	n	$\bar{x}$±SE	n	$\bar{x}$±SE	n	$\bar{x}$±SE
All	1977	42.6± 0.26	305	42.2±0.63	1672	42.7±0.28
Women	1115	43.3±0.36	209	42.8±0.79	906	43.5±0.40
Men	862	41.7±0.36	96	40.9±1.03	766	41.7±0.39

No significant differences were found.

Table 5: Mean (SE) HEI-2010 scores by type of anemia among anemic HANDLS study participants (n=305)

			HEI- 2010 Scores					
	Overall		1st tertile		2nd tertile		3rd tertile	
	N	$\bar{x}$±SE	%	$\bar{x}$±SE	%	$\bar{x}$±SE	%	$\bar{x}$±SE
General Anemia	305	42.21 ±0.63	33.4	31.00 ± 0.43^{ab}	34.1	41.17 ± 0.27^{ac}	32.5	54.87 ± 0.74^{bc}
Anemia Subtypes								
Nutritional Anemia	69	40.2 ±1.36	42	30.12 ± 0.86^{ab}	30.4	40.86 ± 0.62^{ac}	27.5	55.12 ± 1.53^{bc}
Anemia of Inflammation	145	43.4 ±0.93	27.6	31.02 ± 0.70^{ab}	36.6	41.31 ± 0.36^{ac}	35.9	55.12 ± 1.11^{bc}
Unexplained Anemia	91	41.79 ±1.09	36.3	31.74 ± 0.69^{ab}	33.0	41.24 ± 0.52^{ac}	30.8	54.23 ± 1.31^{bc}

Within Rows: Superscripts with the same letter within a row are significantly different at the level $p<0.05$.

Table 6: Spearman Correlation coefficients between anemia status and demographic variables.

Correlation between predictors and prevalence of anemia.	**Correlation Coefficient**	**p-value**
Sex; men	-.107	**<0.01**
Age, y	-.008	.713
Race; African American	0.216	**<0.01**
BMI (kg/m^2)	0.078	**<0.01**
<125% PIR	0.061	**<0.01**
CRP	0.163	**<0.01**
Current Smoker	-0.054	**<0.05**
Hand Grip Strength, kg	-0.075	**<0.01**
Grade Literacy, <8th grade*	.090	**<0.01**

*Determined by WRAT scores

Table 7: Prevalence of anemia, crude odds ratio confidence intervals (95%) according to demographic characteristics.

Demographic Characteristics	**N**	**Anemia**		**Crude Odds Ratio**	**95% CI**	**p-value**
		Yes N(%)	**No N(%)**			
Sex Women	1977	209(10.6)	906 (45.8)	0.535	0.41, 0.69	<**0.01**
Men		96 (4.9)	766 (38.7)			
Race African American	1977	249 (81.6)	869 (52)	4.109	3.03, 5.58	<**0.01**
White		56 (18.4)	803 (48)			
Poverty Status <125% PIR	1977	150 (49.2)	682 (40.8)	1.405	1.10, 1.79	<**0.01**
>125% PIR		155 (50.8)	990 (59.2)			
Grade Literacy <8th grade	1922	139 (47.1)	571 (35.1)	1.65	1.28, 2.12	<**0.01**
>8th grade		156 (52.9)	1056 (64.9)			
HEI-2010 Score; $\bar{x}$±SE	1977	42.21 ±0.63	42.70±0.28	0.99	0.98, 1.01	0.496
CRP; $\bar{x}$±SE	1896	8.87±1.03	4.27±0.20	1.04	1.02, 1.05	<**0.01**
Current Smoker Yes	1814	114 (41.6)	756 (49.1)	0.74	0.57, 0.96	<**0.05**
No		160 (58.4)	784 (50.9)			
Hand Grip Strength; $\bar{x}$±SE	1456	32.1 ±0.62	35.6±0.49	0.97	0.96, 0.99	<**0.01**
BMI; $\bar{x}$ ±SE	1975	31.25±0.5	29.59±0.18	1.03	1.01, 1.04	<**0.01**

Table 8: Adjusted odds ratio confidence intervals (95%) for prevalence of anemia according to demographic characteristics

Demographic Characteristics	**Model 1**			**Model 2**		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Sex; men	0.51	0.37, 0.71	**<0.01**	0.75	0.48, 1.15	0.19
Race; African American	4.64	3.13, 6.87	**<0.01**	4.86	3.27, 7.22	**<0.01**
<125 % PIR	0.92	0.66, 1.27	.598	0.92	0.66, 1.29	0.63
Grade Literacy Level; <8th grade	1.24	0.89, 1.72	0.20	1.19	0.86, 1.67	0.30
HEI- 2010 Scores	0.99	0.97, 1.00	0.115	0.99	0.97, 1.00	0.08
Current Smoker				0.92	0.65, 1.29	0.63
Hand Grip Strength				0.96	0.96, 1.00	**<0.05**
BMI				1.01	0.99, 1.03	0.56

Model 1: adjusted for sex, race, poverty status, literacy, and HEI-2010 scores
Model 2: model 1+ BMI, hand grip strength, and smoking status

Table 9: Logistic regression models of anemia related to HEI-2010 score tertiles.

Variable	Unadjusted (n=1977)		Model 1		Model 2	
	OR	**95 %CI**	**OR**	**95% CI**	**OR**	**95% CI**
HEI- 2010 tertiles						
1st tertile	1.00		1.00		1.00	
2nd tertile	1.07	0.71, 1.60	1.40	0.91, 2.14	1.45	0.93, 2.25
3rd tertile	1.10	0.73, 1.66	1.21	0.79, 1.86	1.24	0.81, 1.92

Model 1: adjusted for HEI, sex, race, poverty status, and literacy
Model 2: model 1+ BMI, hand grip strength, and smoking status

Table 10: Logistic regression models of anemia status based on race.

Variable	Unadjusted		Model 1		Model 2	
	OR	**95 %CI**	**OR**	**95% CI**	**OR**	**95% CI**
Race; African American	4.61	3.14, 6.78	4.72	3.18, 7.01	4.95	3.32, 7.37

Model 1: adjusted for HEI, sex, poverty status, and literacy
Model 2: model 1+ BMI, hand grip strength, and smoking status

Table 11: Logistic regression models of anemia status based on HEI-2010 scores of White HANDLS participants.

	Unadjusted (n=859)		**Model 1**		**Model 2**	
	OR	**95 %CI**	**OR**	**95% CI**	**OR**	**95% CI**
HEI- 2010 Scores	0.97	0.93, 1.00	0.97	0.93, 1.00	0.97	0.93, 1.00

Model 1: adjusted for HEI, sex, poverty status, and literacy
Model 2: model 1+ BMI, hand grip strength, and smoking status

Table 12: Logistic regression models of anemia status based on HEI-2010 scores of African American HANDLS participants.

	Unadjusted (n=1118)		**Model 1**		**Model 2**	
	OR	**95 %CI**	**OR**	**95% CI**	**OR**	**95% CI**
HEI- 2010 Scores	1.01	0.99, 1.02	1.00	0.99, 1.02	1.00	0.98, 1.02

Model 1: adjusted for HEI, sex, poverty status, and literacy
Model 2: model 1+ BMI, hand grip strength, and smoking status

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Appendix A

HANDLS PARTICIPANT CONSENT FORM

MedStar Research Institute

Informed Consent for Clinical Research – HANDLS Phase 1

SITE: Mobile Medical Research Vehicles (MRVs) -- 13 neighborhoods in Baltimore City

PRINCIPAL INVESTIGATOR: Michele K. Evans, M.D. & Alan B. Zonderman, Ph.D.

Co-INVESTIGATORS: D.R. Abernethy, M. Brock, N. Ejiogu, K. Foster, M.C. Gibbons, J. Kelley-Moore, M.H. Kitner-Triolo, M.T. Fanelli Kuczmarski, T.A. LaVeist, J. Lepkowski, S. Ling, E. Nagababu, S. Najjar, N.R. Powe, J.M.Rifkind, J.F. Thayer, A. Trzeciak

INTRODUCTION

We invite you to take part in an observational research study called Healthy Aging in Neighborhoods of Diversity across the Life Span. You were selected as a possible participant in this study because we are looking for residents from your neighborhood between the ages of 30 and 64 years old. Please take your time to read this form, ask any questions you may have and make your decision. We encourage you to discuss your decision with your family and friends.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to learn about changes in health over time. We want to study as many people in different neighborhoods as we can. Our goal is to study health change as people grow older. We plan to do this by studying the same people over many years. This gives us the information we want about how peoples' bodies change over time. We also want to study why some people are healthier than others as they get older. We want to discover if we can predict the causes of good health with aging. If we can find the causes of good health, then we might find the cures for some of the diseases related to aging. This is an observational study where we will follow you over the next twenty years to see how you age. This will help us learn about the natural course of diseases like heart disease, Alzheimer's disease, hypertension, diabetes and stroke. We are trying to understand why some Americans have higher rates of certain diseases and more severe diseases than other Americans. This research is being done so that we can discover better ways to prevent and treat disease.

WHAT ELSE SHOULD I KNOW ABOUT THIS RESEARCH STUDY?

It is important that you read and understand several points that apply to all who take part in our studies:

- Taking part in the study is entirely voluntary and refusal to participate will not affect any rights or benefits you normally have;
- You may or may not benefit from taking part in the study, but knowledge may be gained from your participation that may help others; and
- You may stop being in the study at any time without any penalty or losing any of the benefits you would have normally received.

The nature of the study, the benefits, risks, discomforts and other information about the study are discussed further below. If any new information is learned, at any time during the research, which might affect your participation in the study, we will tell you. We urge you to ask any questions you have about this study with the staff members who explain it to you and with your own advisors before agreeing to participate.

WHO IS IN CHARGE OF THIS STUDY?

The research is being conducted and sponsored by the National Institute on Aging with Michele K. Evans, M.D. and Alan B. Zonderman, Ph.D. as the primary investigators.

WHO CANNOT PARTICIPATE IN THIS STUDY?

You cannot be in this study if any of the following apply to you;

If you:

- Do not have a valid picture ID
- Are unable to give informed consent
- Are under 30 years old
- Are older than 64 years old
- Are pregnant
- Are currently undergoing cancer treatment (chemotherapy or radiation)
- Have undergone cancer treatment (chemotherapy or radiation) within the last 6 months

WHAT IF I AM PRESENTLY PARTICIPATING IN ANOTHER RESEARCH STUDY?

Are you presently participating in any other research studies? Yes ☐ No ☐

If yes, please state which study(ies):_____

While participating in this study, you should not take part in any other research project without approval from the people in charge of each study. This is to protect you from possible injury arising from such things as extra blood drawing, extra x-rays, interaction of research drugs, or similar hazards.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About **4000** people will take part in this study, **around 335** from your neighborhood.

WHAT HAPPENS IF I AGREE TO BE IN THE STUDY?

The study data will be collected in two parts. This is a consent form for the first part. You are required to give your consent for both parts.

This first part of the study consists of a household interview. This interview includes questions about your age, occupation, and neighborhood. We also want to know about your physical activities, use of dental and health services,

and stress that you might experience and how you deal with it. We will also ask you to remember all of the food that you ate in the past day. We will discuss the way the household interview will be conducted below.

In the second part of the study, you will spend a day at our Mobile Medical Research Vehicles (MRVs). While you are there, we will ask you for additional information and we will do additional tests. You will be asked about your medical history and you will receive a physical examination. We will ask what you ate during the last 24 hours. You will receive memory testing. We will also measure your emotions and heart rate changes, muscle strength, bone density and test for hardening of the arteries. We will also take blood, tissue and urine samples. You will be offered a test for HIV. If you decide to have the test, you will be asked to sign a separate consent form that explains the HIV testing procedures for the HANDLS study. You will also be asked to give a DNA sample by using a method called Buccal Mucosa smear. Before you agree to give the DNA sample you will be required to sign a separate consent that explains the procedures and risks of providing DNA samples. More details about the tests for the second part of the study are described in the attached **Consent Form Booklet**.

This first part of the study will take place in your home. We will ask you to answer questions about you and your physical activity, use of dental and health services, stress and how you deal with it and a few questions about your neighborhood. We will also ask you to complete a dietary recall questionnaire that asks you to remember what you had to eat and drink in the last 24 hours. We will use pictures to help you give us information about how much food and drink you had in the last 24 hours. We expect this household interview to take about 90 minutes.

All of the questionnaires collect information about our research. They are not designed to improve your health at this time. We perform these questionnaires free of charge. You may participate in both of the questionnaires, but you do not have to. You may stop any questionnaire after it starts. This will not affect your right to participate in this study. This is a longitudinal study. Our Mobile Medical Research Vehicles will return to your neighborhood every three years and we will ask you again at that time to participate in this study.

HOW LONG WILL I BE IN THE STUDY?

We think you will be in the study for the next 20 years because this is a longitudinal study that follows your health over time as you age. This is a study that provides long-term follow up. The study doctor or the National Institute on Aging may stop your participation in this study at any time without your consent. Any information (data) or blood collected until that point in time would remain part of the study. You can stop participating at any time. However, if you decide to stop participating in the study, we ask you to talk to the researchers first.

WHAT ARE THE RISKS AND SIDE EFFECTS OF THIS STUDY?

If you decide to participate in this study, you should know there may be risks. The risks for this study are minimal. The descriptions of the tests given on the Mobile Medical Research Vehicles include any risks and other possible side effects. They are also explained in the **Consent Booklet** under the Assessment of Risks section. For more information about risks and side effects, you should call the Principal Investigator, Michele K. Evans, M.D. at 410-558-8573.

As part of this study, you will be asked to sign a separate consent form to be in the part of this study involving genetic testing. Risks of genetic testing include the misuse of personal, genetic information. Although rare, misuse of such information has caused problems for persons related to employment, life, or health insurance benefits and right. There is a risk that being in a genetics study can cause psychological distress or tension with other family members. Although

there can be no absolute guarantees, every reasonable effort will be made to keep your personally identifiable information secret so that there will be no misuse. Even when the information is kept secret, if you are asked if you have ever been tested for a genetic disorder, answering "yes" could cause benefits to be denied or could cause other problems including discrimination.

ARE THERE ANY BENEFITS TO TAKING PART IN THE STUDY?

This study is not designed to provide direct benefits to any participants. If you agree to take part in this study, there may or may not be direct medical benefits to you. We hope the information learned from this study will benefit others in the future. There is no charge for any of the testing described. You may benefit by learning more about your health, or possibly from learning that you have a condition or problem. You will receive a Participant Report Package in the mail, with results of your visit to the MRVs. If the study doctor discovers any condition or problem, the information will be provided to you and your doctor, if you authorize it. To authorize the reporting of results to your physician you will need to sign a form called "Release of Medical Information". You will be asked to sign this form only if you want us to communicate with your physician. The study doctors do not provide medical treatment. The information gained from this research may benefit others in the future.

WHAT OTHER OPTIONS ARE THERE?

There are no other options associated with your participation in this study. You may choose either to participate or not to participate in this research. Taking part in this study is entirely voluntary. You may choose to withdraw from the study at any time.

WHAT ABOUT CONFIDENTIALITY?

Your personal health information (PHI) will be kept private to the extent allowed by law. You will not be identified by name in any publications resulting from this study. You will be asked to sign a separate form that will give permission to the investigator, the sponsor, and certain other people, agencies or entities to look at and review the records related to this study including your personal health information and the information discovered during this study. If you do not wish to sign this permission form you will not be allowed to participate in this study.

Personal Health Information (PHI) is stored in secure databases. These databases are password protected and maintained on a secure NIA/NIH system with access limited to authorized NIA staff. All NIA staff that has access to these databases has the proper training on patient confidentiality as well as the required Human Subject Protection Training. The system is administered using the security policies and regulations required by the National Institutes of Health consistent with the Health and Human Services Privacy Rule and HIPAA.

Organizations that may request, inspect and/or copy your research and medical records for quality assurance and data analysis include groups such as: the National Institute on Aging, Office of Human Research Protection, MedStar Research Institute, Institutional Review Board (IRB), Coda and Westat.

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this certificate the researchers cannot be forced to disclose information that may identify you, 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 you, 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.

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your 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 researcher from disclosing voluntarily, without your consent, information that would identify you 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 doctor may be required to report certain cases of abuse, neglect, or suicidal or homicidal intent to the appropriate authorities.

WILL I BE PAID FOR PARTICIPATING IN THIS STUDY?

You will be paid $100 for participating in this study. You will receive your payment in the form of an ATM debit card at the end of the MRV visit. **If you are unable to complete all of the tests you will receive a portion of the payment.** The ATM card will be activated before you leave the vehicle. You will be able to take the card to an ATM machine in your neighborhood to withdraw your payment. We will provide round-trip transportation from your home to our mobile testing center if you want it. We will serve a box breakfast and box lunch if you are participating in tests during mid-day. We will do our best to meet your dietary needs if you have any.

WHAT ARE THE COSTS?

You do not have to pay anything to be in this study. However, if taking part in this study leads to procedures or care not included in the study, it may lead to added costs for you or your insurance company. You will not be charged for any tests or procedures that are part of this research study.

WHAT IF I'M INJURED OR BECOME ILL DURING THE STUDY?

We will make every effort to prevent injuries and illness from being in the study. If you have any adverse experience resulting directly from the study, the National Institute on Aging *will* provide or pay for short-term medical care for any injury resulting from participation in research here as long as the costs are not covered by your medical or hospital insurance. You should not expect any one to pay you for pain, worry, lost income, or non-medical care costs that occur from taking part in this research study. No other form of compensation is available for any adverse experience. *The National Institute on Aging, National Institutes of Health, the Federal Government, the MedStar Research Institute, MedStar Health, CODA or Westat* do not have money set aside to repay you in case of injury.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

You have the right to be told about the nature and purpose of the study;
You have the right to be given an explanation of the exactly what will be done in the study and given a description of potential risks, discomforts, or benefits that can reasonably be expected;

You have the right to be informed of any appropriate alternatives to the study, including, if appropriate, any drugs or devices that might help you, along with their potential risks, discomforts and benefits;
You have the right to ask any questions you may have about the study;
You have the right to decide whether or not to be in the study without anyone misleading or deceiving you; and
You have the right to receive a copy of this consent form.

By signing this form, you will not give up any legal rights you may have as a research participant. You may choose not to take part in or leave the study at any time. If you choose to not take part in or to leave the study, you will not lose any of the benefits you would have received normally. We will tell you about new information that may affect your health, welfare, or willingness to be in this study.

WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact the investigator, Michele K. Evans, M.D., at (410)-558-8573. For medical assistance during the evening or on weekends, call the NIA Security Office at (410) 558-8119 and request that they contact the NIA Physician-on-Call.

If you are having a medical emergency, you should call 911 or go directly to the nearest emergency room.

If you are injured as a result of being in a study, or think you have not been treated fairly, please contact the NIA Clinical Director or Deputy Clinical Director at (410) 350-3922.

For questions about your rights as a research participant, you can call or write the following:

NIA Clinical Director
3001 S. Hanover Street, 5th Floor
Baltimore, MD 21225
Phone (410) 350-3922

NIA Clinical Research Protocol Office
3001 S. Hanover Street, Room 539
Baltimore, MD 21225
Phone: (410) 350-3947
Fax: (410) 350-3979.

MedStar Research Institute
Office of Regulatory Affairs
6495 New Hampshire Avenue, Suite 201
Hyattsville, MD 20783
Phone: (301) 560-7339
Toll Free: (800) 793-7175
Fax: (301) 560-7336

SIGNATURES

As a representative of this study, I have explained the purpose, the procedures, the possible benefits and risks that are involved in this research study. Any questions that have been raised have been answered to the individual's satisfaction.

Signature of Person Obtaining Consent _____ Date of Signature _____

I, the undersigned have been informed about this study's purpose, procedures, possible benefits and risks, and I have received a copy of this consent. I have been given the opportunity to ask questions before I sign, and I have been told that I can ask other questions at any time. I voluntarily agree to be in this study. I am free to stop being in the study at any time without need to justify my decision and if I stop being in the study I understand it will not in any way affect my future treatment or medical management. I agree to cooperate with Dr. Michele K. Evans, Dr. Alan B. Zonderman, and the research staff and to tell them immediately if I experience any unexpected or unusual symptoms.

Participant's Signature _____ Date of Signature _____

Signature of Witness _____ Date of Signature _____

Signature of Legally Authorized Representative (When Appropriate) _____ Date of Signature _____

Relationship to Participant (When Appropriate) _____ Date of Signature _____

IRB Approval Stamp
(ORP USE ONLY - DO NOT CHANGE ANY INFORMATION IN THIS SECTION)

NOV 0 8 2006
OCT 24 2006

Form Revision Date: 05/10/04

MedStar Research Institute

Informed Consent for Clinical Research – HANDLS Phase 2

SITE: Mobile Medical Research Vehicles (MRVs) -- 13 neighborhoods in Baltimore City

PRINCIPAL INVESTIGATOR: Michele K. Evans, M.D. & Alan B. Zonderman, Ph.D.

Co-INVESTIGATORS: D.R. Abernethy, M. Brock, N. Ejiogu, K. Foster, M.C. Gibbons, J. Kelley-Moore, M.H. Kitner-Triolo, M.T. Fanelli Kuczmarski, T.A. LaVeist, J. Lepkowski, S. Ling, E. Nagababu, S. Najjar, N.R. Powe, J.M.Rifkind, J.F. Thayer, A. Trzeciak

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We invite you to take part in an observational research study called Healthy Aging in Neighborhoods of Diversity across the Life Span. You were selected as a possible participant in this study because we are looking for residents from your neighborhood between the ages of 30 and 64 years old. Please take your time to read this form, ask any questions you may have and make your decision. We encourage you to discuss your decision with your family and friends.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to learn about changes in health over time. We want to study as many people in different neighborhoods as we can. Our goal is to study health change as people grow older. We plan to do this by studying the same people over many years. This gives us the information we want about how peoples' bodies change over time. We also want to study why some people are healthier than others as they get older. We want to discover if we can predict the causes of good health with aging. If we can find the causes of good health, then we might find the cures for some of the diseases related to aging. This is an observational study where we will follow you over the next twenty years to see how you age. This will help us learn about the natural course of diseases like heart disease, Alzheimer's disease, hypertension, diabetes and stroke. We are trying to understand why some Americans have higher rates of certain diseases and more severe diseases than other Americans. This research is being done so that we can discover better ways to prevent and treat disease.

WHAT ELSE SHOULD I KNOW ABOUT THIS RESEARCH STUDY?

It is important that you read and understand several points that apply to all who take part in our studies:

- Taking part in the study is entirely voluntary and refusal to participate will not affect any rights or benefits you normally have;
- You may or may not benefit from taking part in the study, but knowledge may be gained from your participation that may help others; and
- You may stop being in the study at any time without any penalty or losing any of the benefits you would have normally received.

The nature of the study, the benefits, risks, discomforts and other information about the study are discussed further below. If any new information is learned, at any time during the research, which might affect your participation in the

study, we will tell you. We urge you to ask any questions you have about this study with the staff members who explain it to you and with your own advisors before agreeing to participate.

WHO IS IN CHARGE OF THIS STUDY?

The research is being conducted and sponsored by the National Institute on Aging with Michele K. Evans, M.D. and Alan B. Zonderman,Ph.D. as the primary investigators.

WHO CANNOT PARTICIPATE IN THIS STUDY?

You cannot be in this study if any of the following apply to you;

If you:

- Do not have a valid picture ID
- Are unable to give informed consent
- Are under 30 years old
- Are older than 64 years old
- Are pregnant
- Are currently undergoing cancer treatment (chemotherapy or radiation)
- Have undergone cancer treatment (chemotherapy or radiation) within the last 6 months

WHAT IF I AM PRESENTLY PARTICIPATING IN ANOTHER RESEARCH STUDY?

Are you presently participating in any other research studies? Yes ☐ No ☐

If yes, please state which study(ies):_____

While participating in this study, you should not take part in any other research project without approval from the people in charge of each study. This is to protect you from possible injury arising from such things as extra blood drawing, extra x-rays, interaction of research drugs, or similar hazards.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About **4000** people will take part in this study, **around 335** from your neighborhood.

WHAT HAPPENS IF I AGREE TO BE IN THE STUDY?

The study data will be collected in two parts. This is a consent form for the second part. You are required to give your consent for both parts. The first part of the study included a household interview with questions about your age, occupation and neighborhood. We asked about your physical activity, use of dental and health services, and stress you may have experienced. We also asked you to complete a dietary recall questionnaire during the home visit.

This second part of the study will require you to spend a day at our Mobile Medical Research Vehicles (MRVs) to have testing. You will be asked about your medical history and you will receive a physical examination. We will ask you to remember all of the food you ate in the last day. We will measure your emotions and heart rate changes, muscle

strength, bone density and you will have a test for hardening of the arteries. We will also take blood, tissue and urine samples. You will be offered a test for HIV. If you decide to have the test, you will be asked to sign a separate consent form that explains the HIV testing procedures for the HANDLS study. You will also be asked to give a DNA sample by using a method called Buccal Mucosa smear. Before you agree to give the DNA sample you will be required to sign a separate consent that explains the procedures and risks of providing DNA samples. More details about the specific testing for this part of the study are described below and in the attached **Consent Booklet**.

The tests involved in this study are described in the attached **Consent Booklet**. All of the tests are performed for the purpose of research and are not designed to improve your health at this time. There are no experimental tests or procedures in this study. We perform these tests free of charge. If, after reading the **Consent Booklet**, there are tests in which you do not wish to participate, please list them on the back of this form.

Below is a chart that shows the tests you will be expected to complete today. This chart also tells you how long we think it will take each test to be done and in which vehicle it will be given. You will find more details about each test in the **Consent Booklet** that goes with this form.

Procedure	Where	Expected Time	Maximum Time
Tests of Heart Function: Carotid Doppler Ultrasonography, Pulse Wave Velocity, and EKG	MRV I	50 minutes	60 minutes
Bone Density and Body Composition Tests: DEXA Scanner, Weight and Body Measurements	MRV I	30 minutes	35 minutes
Muscle Strength Function Testing: Grip Strength, Chair Stand, and Balance Tests	MRV I	10 minutes	15 minutes
Blood, Tissue and Urine Sampling	MRV I	10 minutes	15 minutes
Medical History & Physical Examination	MRV I	50 minutes	60 minutes
Nutritional Dietary Recall	MRV I	20 minutes	30 minutes
Emotions and Heart Rate Testing	MRV II	45 minutes	55 minutes
Problem Solving and Memory Testing	MRV II	50 minutes	60 minutes
Audio-administered Questionnaire	MRV II	20 minutes	30 minutes

You may participate in any of the tests, but you do not have to participate in all of the tests. This will not affect your right to participate in this study. You may stop any test after it starts.

This is a long-term study, our Mobile Medical Research Vehicles will be back in your neighborhood every three years and we will ask you again at that time to participate in this study.

HOW LONG WILL I BE IN THE STUDY?

We think you will be in the study for the next 20 years because this is a longitudinal study that follows your health over time as you age. This is a study that provides long-term follow up. The study doctor or the National Institute on Aging may stop your participation in this study at any time without your consent. Any information (data) or blood collected until that point in time would remain part of the study. You can stop participating at any time. However, if you decide to stop participating in the study, we ask you to talk to the researchers first.

WHAT ARE THE RISKS AND SIDE EFFECTS OF THIS STUDY?

If you decide to participate in this study, you should know there may be risks. The risks for this study are minimal. The descriptions of the tests given on the Mobile Medical Research Vehicles include any risks and other possible side effects. They are also explained in the **Consent Booklet** under the Assessment of Risks section. For more information about risks and side effects, you should call the Principal Investigator, Michele K. Evans, M.D. at 410-558-8573.

As part of this study, you will be asked to sign a separate consent form to be in the part of this study involving genetic testing. Risks of genetic testing include the misuse of personal, genetic information. Although rare, misuse of such information has caused problems for persons related to employment, life, or health insurance benefits and right. There is a risk that being in a genetics study can cause psychological distress or tension with other family members. Although there can be no absolute guarantees, every reasonable effort will be made to keep your personally identifiable information secret so that there will be no misuse. Even when the information is kept secret, if you are asked if you have ever been tested for a genetic disorder, answering "yes" could cause benefits to be denied or could cause other problems including discrimination.

ARE THERE ANY BENEFITS TO TAKING PART IN THE STUDY?

This study is not designed to provide direct benefits to any participants. If you agree to take part in this study, there may or may not be direct medical benefits to you. We hope the information learned from this study will benefit others in the future. There is no charge for any of the testing described. You may benefit by learning more about your health, or possibly from learning that you have a condition or problem. You will receive a Participant Report Package in the mail, with results of your visit to the MRVs. If the study doctor discovers any condition or problem, the information will be provided to you and your doctor, if you authorize it. To authorize the reporting of results to your physician you will need to sign a form called "Release of Medical Information". You will be asked to sign this form only if you want us to communicate with your physician. The study doctors do not provide medical treatment. The information gained from this research may benefit others in the future.

WHAT OTHER OPTIONS ARE THERE?

There are no other options associated with your participation in this study. You may choose either to participate or not to participate in this research. Taking part in this study is entirely voluntary. You may choose to withdraw from the study at any time.

WHAT ABOUT CONFIDENTIALITY?

Your personal health information (PHI) will be kept private to the extent allowed by law. You will not be identified by name in any publications resulting from this study. You will be asked to sign a separate form that will give permission to the investigator, the sponsor, and certain other people, agencies or entities to look at and review the records related to this study including your personal health information and the information discovered during this study. If you do not wish to sign this permission form you will not be allowed to participate in this study.

Personal Health Information (PHI) is stored in secure databases. These databases are password protected and maintained on a secure NIA/NIH system with access limited to authorized NIA staff. All NIA staff that has access to these databases has the proper training on patient confidentiality as well as the required Human Subject Protection Training. The system is administered using the security policies and regulations required by the National Institutes of Health consistent with the Health and Human Services Privacy Rule and HIPAA.

Organizations that may request inspect and/or copy your research and medical records for quality assurance and data analysis include groups such as: the National Institute on Aging, Office of Human Research Protection, MedStar Research Institute, Institutional Review Board (IRB), Coda and Westat.

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this certificate the researchers cannot be forced to disclose information that may identify you, 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 you, 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.

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your 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 your consent, information that would identify you 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 doctor may be required to report certain cases of abuse, neglect, or suicidal or homicidal intent to the appropriate authorities.

WILL I BE PAID FOR PARTICIPATING IN THIS STUDY?

You will be paid $100 for participating in this study. You will receive your payment in the form of an ATM debit card at the end of the MRV visit. **If you are unable to complete all of the tests you will receive a portion of the payment.** The ATM card will be activated before you leave the vehicle. You will be able to take the card to an ATM machine in your neighborhood to withdraw your payment. We will provide round-trip transportation from your home to our mobile testing center if you want it. We will serve a box breakfast and box lunch if you are participating in tests during mid-day. We will do our best to meet your dietary needs if you have any.

WHAT ARE THE COSTS?

You do not have to pay anything to be in this study. However, if taking part in this study leads to procedures or care not included in the study, it may lead to added costs for you or your insurance company. You will not be charged for any tests or procedures that are part of this research study.

WHAT IF I'M INJURED OR BECOME ILL DURING THE STUDY?

We will make every effort to prevent injuries and illness from being in the study. If you have any adverse experience resulting directly from the study, the National Institute on Aging *will* provide or pay for short-term medical care for any injury resulting from participation in research here as long as the costs are not covered by your medical or hospital insurance. You should not expect any one to pay you for pain, worry, lost income, or non-medical care costs that occur from taking part in this research study. No other form of compensation is available for any adverse experience. *The National Institute on Aging, National Institutes of Health, the Federal Government, the MedStar Research Institute, MedStar Health, CODA or Westat* do not have money set aside to repay you in case of injury.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

You have the right to be told about the nature and purpose of the study;
You have the right to be given an explanation of the exactly what will be done in the study and given a description of potential risks, discomforts, or benefits that can reasonably be expected;
You have the right to be informed of any appropriate alternatives to the study, including, if appropriate, any drugs or devices that might help you, along with their potential risks, discomforts and benefits;
You have the right to ask any questions you may have about the study;
You have the right to decide whether or not to be in the study without anyone misleading or deceiving you; and
You have the right to receive a copy of this consent form.

By signing this form, you will not give up any legal rights you may have as a research participant. You may choose not to take part in or leave the study at any time. If you choose to not take part in or to leave the study, you will not lose any of the benefits you would have received normally. We will tell you about new information that may affect your health, welfare, or willingness to be in this study.

WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, contact the investigator, Michele K. Evans, M.D., at (410)-558-8573. For medical assistance during the evening or on weekends, call the NIA Security Office at (410) 558-8119 and request that they contact the NIA Physician-on-Call.

If you are having a medical emergency, you should call 911 or go directly to the nearest emergency room.

If you are injured as a result of being in a study, or think you have not been treated fairly, please contact the NIA Clinical Director or Deputy Clinical Director at (410) 350-3922.

For questions about your rights as a research participant, you can call or write the following:

NIA Clinical Director
3001 S. Hanover Street, 5th Floor
Baltimore, MD 21225
Phone (410) 350-3922

NIA Clinical Research Protocol Office
3001 S. Hanover Street, Room 539
Baltimore, MD 21225
Phone: (410) 350-3947
Fax: (410) 350-3979.

MedStar Research Institute
Office of Regulatory Affairs
6495 New Hampshire Avenue, Suite 201
Hyattsville, MD 20783
Phone: (301) 560-7339
Toll Free: (800) 793-7175
Fax: (301) 560-7336

SIGNATURES

As a representative of this study, I have explained the purpose, the procedures, the possible benefits and risks that are involved in this research study. Any questions that have been raised have been answered to the individual's satisfaction.

Signature of Person Obtaining Consent _____ Date of Signature _____

I, the undersigned have been informed about this study's purpose, procedures, possible benefits and risks, and I have received a copy of this consent. I have been given the opportunity to ask questions before I sign, and I have been told that I can ask other questions at any time. I voluntarily agree to be in this study. I am free to stop being in the study at any time without need to justify my decision and if I stop being in the study I understand it will not in any way affect my future treatment or medical management. I agree to cooperate with Dr. Michele K. Evans, Dr. Alan B. Zonderman, .and the research staff and to tell them immediately if I experience any unexpected or unusual symptoms.

Participant's Signature _____ Date of Signature _____

Signature of Witness _____ Date of Signature _____

Signature of Legally Authorized Representative (When Appropriate) _____ Date of Signature _____

Relationship to Participant (When Appropriate) _____ Date of Signature _____

IRB Approval Stamp
(ORP USE ONLY - DO NOT CHANGE ANY INFORMATION IN THIS SECTION)
MedStar Research Institute
APPROVAL DATE NOV 0 8 2005
EXPIRATION DATE OCT 24 2006
IRB ASSIGNMENTS
Form Revision Date: 05/10/04

IRB Approval Stamp
(ORP USE ONLY - DO NOT CHANGE ANY INFORMATION IN THIS SECTION)

NOV 0 8 2005
OCT 24 2006

Form Revision Date: 05/10/04

Appendix B

HEALTHY EATING INDEX-2010 COMPONENTS AND STANDARDS FOR SCORING

Component	Maximum points	Standard for maximum score	Standard for minimum score of zero
HEI-2010[1]			
Adequacy:			
Total Fruit[2]	5	≥0.8 cup equiv. per 1,000 kcal	No Fruit
Whole Fruit[3]	5	≥0.4 cup equiv. per 1,000 kcal	No Whole Fruit
Total Vegetables[4]	5	≥1.1 cup equiv. per 1,000 kcal	No Vegetables
Greens and Beans[4]	5	≥ 0.2 cup equiv. per 1,000 kcal	No Dark Green Vegetables or Beans and Peas
Whole Grains	10	≥1.5 oz equiv. per 1,000 kcal	No Whole Grains
Dairy[5]	10	≥1.3 cup equiv. per 1,000 kcal	No Dairy
Total Protein Foods[6]	5	≥2.5 oz equiv. per 1,000 kcal	No Protein Foods
Seafood and Plant Proteins[6,7]	5	≥0.8 oz equiv. per 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids[8]	10	(PUFAs + MUFAs)/SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 oz equiv. per 1,000 kcal	≥4.3 oz equiv. per 1,000 kcal
Sodium	10	≤1.1 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
Empty Calories[9]	20	≤19% of energy	≥50% of energy

[1]Intakes between the minimum and maximum standards are scored proportionately.
[2]Includes fruit juice.
[3]Includes all forms except juice.
[4]Includes any beans and peas (called legumes in HEI-2005) not counted as Total Protein Foods (called Meat and Beans in HEI-2005).
[5]Includes all milk products, such as fluid milk, yogurt, and cheese, and fortified soy beverages.
[6]Beans and peas are included here (and not with vegetables) when the Total Protein Foods (called Meat and Beans in HEI-2005) standard is otherwise not met.
[7]Includes seafood, nuts, seeds, soy products (other than beverages) as well as beans and peas counted as Total Protein Foods.
[8]Ratio of poly- and monounsaturated fatty acids to saturated fatty acids.
[9]Calories from solid fats, alcohol, and added sugars; threshold for counting alcohol is >13 grams/1000 kcal.

Appendix C

HEALTHY EATING INDEX 2010 CALCULATION

The basic steps in calculating the Healthy Eating Index (HEI)-2010 component and total scores for individual food intake are described at National Cancer Institute's Applied Research website, where the SAS code is also archived. For this study the code for HEI-2010 for 24-hour recalls was used.

For a complete food equivalent data set, the MyPyramid Equivalents Database (MPED), version 2.0, the USDA's Center for Nutrition Policy and Promotion (CNPP) MPED Database for Whole Fruit and Fruit Juice for NHANES 2003-04, and the CNPP Addendum 2.0B were first combined. The CNPP Addendum to the MPED, version 2.0B was used since it contained food codes reported NHANES 2005-06, the same time period data were collected in HANDLS study. Then these data were merged with HANDLS baseline individual food intake data to calculate participants' 1-day food-group intake. The number of equivalents per 100 grams of food was generated for the following food groups: Total Fruit, Total Vegetables, Dark Green Vegetables, Legumes, and Total Milk expressed in cup equivalents; Whole Grains, Meat, Poultry, and Fish, Eggs, Nut and Seeds, Soybean products, Fish and shellfish high in n-3 fatty acids, Fish and shellfish low in n-3, and Non-whole/refined Grains expressed in ounce equivalents; Discretionary solid fat expressed in grams; and Added sugars expressed in teaspoon equivalents. Foods containing fruit were assigned to

either Whole Fruit or Fruit Juice in cup equivalents per 100 grams of foods. For foods which contained both whole fruit and fruit juice, Total Fruit equivalents were assigned to either Whole Fruit or Fruit Juice, based on the majority ingredient according to its description or recipe USDA's FNDDS, Version 2.0. The data were reviewed by Registered Dietitian who assigned the appropriate food group for 53 items reported in the HANDLS study that did not match an 8-digit code in MPED and verified the assignment of missing total fruit items into either the whole fruit or fruit juice group.

In general, the initial step used to obtain the required variables to compute the components of the HEI-2010 as well as the total score, was to estimate each day's intake using the grams consumed of each food code that was assigned with either the FNDDS (per gram of food: grams×nutrient.gram-1) or MPED database (per 100 g of food: (grams×100×(equivalent per 100g)). Daily intake was obtained by summing the grams per day of food groups and nutrients across food codes but within individuals for each day of intake. Special preliminary calculations were required for selected groups, such as empty calories and soy beverages. For instance, in the HEI-2010 calories from alcohol are considered to be empty calories, but only when alcohol is consumed beyond moderate amounts. Soy beverages were assigned to the Dairy component of the HEI-2010. However, in the MPED soy beverages were grouped with other soy products in the Meats and Beans group. Detailed descriptions of essential modifications to calculations can be found at HEI Tools for researchers.

Since the HEI-2010 is a multi-dimensional construct involving 12 densities (amounts of food per 1,000 calories and ratios of fatty acids), the use of a Monte Carlo

simulation step was required for the calculation of standard errors. The SAS code for the generation of a Monte Carlo dataset was provided at the National Cancer Institute's Applied Research website.

Using the Monte Carlo data set, a SAS macro was run for the allocation of beans and peas. Legume intake was first allocated to Total Proteins group and once this standard was met, the additional intake was allocated to Total Vegetables, and Greens and Beans components. Then using the Monte Carlo data set with the beans and peas allocated, the HEI-2010 scoring macro was used to calculate densities for each HEI-2010 component and to apply the scoring algorithm. The total and component HEI-2010 scores were calculated for each recall day (day 1 and day 2) and then were averaged to obtain the mean HEI-2010 total and component scores for both days combined.

Appendix D

DIAGNOSIS OF ANEMIA

Type of Anemia	Blood Parameters	Diagnosis Criteria
General Anemia	Hemoglobin	Based on WHO criteria: Female; Hemoglobin<12g/dL Male; Hemoglobin<13g/dL
Nutritional Anemias*		
Iron deficiency	Serum Ferritin Transferrin Saturation (fesat)	Ferritin ≤12ng/mL & fesat ≤15%
B12 deficiency	Serum B_{12}	B_{12} <200 pg/mL
Folate deficiency	Serum Folate	Folate <4 ng/mL
Anemia of Inflammation	Serum Ferritin Transferrin Saturation (fesat)	Ferritin >12 & fesat >15%
Unexplained Anemia		Low hemoglobin but does not fit into any other category

Appendix E

CORRELATION COEFFICIENTS BETWEEN ANEMIA STATUS AND DISEASE STATES

Disease State	**Correlation Coefficient**	**P-value**
Cardiovascular	0.022	0.322
Diabetes	0.071	**<0.01**
Arthritis	0.017	0.494
Thyroid Disorder	0.037	0.141

Appendix F

PREVALENCE OF ANEMIA, CRUDE ODDS RATIO CONFIEDENCE INTERVALS (95%) ACCORDING TO DISEASE STATE

Health Status Variables	N	Anemia		Crude Odds Ratio	95% CI	p-value
		Yes N(%)	No N(%)			
Cardiovascular	1977			1.13	0.89, 1.45	0.322
Yes		167 (54.8)	864 (51.7)			
No		138 (45.2)	808 (48.3)			
Diabetes	1601			1.65	1.16, 2.30	**<0.01**
Yes		53 (21.9)	199 (14.6)			
No		189 (78.1)	1160 (85.4)			
Arthritis	1622			1.12	0.82, 1.53	0.493
Yes		63 (26)	331 (24)			
No		179 (74)	1049 (76)			
Thyroid	1601			1.43	0.89, 2.31	0.143
Yes		23 (9.5)	93 (6.8)			
No		219 (90.5)	1266 (93.2)			

Appendix G

MEAN (SE) CRP LEVELS BY TYPE OF ANEMIA

Type of Anemia	**CRP level $\bar{X}$±SE**
No Anemia	4.27±0.20[a]
Nutritional Anemia	6.27±1.08[b]
Anemia of Inflammation	5.32±0.76[c]
Unexplained Anemia	16.44±2.98[abc]

Superscripts with the same letter within a column are significantly different at the level $p<0.05$.