# THE EFFECT OF IRON STATUS ON THE RISK FOR DEVELOPING CARDIOVASCULAR DISEASE IN THE HEALTHY AGING IN NEIGHBORHOODS OF DIVERSITY ACROSS THE LIFE SPAN (HANDLS) STUDY SAMPLE

by
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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Human Nutrition.

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## ABSTRACT

*Background* – Over the past three decades there has been considerable and conflicting debate on the impact of iron mediated oxidative damage on coronary heart disease.

*Objective* - To determine if the baseline participants of the *Healthy Aging in Neighborhoods of Diversity across the Life Span* (HANDLS) study, are at risk for coronary heart disease (CHD), as determined by the Framingham Heart Study's risk equations, and, atherosclerosis, as measured by carotid intima-media thickness (IMT), due to variations in iron status. Thereafter, to identify whether race, and/or socioeconomic status impacts the iron status of participants.

*Design* – Participants (n = 1874) were from the HANDLS baseline sample, which included African American and white men and women ages 32-64 years old, living in Baltimore, MD and categorized by race, sex, and menopausal status, as well as socioeconomic status. Pearson correlation and stepwise regression analyses were used to determine the differences in iron status, 10-year risk for development of CHD, and IMT for these groups, in order to determine the impact of iron stores on heart disease.

*Results* – Stepwise general linear regression found serum ferritin was one of the most significant predictors ($P$ <0.0001) of 10-year CHD risk ($R^2$ =0.42). Although significant correlations were found between IMT and serum ferritin ($P$ <0.0001), further analysis

with regression modeling found serum ferritin was not a significant predictor of IMT variance ($P = 0.17$) ($R^2 = 0.21$).

*Conclusions* – Elevated serum ferritin was one of the strongest predictors of 10-year CHD risk in a low to middle- income urban population. Elevated serum ferritin was not a significant predictor of atherosclerosis as determined by IMT.

Chapter 1

# INTRODUCTION

## Introduction

In the United States, cardiovascular disease is the leading cause of death for men and women of all ages (1-2). In fact, the American Heart Association recently reported that the US spent more than $475.3 billion treating cardiovascular disease in 2008 (3). The most recent mortality data show approximately 27% of the 2.4 million deaths nationwide were related to diseases of the heart (4). More specifically, coronary heart disease, the most common form of heart disease in the US, comprised 68.3% of heart disease related deaths (5), and had a prevalence of 16.8 million Americans in 2006 (3). Coronary heart disease occurs when the coronary arteries, which are the suppliers of blood to the heart, become blocked due to plaque buildup, causing them to narrow and harden. This buildup of plaque is referred to as atherosclerosis. Regarding prevalence, 8.7 million were male and 8.1 million, female. African American males had the highest prevalence, followed by African American females, white males, and finally, white females (3).

Due to the significant impact of coronary heart disease, it is important for prevention purposes to identify the determinants of risk for developing this disease. This can be done by utilization of risk factor equations. The Framingham Heart Study has developed coronary heart disease prediction algorithms to assess absolute and relative

risk scores for the development of disease in the following 10 years (6-7). Relative risk is calculated as a ratio between absolute risk and that of a low risk group. Although, these equations were created based on a primarily white population, they have since been validated in both African American and white populations (8).

In addition to predictive risk equations, carotid intima-media thickness (IMT), assessed by B-mode ultrasound, has also been widely used as a marker for generalized atherosclerosis and has shown to predict the occurrence of cardiovascular events (9). Thickening for the carotid artery can be from intimal and/or muscular thickening, although there is large agreement that beyond 900μm generally represents atherosclerosis.

While it is recognized that elevated HDL, normal blood pressure, not smoking and a healthy body weight may have a protective effect with respect to risk for cardiovascular disease, less is known about iron status. In the early 1980's, Jerome Sullivan hypothesized that iron depletion might provide a protective mechanism in warding off heart disease (10). He proposed the iron hypothesis based upon three logical reasons; myocardial failure was seen in iron storage diseases, increased stored iron was seen in men as they aged, and also in women after menopause (10). Since then, the iron and heart disease debate has generated some considerably conflicting results.

In explanation, iron is a mineral, vital to the body due to its role in a number of metabolic reactions. Functional iron within the cells must be bound to proteins, which is necessary for the promotion of oxygen transport, cell growth and differentiation, electron transport, and energy metabolism, as well as antioxidant and pro-oxidant reactions (11-

12). Iron is needed in relatively high amounts in the body for the proper functioning of many metabolic processes. However, when too much iron is taken into the body or when overload occurs due to another disorder, such as hemochromatosis, toxicities can result. This overload occurs when formerly discussed iron-protein complexes become unbound within the cell. Iron is freed due to the saturated binding capacity of transferrin, as well as ferritin and hemosiderin (11). Free iron, or ferrous iron (Fe+2) is harmful to the body because of its role in oxidative damage. When the iron transport and storage proteins (transferrin and ferritin) become saturated, free iron (Fe+2) reacts with hydrogen peroxide forming ferric iron (Fe+3) and free radicals, also known as the Fenton reaction. Free radicals, especially the hydroxyl radical are extremely reactive and initiate tissue damage and lipid peroxidation (11).

Consequently, when excess iron is consumed and transferrin becomes saturated, free iron can be released (11). The production of free radicals by free iron have been found in some studies to cause oxidative damage to the coronary arteries, and possibly oxidize low-density lipoprotein cholesterol, resulting in even more coronary damage (12). Significant injury can then progress to atherosclerotic disease. Moreover, an increase in free radical production causes oxidative stress, which may possibly cause thrombosis and interfere with typical vasomotor regulation. Thus it is biologically plausible that iron may play a role in the development of a heart disease.

In fact, several studies have found significant associations between iron storage and atherosclerosis (13-15). In addition, some studies are suggestive of a link between iron overload and myocardial infarction (16-17). Nonetheless, many studies

have found evidence that does not support the link between iron storage and coronary heart disease (18-19), or atherosclerosis (20-21). Finally, most past reviews have concluded that there is not enough evidence to determine the extent of involvement of stored iron on coronary heart disease and/or atherosclerosis (22-24).

However, for many years iron deficiency has been the most common nutrient deficiency worldwide, leading to microcytic and hypochromic anemia (11). Still, excluding individuals with the iron overload condition, hemochromatosis, as well as anemic conditions associated with iron overload (thalassemias); limited attention has been paid to iron status in the absence of severe deficiency or overload. It is understandable that these conditions have presented themselves with very serious and harmful characteristics, and thus have been taken more seriously. However, if iron status is associated with coronary heart disease, a set of standardized measures of iron status that promote optimal heart health has yet to be created.

The scope of the effect on the body from excess iron saturation is extremely important in light of the results from national consumption surveys. In the United States, the Recommended Dietary Allowance (RDA) for iron is set at 8mg/day in adult males and women over 50 years of age, and 18mg/day for premenopausal women aged 19-50 years (25). The RDA for premenopausal women is higher due to the monthly loss of blood, and subsequently, iron during menstruation. The What We Eat in America - National Health and Nutrition Examination Survey, 2005-2006 found that the average intake for men >20 years, is more than double (~19mg/day) the recommended allowance for iron (26). On the other hand, the same data have shown women, age 20-49 years are

consuming ~13-15mg/day, and women, ≥ 50 years, consume ~13mg of iron per day. Thus, while premenopausal women consume less than the RDA, many men and postmenopausal women consume much more. In addition, since these are consumption values, supplement usage may notably increase the mean intake for these groups. Although these intakes do not reach levels of toxicity, (tolerable upper intake level for adults 19+ equals 45mg/day) little research as been done on the long-term effects of iron intake above the RDA. Considering men and postmenopausal women are the most at risk for developing cardiovascular disease, intakes may warrant concern (1-2). It is also important to note that the Dietary Reference Intakes do suggest men and postmenopausal women avoid iron supplementation and highly fortified foods. However support of this recommendation is limited by current research, so little attention has been given to promotion of this message to the consumer.

It seems that optimal iron intake may require a delicate balancing act in order to achieve an iron status that prevents deficiency, and at the same time reduces the risk of deleterious cardiovascular outcomes. Given the disconnect between iron status and heart disease, the enormous impact heart disease has on the US population, and the current consumption rates of iron in the US diet, it is extremely important for future research to determine what role, if any, iron has on heart disease. Unsurprisingly, most researchers, regardless of outcome supported and urged the need for future research, especially in regards to large-scale epidemiological studies, which would focus on the variability of iron status between race, sex, and age. Results supporting these suggestions include findings from a recent study, which found significantly higher iron stores in African

American men, compared to white men (27). Another study supported this finding between both races, regardless of sex (28). Other recommendations support research of the role menopause plays in regards to coronary heart disease due to the increased iron storage found after amenorrhea. To our knowledge, there have been no studies published to date, which examined the difference between race, sex, age and menopausal status, as well as, socioeconomic status in relation to iron status and coronary heart disease risk. It is essential to determine these associations, since this area of research could potentially impact millions of lives

In 2004, data collection began for a new 20-year prospective study sponsored by the National Institute of Aging (29-30), - the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. This large-scale epidemiological study is designed to examine the influence of aging, race, and socioeconomic status on the risk for development of cerebrovascular and cardiovascular disease. The study is focused on low to low-middle income sample of white and African Americans, aged 30-64 years, living in Baltimore, Maryland.

The main objective of this study is to determine the role iron status may play in the risk for development of atherosclerotic disease, (as determined by carotid intima-media thickness), and coronary heart disease, (as determined by the utilization of the Framingham Heart Study's coronary heart disease risk equations), in the baseline sample of the HANDLS study. Another aim is to determine whether or not the association between iron status and risk for heart disease is affected by the race and socioeconomic status of the participants.

## Statement of Problem

It has yet to be determined the extent iron levels in the body have on heart health, either due to unknown mechanisms or insufficient study of the problem. After an extensive review of the literature no publications were found regarding this topic in a population comprised of individuals from an urban population, who also live with low to low-middle socio-economic means. It is apparent that this population has been extremely underrepresented in large epidemiological studies. If more evidence is found either positively or negatively regarding iron's effect on heart health, especially in minority and low income populations, it would be a great asset, not only to help understand heart disease and it's progression, but also to be able to help develop new recommendations in order to educate the general public.

**Research Questions**

1) Are there significant differences in measures used to characterize iron status (serum ferritin, total iron binding capacity (TIBC), transferrin saturation percentage (Tsat%), and serum iron) between race, by sex, and across socioeconomic status?

2) Is there a correlation between carotid intima-media thickness (IMT) and the Framingham Heart Study's coronary heart disease 10-year risk equations?

3) Is there a correlation between iron status, as defined by serum ferritin and Tsat%, and risk for developing coronary heart disease, as determined by the Framingham Heart Study's 10-year coronary heart disease risk equations, and by IMT?

4) Is there a correlation between iron status and obesity as measured by BMI?

5) Is the risk for developing coronary heart disease, as determined by Framingham Heart Study's 10-year coronary heart disease risk equations and IMT values, affected by iron status when controlling for socioeconomic status, sex, and race?

**Dependent Variables:**

10-year coronary heart disease risk & Carotid intima-media thickness

**Delimitations**

The main delimitation of this study is that the relationship between iron status and overall nutritional health, which can impact risk for heart disease, was not explored.

The reason for having chosen this topic was due to the intense debate reviewed in the literature. There are many conflicting results, which lead one to think there must be some kind of connection between iron and heart disease but the answer has not been found, yet. With that said, new studies regarding this topic need to go above and beyond what has already been done. Critical thought lent to the topic to determine what factors may be leading researchers to find significant relationships in some studies and no significance in others is needed.

After review of research it seemed very fitting to conduct the study on the HANDLS population. There were studies showing the differences in iron status between African Americans and whites. There were no studies, which mentioned or to our knowledge included individuals from low socioeconomic status living in an urban location. In addition, heart disease is a disease that mainly affects individuals as they age. Therefore, utilization of an aging population, as well as the differences in race and socioeconomic status, provided us with a sample of individuals not previously studied, which could possibly provide some insight into the contradictory findings.

It was decided to split the sample into subgroups based on race, sex, and menopausal status. There was no discussion about division of race and sex. However, the division of menopausal status was decided upon due to the monthly loss of blood and subsequently iron in premenopausal women. Later, individuals were grouped further by BMI status since studies have shown iron stores can be different across various weight ranges.

At first there was discussion of using dietary iron intake since 24-hour dietary recalls are being collected from the HANDLS participants. However, dietary iron intake does not reflect what is absorbed and utilized by the body. Consequently, the three iron blood values and the calculated transferrin saturation percentage were used.

Coronary heart disease risk scores were studied. The Framingham Heart Study's equations were chosen because previous studies had validated its use in white and African American populations.

Carotid intima-media thickness was used due to the review of research, which showed this to be the best measure of atherosclerotic risk.

**Definition of Terms and Abbreviations**

| Variables | Will hereafter be referred to as: |
|---|---|
| Self-reported whites | Whites |
| Self-reported African Americans | African Americans |
| White, pre-menopausal females | WF-Pre |
| African American, premenopausal females | AAF-Pre |
| White, post-menopausal females | WF-Post |
| African American, post-menopausal females | AAF-Post |
| White males | WM |
| African American Males | AAM |
| Carotid intima-media thickness | IMT |
| 10-year coronary heart disease risk (As determined by utilization of Framingham Heart Study's 10-year risk equations) | 10-year CHD risk |
| Transferrin saturation percentage | Tsat% |
| Total iron binding capacity | TIBC |

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# Chapter 2

# LITERATURE REVIEW

## Literature Review

In order to more easily review the research articles written on the role of iron in heart disease, the literature review is broken down into sub-categories to identify results that support the role of iron in heart disease, and those that do not. Thereafter, they will are grouped by those who researched iron stores and heart disease in general, and those that aimed to determine the relationship between stored iron and atherosclerosis. Unless otherwise noted, most studies used serum ferritin as the marker to quantify iron stores.

### Results supporting a role of iron in heart disease

*Iron and Heart Disease*

One of the most widely cited papers on iron and coronary heart disease was conducted by Salonen and colleagues (1). The reason for its popularity is due to the fact that it was the first study to show support for Jerome Sullivan's iron hypothesis proposed in 1981 (1-2). Salonen et al. based their study on Eastern Finnish men (n = 1,931), ages 42-60 years from the Kuopio Ischemic Heart Disease Risk Factor Study. This study was developed since men from this area had the highest recorded incidence of mortality from coronary heart disease in the world. Men were included if they had not previously shown symptoms of coronary heart disease. The sample was followed for three years after baseline collection. During that time 51 men experienced an acute myocardial infarction.

In addition to the iron storage protein-serum ferritin, age, examination year, number of cigarettes smoked, ischemic ECG in exercise test, maximal oxygen uptake, systolic blood pressure, blood glucose, serum copper, blood leukocyte count, high density lipoprotein cholesterol, apolipoprotein B, and triglycerides were assessed. Interestingly, results found that men with serum ferritin levels >200 ug/L had a 2-3 fold increase in risk for having a myocardial infarction when compared to men with the lowest levels of serum ferritin. In addition, serum ferritin was significantly associated with the risk of acute myocardial infarction in all models.

Six years later, Tuomainen et al. studied some of the same men included in the Kuopio Ischemic Heart Disease Risk Factor Study as listed above (3). The new results supported the findings from the above research in that elevated iron stores were associated with acute myocardial infarction in their sample. However, these researchers used a new ratio (at that time) to quantify iron storage, the serum transferrin receptor to serum ferritin or sTfR:ferritin ratio.

A more recent study using data from the National Health and Nutrition Examination Survey (NHANES III), 1988-1994, analyzed the association between cardiovascular disease risk factors and serum ferritin in 3,400 American women aged 20-49 years (4). Women were divided into race-ethnicity groups and include; non-Hispanic whites (NHW), non-Hispanic blacks (NHB), and Mexican American (MA) women. Cardiovascular risks factors included: body mass index, serum triacylglycerol, total cholesterol, high density lipoprotein cholesterol, blood glucose, and systolic blood pressure. The 25th and 75th percentiles of serum ferritin were used to determine three

categories of iron stores; low (<25th percentile), medium (25th to 75th percentile), and high classes (>75th percentile). Results found cardiovascular risk was significantly higher in women who fell into the highest percentile for serum ferritin for all groups. However, significance was only seen in the NHB and MA groups after control for confounders. The strongest association was seen in MA women in the highest serum ferritin percentile. In addition, it is important to emphasize that this was the first study, which found a significant, positive association regarding iron storage and heart disease risk.

Moving in a slightly different direction, C-reactive protein (CRP) is a marker of inflammation and has been positively linked with coronary heart disease. As of 2003, however, its relation to iron status was unknown (5). In an attempt to determine if there was an association between iron status, cardiovascular disease, and CRP, Sung and colleagues proposed that the oxidation of low density lipoprotein cholesterol (LDL) by iron would induce inflammation, and thereby increase the inflammatory marker CRP. This study examined 808 men and women, mean age 47 ± 11.1 years, from the Kangbuk Samsung Hospital in Seoul, Korea. High levels of each variable where defined as; CRP levels >3.0 mg/L, serum ferritin levels >200 ng/mL, total cholesterol levels >200mg/dL, and LDL levels >160 mg/dL. Results showed elevated LDL levels and CRP levels, which in turn showed a significant and strong correlation ($P = 0.002$) between CRP and serum ferritin. A point of note is that 43% of the subjects were >50 years old which may support the basis of Sullivan's hypothesis of iron stores increasing as men and women age, along with heart disease risk (2).

*Iron and Atherosclerosis*

The objective in a cross-sectional survey conducted by Wolff and colleagues was to analyze the association between serum ferritin levels and carotid atherosclerosis (6). Close to 2,500 participants, aged ≥45 years were included in this sample, taken from the Study of Health in Pomerania (SHIP) of Northeast Germany. Carotid intima-media thickness (IMT) was assessed by B-mode ultrasound. Linear regression did not find an association with iron stores and IMT for men and women overall, or in age-adjusted groups. However, results did show, elevated serum ferritin levels had an increased odds ratio for the development of carotid plaques in men, as well as in postmenopausal women. Interestingly, there was an association found between serum ferritin and plaque prevalence strengthened by an interaction between serum ferritin and LDL cholesterol, in men only ($P = 0.039$). Nevertheless, although some findings were strong within the sample, limitations included inability to capture the variance in serum ferritin levels over time due to the cross-sectional nature of the study design, as well as the impact inflammation and liver disease has on serum ferritin.

Finally, another cross-sectional study conducted by Kiechl et al. was comprised of men and women (n = 1000), aged 40-79 years from the Bruneck Ischemic Heart Disease and Stroke Prevention Study (7). Exclusion criteria included previous transient ischemic attack or ischemic stroke. Smoking status, blood pressure measures, and body mass index were collected during the clinical history and exam. A wide variety of laboratory measures were done, including serum ferritin. The coronary arteries were assessed using a duplex ultrasound system and a plaque score was created to quantify

carotid atherosclerosis. Results found serum ferritin was highly indicative of atherosclerosis in both sexes of the sample. A limitation to this study included the low variability in serum ferritin levels throughout the group. However, there was also a high prevalence of atherosclerotic lesions, which the researchers suggested were possibly due to the high iron storage.

**Results weakening a role of iron in heart disease**

*Iron & Heart Disease*

A large sample of participant data from the NHANES II Mortality Study was assessed to determine if serum ferritin levels were associated with death from all causes and more specifically cardiovascular disease, coronary heart disease, and myocardial infarction (8). Serum ferritin was collected once during baseline collection. Cause of death was determined in black and white adults' ages 45-74 years. Individuals were chosen from the main cohort of the National Health and Nutrition Examination Study (NHANES II). The sample included 128 black men, 658 white men, 100 black women, and 718 white women due to the absence of coronary heart disease at baseline. The relative risk of death for participants with serum ferritin levels: <50 ug/L; or 100-100 ug/L; or >200 ug/L was compared to a serum ferritin reference group of: 50-99 ug/L, using Cox proportional-hazards model. Black men had the highest serum ferritin levels and white women had the lowest levels, however there were no statistically significant estimates of relative risk found.

The next study by Sun et al. also utilized the transferrin receptor/serum ferritin ratio (sTfR:ferritin) to assess body iron stores in a sample of women from the

Nurses' Health Study (9). Women ages 30-55 years were followed 9 years after baseline collection to assess iron storage on coronary heart disease risk. Cases of coronary heart disease (n = 242) were identified and matched with two controls (n = 483) for similar age, smoking status, and fasting status. Although women with coronary heart disease had significantly higher sTfR levels, multivariate analysis showed no significant association with sTfR:ferritin ratio for coronary heart disease risk.

*Iron & Atherosclerosis*

Moore and colleagues studied participants in the Atherosclerosis Risk in Communities (ARIC) Study in order to examine a relation between carotid IMT and serum ferritin levels (10). The sample consisted of case-controlled pairs (n = 365) of adults aged 45 – 65 years, living in four communities in the United States. Methods included measures of carotid IMT, and serum ferritin levels, along with dietary iron, LDL cholesterol, waist/hip ratios hypertension status, current vs. former smoker, and diabetic status. This study found no support that elevated serum ferritin had an effect on increased atherosclerosis, as measured by carotid IMT. Researchers also examined 124 case-control pairs with dietary data and found no support that increased dietary intake of iron was associated with increased risk of atherosclerosis.

**Summary**

In summary, many studies have shown very compelling results which support the hypothesis that iron-mediated oxidative stress can cause serious detriment to the heart (1, 3-7). In many of these studies elevation of the main iron storage protein, serum ferritin, was significantly associated with coronary heart disease risk and/or

atherosclerosis. This relationship was evident in a study, which found higher prevalence of carotid plaques in men, as well as an increased odds ratio for plaque prevalence in postmenopausal women (6). In fact, the former association was strengthened by an interaction between elevated serum ferritin and LDL, in men. Another study found the relationship to be even more complex when results showed elevated serum ferritin was highly correlated with elevated LDL and CRP (5). In addition, a 2-3 fold increased risk of the having a myocardial infarction was seen Eastern Finnish men with iron stores starting within the normal range; serum ferritin, ≥200 ug/L (1). Finally, one study was the first to show significant cardiovascular risk with elevated serum ferritin stores in pre-menopausal women, although this risk was significantly higher in non-Hispanic black and Mexican American, compared to non-Hispanic white women (4).

On the other hand, several studies did not conclusively support this hypothesis (8-10). First, in the same study that found an association between serum ferritin and higher prevalence of carotid plaques in men and postmenopausal women, researchers did not see any association between carotid IMT and iron stores (6). In addition, no association was found regarding serum ferritin levels and carotid IMT in participants from the ARIC study (10). Furthermore, no associations were seen in serum ferritin levels and death all causes or from disease of the heart (cardiovascular disease, coronary heart disease, myocardial infarction), in a large sample of participants from the NHANES II (8).

As reviewed, the results regarding iron-mediated oxidative stress to the heart are conflicting. Unsurprisingly, most researchers urge the need for further research.

Although suggestions have been made regarding the best way to determine iron status, the most appropriate method is yet to be determined and/or agreed upon by the scientific community. More research is especially needed to explain the variability of iron status between different races and ethnicities (11-12). In fact, articles have highlighted the need for large-scale epidemiological studies to focus on specific populations such as, minority groups, women, and older adults since the prevalence of heart disease increases with increasing age. This is due to the fact that non-Hispanic black and Mexican American women had increased cardiovascular risk with elevated iron stores (4). In addition, studies have shown that black men have much higher iron stores in comparison to white counterparts (11). Focus on women in general should delve into the role that menopause plays in regards to coronary heart disease due to increased iron storage after amenorrhea (6). It is also a necessity for future studies to focus on the best measures of iron status to ensure we are drawing the correction conclusions from research.

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# Chapter 3

## JOURNAL MANUSCRIPT

**The effect of iron status on the risk for developing coronary heart disease in The Healthy Aging in Neighborhoods of Diversity Across the Life Span (HANDLS) study sample**

Running Head: Iron status and heart disease risk

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**Abstract**

*Background* – Over the past three decades there has been considerable and conflicting debate on the impact of iron mediated oxidative damage on coronary heart disease.

*Objective* - To determine if the baseline participants of the *Healthy Aging in Neighborhoods of Diversity across the Life Span* (HANDLS) study, are at risk for coronary heart disease, as determined by the Framingham Heart Study's risk equations, and, atherosclerosis, as measured by carotid intima-media thickness (IMT), due to variations in iron status. Thereafter, to identify whether race and/or socioeconomic status impacts the iron status of these participants.

*Design* – Participants (n = 1874) were from the HANDLS baseline sample, which included African American and white men and women ages 32-64 years old, living in Baltimore, MD and categorized by race, sex, and menopausal status, as well as socioeconomic status. Pearson correlation and stepwise regression analysis were used to determine the differences in iron status, 10-year risk for development of coronary heart disease (CHD), and IMT for these groups, in order to determine the impact of iron stores on heart disease.

*Results* – Stepwise general linear regression found serum ferritin was one of the most significant predictors ($P$ <0.0001) of 10-year CHD risk ($R^2$ =0.42). Although significant correlations were found between IMT and serum ferritin ($P$ <0.0001), further analysis with regression modeling found serum ferritin was not significant predictor of IMT variance ($P$ = 0.17) ($R^2$ = 0.21).

*Conclusions* – Elevated serum ferritin was one of the strongest predictors of 10-year CHD risk in a low to middle- income urban population. Elevated serum ferritin was not a significant predictor of atherosclerosis as determined by IMT.

## Introduction

In the United States, cardiovascular disease is the leading cause of death for men and women of all ages accounting for approximately 27% of the 2.4 million deaths nationwide (1-3). More specifically, coronary heart disease, the most common form of heart disease in the US, comprised 68.3% of heart disease related deaths (4), and had a prevalence of 16.8 million Americans in 2006 (5). Regarding prevalence, 8.7 million were male and 8.1 million, female. African American males had the highest prevalence, followed by African American females, white males, and finally, white females.

Due to the significant impact of coronary heart disease on health, the utilization of predictive risk equations is important for prevention purposes. The Framingham Heart Study has developed coronary heart disease prediction algorithms to assess absolute and relative risk scores for the development of disease in the following 10 years (6-7). Although, these equations were created based on a primarily white population, they have since been validated in both African American and white populations (8).

Carotid intima-media thickness (IMT), assessed by B-mode ultrasound, has also been widely used as a marker for generalized atherosclerosis and has shown to predict the occurrence of cardiovascular events (9). Thickening for the carotid artery can be from intimal and/or muscular thickening, although there is large agreement that beyond 900μm generally represents atherosclerosis.

While it is recognized that elevated HDL, normal blood pressure, not smoking and a healthy body weight may have protective effect with respect to risk for cardiovascular disease, less is known about iron status. In the early 1980's, Jerome

Sullivan hypothesized that iron depletion might provide a protective mechanism in warding off heart disease (10). Sullivan based this hypothesis on the fact that myocardial failure was seen in iron storage diseases, and increased stored iron was seen in men as they aged, and also in women after menopause.

Iron may initiate or progress cardiovascular disease based upon its role in the Fenton reaction (11). When the iron transport and storage proteins (transferrin and ferritin) become saturated, free iron ($Fe^{+2}$) reacts with hydrogen peroxide forming ferric iron ($Fe^{+3}$) and free radicals. These free radicals, especially the hydroxyl radical, are known to initiate tissue damage and lipid peroxidation, which can cause oxidative damage to the coronary arteries, and may oxidize low-density lipoprotein cholesterol, resulting in even more coronary damage (12). Significant injury can then progress to atherosclerotic disease. Several studies have found significant associations between iron storage and atherosclerosis (13-15). In addition, some studies are suggestive of a link between iron overload and myocardial infarction (16-17). Nonetheless, many studies have found evidence that does not support the link between iron storage and coronary heart disease (18-19), or atherosclerosis (20-21). More evidence is required to determine the extent of involvement of stored iron on coronary heart disease and/or atherosclerosis (22-23).

To our knowledge, there have been no studies published to date, which examined the difference between race, sex, age and menopausal status, as well as, socioeconomic status in relation to iron status and coronary heart disease risk. Significantly higher iron stores have been found in African American men, compared to white men (24). Another

study supported this finding between both races, regardless of sex (25). Increased iron storage found after amenorrhea may increase the risk coronary heart disease of post-menopausal women. Yet, one study found significantly increased cardiovascular risk with elevated serum ferritin levels in pre-menopausal women. This risk was significantly higher in non-Hispanic black and Mexican American, compared to non-Hispanic white women (26).

In 2004, data collection began for a new 20-year prospective study sponsored by the National Institute of Aging (27 - 28), - the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. This large-scale epidemiological study is designed to examine the influence of aging, race, and socioeconomic status on the risk for development of cerebrovascular and cardiovascular disease. The study is focused on low to low-middle income sample of white and African Americans, aged 30-64 years, living in Baltimore, Maryland.

The purpose of this study is to determine the role iron status may play in the risk for development of coronary heart disease, (as determined by use of the Framingham Heart Study's Global Risk Assessment Model), and atherosclerotic disease, (as determined by assessment of carotid intima-media thickness), using baseline participants enrolled in the HANDLS study. In addition the impact of age, race, menopausal status, and socioeconomic status of the participants on the iron hypothesis will be assessed.

## Methods

### *HANDLS Background*

The HANDLS study began baseline collection in August 2004 and ended March 2009, with a total of 3723 participants. There were two phases of the baseline study. The first phase was done in the participant's home and consists of a 24-hour dietary recall and an interview about their health status, health services, psychosocial factors, and personal and neighborhood characteristics. The second phase was completed usually 3 to 10 days after the first, on a Mobile Research Vehicles (MRV), located in areas of preselected census tracts where participants reside. This phase included a medical history, a second 24-hour dietary recall, physical examination, cognitive measures and physiology assessments including heart rate variability, arterial thickness, assessments of muscle strength and bone density, and laboratory measurements including fasting blood chemistries and hematology. Extensive methodology specific to the entire study can be found elsewhere (27 - 28).

### *HANDLS Participants*

The participants of the HANDLS study consist of African Americans and white individuals, between the ages of 30-64 years old living in 13 census tracts in Baltimore, Maryland (28). These tracts were selected because they are likely to yield representative distributions of individuals between 30 and 64 years old who are African American and white, men and women, and having either a SES <125% or ≥125% the Federal poverty level. To be included in the HANDLS study, participants must have been, 1) within ages 30-64 years at baseline, 2) able to give informed consent, 3) able to perform at least five

measures of the following evaluations: labs, medical history, physical examination, physical performance, cognitive testing, dietary recall, audio questionnaire, body composition, carotid Doppler, or pulse wave velocity assessment, 4) able to provide valid picture identification, and 5) able to verifiable address at time of entry. Exclusion criteria included, pregnancy, and having undergone or were currently being treated for cancer, (chemotherapy, biologic, radiation), within the past 6 months before recruitment. Multi-ethnic individuals were placed into the group (African American or white), which they identify with the most. The human investigation review boards at both Medstar Research Institute and the University of Delaware approved the study protocol. All participants provided written informed consent.

***Study Participants***

The participants of this study include only those individuals who completed two dietary recalls and had no missing values with respect to iron status indicators [serum ferritin, transferrin saturation % (Tsat%), serum iron], fasting blood glucose, systolic blood pressure, high density lipoprotein cholesterol, total serum cholesterol, and demographic variables. Participants with a prior hysterectomy; participants who had a history of coronary artery disease or blockage (self-report); and participants who had undergone prior coronary artery bypass surgery were excluded from the analyses. A total of 1874 participants remained after exclusions.

For this analysis, participants were grouped into six categories in order to assess the differences between race, sex, and for women, menopausal status. Males were grouped into two categories based on race, since iron recommendations do not change

over their lifespan. Females were grouped into four categories, by race and then menopausal status, due to the difference in recommended iron intake by menopausal status. These groupings were determined from the self-reported answers regarding menopausal status on the medical history questionnaire. The sub-groups will hereafter be referred to as:

1. WM - (White Male)
2. WF-Pre - (White Female – Premenopausal)
3. WF-Post – (White Female – Postmenopausal)
4. AAM – (African American Male)
5. AAF-Pre – (African American Female – Premenopausal)
6. AAF-Post – (African American Female – Postmenopausal)

***Medical History***

Questions from the medical history that were included regarded cardiovascular health, history of certain diseases (cancer, diabetes, etc.), age at the beginning of menopause, information regarding past hysterectomy, and smoking status.

***Anthropometrics***

At the mobile research vehicle visit, fasting participants were weighed (kg) without shoes and coats using a calibrated Health O Meter digital scale. Height (cm) was obtained with the subject's heels and back against a height meter by Novel products, Inc. These measures were used to calculate body mass index with the following equation: Weight (kg)/Height $(m)^2$.

Body mass index (BMI) was used to classify people as normal, overweight, or obese (28). For this study the range for underweight ($<18.5$ kg/$m^2$) was combined with normal weight (18.5-24.9) due to the small number of participants who fell into

underweight category. Overweight, obese, and morbidly obese categories were defined as, 25.0-29.9 kg/$m^2$, and 30.0-39.9 kg/$m^2$, ≥40.0 kg/$m^2$, respectively (29).

***Socioeconomic Status (SES)***

Socioeconomic status was determined by using information collected from the household questionnaire. Groupings are as follows:

1. Education: <12 years or ≥12 years
2. Employment in the past month: Yes or No
3. Poverty Income Ratio (PIR)[30]: <125% or ≥125%

***Blood Values & Blood Pressure***

Fasting blood samples were taken on the mobile research vehicles (28). A total of ~62 milliliters of blood was drawn from each participant. Laboratory analysis was done in the NIA Clinical Core Laboratory and at the Harbor Hospital Laboratory (27).

Fasting blood values utilized for this study included: measures of serum cholesterol (mmol/L), C-reactive protein (mg/L), and four values for iron status. Iron values were run as continuous variables and included for analysis:

1. Serum Iron, mcg/dL
2. Total iron binding capacity, mcg/dL, (TIBC)
3. Transferrin saturation % - Calculated: (Serum iron/TIBC)) x 100)
4. Serum Ferritin, ng/mL

Blood pressure was assessed using the Portapres ambulatory heart rate and blood pressure monitor (28).

***Carotid Intima-Media Thickness***

Carotid intima-media thickness (IMT) was assessed by Carotid Doppler ultrasonography. The variables of IMT that were included in the original assessment are:

blood pressure values, measurements of left IMT, measurements of left systolic diameter, and measurements of left diastolic diameter. Since, IMT is not constant during systole and diastole measures, agreement has been made that a cross-sectional measure provides a more accurate and constant measure. Therefore, averages of the 5 measurements of left IMT were used for this study. Groupings were defined as (9):

1. Atherosclerotic risk - ≥ 900μm
2. No risk - < 900μm

***Coronary Heart Disease Risk Assessment***

The Framingham Heart Study's coronary heart disease risk assessment equations were used to calculate the individual absolute heart disease risk and relative risks compared to a low risk group. The low risk state is defined according to the Framingham Heart Study (7-8) and utilizes desirable ranges for serum total cholesterol, LDL cholesterol, HDL cholesterol, blood pressure, as well as non-diabetic and non-smoking status to determine risk scores.

***Statistical Analysis***

Data analysis was completed using the SAS 9.1 statistical program (SAS Institute, Cary, NC, USA). Descriptive statistics were calculated for demographic characteristics, IMT measures, and selected biomarkers (**Table 1**). Least square means were run for effects. Relationships were assessed via chi-square and correlation methods for discrete and continuous variables, respectively. Pearson correlations were run for the following variables: all four measures of iron status, age, 10-year risk, relative risk, and BMI. After analysis verified that both the 10-year risk and relative risk values were positively

correlated to serum ferritin ($P$ <0.001), regression models were utilized to assess the impact of iron storage on coronary heart disease risk. BMI was included in all regression analyses after finding significant correlations between BMI and all four measures of iron status, (serum iron, $P$ <0.0001; TIBC, $P$ = 0.007; Tsat%, $P$ <0.0001; serum ferritin, $P$ = 0.002), as well as with 10-year risk and relative risk.

Stepwise regression was performed to assess the variables impacting the dependent variable, 10-year cardiovascular risk. The covariates that entered into the final model for 10-year cardiovascular risk included race, sex, education, race/sex interaction, race/sex/education interaction, employment, serum ferritin, Tsat%, age, and BMI. Comparative risk was also assessed between the HANDLS study baseline sample and the Framingham Heart Study cohort.

Stepwise regression was also performed to assess the influence of iron status on IMT, the dependent variable. For this analyses, a sub-sample of the total participant sample (n=1874) with no missing values for IMT was used (n = 1178). The regression model for IMT, showed no association between IMT and iron status, and therefore will not be discussed in the results.

## Results

### *Study Participants*

Overall, based on BMI, 30% of the sample (n = 1874) were classified as overweight, another 30% as obese, and 10% as morbidly obese. About 47.7% of the population smoked cigarettes at baseline. Diabetes afflicted 14.2% of the study sample. Approximately 1/3 of the sample did not complete high school. Nearly 40% were not

224 employed. Poverty was also a major factor, with 39% falling below 125% of the poverty
225 level.

226 Descriptive statistics, indicators of risk for developing cardiovascular disease, as
227 well as socioeconomic variables, are presented for the sample categorized by race, sex,
228 and menopausal status in Table 1. The approximate percent composition per group
229 yielded: WF-Pre - 11.8%, WF-Post – 8.3%, AAF-Pre – 19.8%, AAF-Post – 9.3%, WM –
230 20.9%, and AAM – 29.8%. The mean age (± SEM) of the participants ranged from 41.0
231 ± 0.5 to 55.3 ± 0.4 years. According to mean BMI values males were classified as
232 overweight, regardless of race. In contrast, mean BMI for all females, in spite of
233 menopausal status was >30, indicating obesity. With the exception of WF-Post (200.3 ±
234 3.1 mg/dL), mean total cholesterol for the participants was below the borderline-high risk
235 range, (200 mg/dL) as defined by the American Heart Association (Table 1). Finally,
236 means for high-sensitivity C-reactive protein (hs-CRP) were found to be >3.0 mg/L
237 across the entire sample.

238 ***Iron Status***

239 There were statistically significant differences ($P$ <0.0001) when comparing men
240 to women for all four indicators of iron status (serum iron, serum ferritin, TIBC, Tsat%).
241 Men had higher values for all measures except TIBC. In addition, there were significant
242 differences between pre- and post-menopausal women, for each iron marker, ($P$ <0.05,
243 serum iron; $P$ <0.0001, serum ferritin, TIBC, Tsat%), with post-menopausal women
244 having higher values for all measures except TIBC. However, when assessing racial

differences African American's had significantly lower serum iron and Tsat% compared to whites ($P$ <0.0001).

The mean (±SEM) for the iron status indicators categorized by the six sample groupings are provided in **Table 2**. It is important to note each group fell within normal reference ranges for all four indicators, with the exception of Tsat% for woman. Both pre and post-menopausal women had slightly lower than reference values (31). There was a significant difference in serum ferritin levels between the WF-Post and AAF-Post categories, with AAF-Post having the higher value. In contrast, among premenopausal women, WF-Pre had significantly higher mean serum iron and Tsat% (Table 2). Mean serum iron and Tsat% were significantly higher for WM compared to AAM (Table 2).

***Socioeconomic Status***

For all individuals below 125% of the poverty level, serum iron values, ($P$ <0.05) and Tsat%, ($P$ = 0.03) were significantly lower than individuals with incomes >125% poverty level. Significantly lower serum iron, ($P$ <0.0001) and significantly lower Tsat%, ($P$ <0.0001), were found for non-employed individuals as compared to employed persons. However, significantly lower serum ferritin levels ($P$ = 0.03) were found for employed individuals compared to non-employed. No significant differences were seen between education level and iron status for the entire sample.

However, when accounting for race, sex, menopausal status, and socioeconomic factors, differing trends emerged. Regarding employment status, there was no significant difference seen between unemployed WF-Post and employed AAF-Post. There was significance found between unemployed AAM and employed WM. In regards to poverty

status, there were no significant differences between males or premenopausal females for serum ferritin. However, significance was found between African American and white postmenopausal for all levels of poverty status excluding WF-Post >125% PIR and AAF-Post <125% PIR. Finally, when split by education level, there were still no significant differences between males or between premenopausal females for serum ferritin. However, post-menopausal grouping showed a significant difference between race for all groups except, AAF-Post who completed high school and WF-Post who did not complete high school.

***Carotid IMT***

Significant positive correlations were found between IMT and serum ferritin ($P$ <0.0001), as well as IMT and 10-year CHD risk. However, further analysis using regression models found that serum ferritin was not a significant predictor of IMT variance ($P = 0.17$) ($R^2 = 0.21$). For this analysis race ($P = 0.0003$), sex ($P$ <0.0001), race/sex interaction ($P = 0.002$), age ($P$ <0.0001), and BMI ($P$ <0.0001) were significant predictors of IMT variance, while race/education interaction ($P = 0.06$), employment ($P$ = 0.22), Tsat% ($P = 0.09$) and serum ferritin ($P = 0.17$) were not.

***Coronary Heart Disease Risk***

Positive correlations between the relative risk and the following three variables; serum ferritin, 10-year risk, and BMI, each yielded significance at $P$ <0.0001. Overall between men and women significant differences, ($P$ <0.0001) were found for both 10-year risk and relative risk. Again, significance at $P$ <0.0001, accounted for the differences seen between race and 10-year risk, as well as, race and relative risks.

Descriptive results of coronary heart disease risk scores to predict 10-year risk and relative risk are presented in **Table 3** for each of the six sample sub-categories. With respect to 10-year risk, no significance was seen between AAF-Pre and WF-Pre women, nor was there significance found between AAF-Post and WF-Post women. Significance was seen, however, between AAM and WM for 10-year risk ($P<0.0001$). The previous three findings were also reflected in relation to relative risks. Only significance between AAM and WM was found ($P<0.0001$). Within both races significant differences were found for women, with postmenopausal women having higher 10-year risk compared to premenopausal women ($P<0.0001$), (Table 3).

**Table 4** shows comparative results for men and women from the Framingham Heart and HANDLS studies. Overall, male HANDLS participants, 30-64 years of age, had lower predicted risk scores compared to the male Framingham population. Further assessment revealed African American HANDLS participants tended to have lower predicted risk scores when compared to their white counterparts.

In contrast, female HANDLS participants, showed similar results to the female Framingham population, for ages 30-44. Overall, HANDLS females, ages 45-64 had lower predictive scores than female Framingham participants. Yet, in comparison to females 60-64 years, African American HANDLS females have equivalent 10-year cardiovascular risk scores to Framingham females. Conversely, white HANDLS females had a 3% lower risk than both groups.

***Regression Analysis***

A stepwise general linear regression model was run to determine the strength of variance upon the dependent variable of 10-year risk ($R^2$ =0.42) **(Table 5)**. The significant covariates stepped into the final model were: race, sex, education, race/sex interaction, race/sex/education interaction, employment, serum ferritin, Tsat%, age, and BMI. Sex, age, BMI, and serum ferritin were all found to be the most significant predictors of variance in 10-year risk ($P$ <0.0001).

## Discussion

*Iron Status*

The findings of this study showed there are significant differences overall between males and females and between pre- and postmenopausal women for all four measures of iron status. Noteworthy was the significant difference ($P$ = 0.0036) seen in serum ferritin levels between post-menopausal women, with AAF-Post having the much higher value (Table 2). Conversely, there was no significant difference found in serum ferritin levels between pre-menopausal women by race, nor was there a significant difference in serum ferritin levels between men by race. This is particularly interesting since previous research with NHANES III data has consistently shown that African Americans males and post-menopausal women have higher serum ferritin concentrations than white individuals from the same groups (25, 32-33). In fact, Pan and colleagues found that non-Hispanic black males were 2.15 times more likely than white males to have increased serum ferritin concentrations with acute inflammation (C-reactive protein ≥1.0 mg/dL), and 1.81 times more likely without inflammation (25). Our results show

that race is not a significant determinant of serum ferritin levels between similar age/sex categories in our sample, despite the elevated means for hs-CRP found in each of the six groups (Table 1).

Interestingly, differences in serum ferritin levels were seen between African American and white post-menopausal women by socioeconomic factors. In fact, post-menopausal African American women who did not complete high school, were unemployed, and <125% of the poverty income ratio, had the highest levels of serum ferritin when compared to all other post-menopausal women. Differences in SES may somewhat account for the variability in serum ferritin levels found when only accounting for the race and menopausal status of these women. To our knowledge this is the first study to examine the influence of race, sex, and in women menopausal status, on serum ferritin levels.

*Carotid IMT*

These findings are consistent with the findings of others which found no association between elevated serum ferritin and increased IMT (20-21). However, findings from a study by Wolff et al. found that although serum ferritin levels were not independently associated with IMT, there was a significant relationship seen between high serum ferritin levels and carotid plaque prevalence in men and women, which seemed to be enhanced by increased LDL cholesterol in men (14). These results suggest future studies should focus on the relationship between serum ferritin, plaque prevalence, and LDL cholesterol in the HANDLS sample, as well as other samples.

*Coronary Heart Disease Risk*

In our sample, the four strongest predictors of 10-year CHD risk, in order of predominance, were age, sex, BMI, and serum ferritin (Table 5). A vast amount of research has shown the significant relationship between the first three variables and heart disease, however our model also shows the importance of stored iron in the risk for developing coronary heart disease. These findings are strengthened by the fact that serum ferritin showed significant positive correlation with both 10-year CHD risk and relative risk. To our knowledge this is the first study to show significance between serum ferritin levels and coronary heart disease by utilization of the Framingham Heart Study's 10-year CHD risk equations.

The significance between serum ferritin and acute myocardial infarction may be attributed to a poor diet quality (34). It has been suggested that a diet high in fat and cholesterol, low in vegetables, and known antioxidants, Vitamin E, and selenium might lead one to find a relationship between serum ferritin and myocardial infarction (34). A recent study by Raffensperger et al. found that overall diet quality of a sample of HANDLS participants was poor (35). Findings showed large proportions of individuals (n = 1990) had low intakes (≤67% of a nutrient adequacy ratio) of the following antioxidants: Vitamin A (67.2%), Vitamin C (56.9%), and Vitamin E (85.3%). If iron-mediated oxidative stress is a risk factor for developing coronary heart disease, then it seems likely that consuming a diet rich in antioxidants would decrease this risk by protecting the body from toxic free radicals. It is possible that the significance between elevated serum ferritin and coronary heart disease risk may in part be due to a diet of the

sample HANDLS participants which has previously been shown to be low in antioxidant rich foods, which are well known to provide defense against pro-oxidants.

In addition, the most significant finding of the study by Raffensperger and colleagues what that education may have been the most important predictor of nutrient-based diet quality of the sample (35). Education was a predictor of CHD risk in this study suggesting an effect of education level on coronary heart disease risk may also be related to a diet low in antioxidant rich foods.

The major limitation of this study is in regards to variability of the iron storage protein, serum ferritin when inflammation is present within the body. Since, serum ferritin is an acute phase reactant it can be elevated due to inflammation, certain type of cancers, and liver disease. Therefore, we decided to take high sensitivity C-reactive protein (hs-CRP) into account. The overall study's exclusion criteria did not allow individuals who had undergone or were currently being treated for cancer, (chemotherapy, biologic, radiation), within the past 6 months before recruitment. However, we did not exclude for liver disease, so any individuals with this disease may have had elevated serum ferritin due to their disease process.

The mean for hs-CRP for the HANDLS sample was >3.0 mg/L, indicating considerable inflammation and high risk for the development of cardiovascular disease (36). The use of hs-CRP as an independent predictor of increased coronary heart disease risk has been recommended by the American Heart Association and the Centers for Disease Control and Prevention (37). The work by Mainous et al., which found that an interaction between elevated serum ferritin and LDL cholesterol or low HDL cholesterol

was significantly related to elevated CRP in adults ≥25 years of age (38). In addition, this relationship was stronger than the relationship seen between serum ferritin and CRP alone. In addition, CRP was not significantly associated with LDL cholesterol in the study (38). A study by Sung et al. showed similar results (13). The previous results show possible support for iron-mediated oxidation of LDL cholesterol to induce inflammation.

Another limitation is participant self-report, which always contains an element of error inherent to the data. Data that may have been affected by self reported answers include questions about coronary artery disease or blockage, prior coronary artery bypass surgery, menopausal status, medical histories questionnaires regarding various diseases, and habits, such as smoking, as well as information on socioeconomic status from the household questionnaire.

Next, due to the small number of participants who fell into the underweight category for BMI, they were combined with normal weight individuals, which did not allow for thorough assessment of this group. Finally, IMT values were not obtained for the entire sample (n = 1874), so results could not be compared between the groups.

Future research should continue to determine the effect that ethnicity, sex, and SES have on this topic. In addition, researchers should begin to focus on overall diet quality, especially in regards to antioxidant levels of individuals with elevated serum ferritin to see if antioxidant rich diets or lack thereof is the reason for so many conflicting results.

In conclusion, elevated serum ferritin levels were not a significant predictor of atherosclerosis as determined by IMT, in baseline HANDLS participants. However,

following three previously known heart disease risk factors, (age, sex, BMI), serum ferritin was one of the most significant predictors of 10-year CHD risk. The four strongest predictors of 10-year CHD risk in a low to middle- income urban population, in order of predominance, were age, sex, BMI, and serum ferritin. Race and socioeconomic status influenced CHD risk but their effect was less than serum ferritin.

**TABLE 1**
Participant characteristics by race, sex, and menopausal status[1]

| | WF-Pre (n = 222) | AAF-Pre (n = 371) | WF-Post (n = 156) | AAF-Post (n = 175) | WM (n = 391) | AAM (n = 559) |
|---|---|---|---|---|---|---|
| Age at baseline (y) | 41.0 ± 0.5 | 41.9 ± 0.4 | 55.3 ± 0.4 | 54.7 ± 0.4 | 48.0 ± 0.5 | 47.5 ± 0.4 |
| BMI (kg/m$^2$) | 31.0 ± 0.6 | 31.7 ± 0.5 | 30.7 ± 0.6 | 32.2 ± 0.6 | 29.3 ± 0.3 | 27.3 ± 0.3 |
| Serum total cholesterol (mmol/L) | 184.8 ± 2.6 | 177.5 ± 1.9 | 200.3 ± 3.1 | 198.3 ± 3.1 | 187.7 ± 2.3 | 181.0 ± 1.9 |
| Serum HDL cholesterol (mmol/L) | 50.4 ± 1.0 | 57.5 ± 0.9 | 55.4 ± 1.1 | 58.4 ± 1.2 | 44.4 ± 0.6 | 53.8 ± 0.8 |
| Plasma glucose (mmol/L) | 100.0 ± 2.6 | 98.3 ± 2.0 | 105.5 ± 2.6 | 105.1 ± 3.4 | 109.3 ± 2.3 | 103.7 ± 1.9 |
| C-reactive protein (mg/L) | 4.6 ± 0.5 | 5.8 ± 0.6 | 5.6 ± 0.7 | 6.1 ± 0.5 | 3.1 ± 0.2 | 4.5 ± 0.6 |
| Diastolic BP (mm Hg) | 70.4 ± 0.7 | 71.2 ± 0.7 | 70.2 ± 1.1 | 73.7 ± 0.9 | 74.5 ± 0.6 | 73.6 ± 0.5 |
| Systolic BP (mm Hg) | 113.8 ± 1.2 | 116.8 ± 1.2 | 121.4 ± 1.9 | 127.4 ± 1.4 | 121.6 ± 0.8 | 121.0 ± 0.7 |
| Smoker (%) | 39.6 | 46.4 | 43.6 | 36.6 | 46.0 | 57.4 |
| Diabetes Mellitus (%) | 10.8 | 11.1 | 19.2 | 21.7 | 14.3 | 13.8 |
| SES Variables (%) | | | | | | |
| Education (<12 years) | 25.2 | 28.8 | 33.3 | 29.7 | 31.2 | 34.4 |
| Employed in the past month | 63.5 | 58.0 | 53.2 | 56.0 | 68.0 | 58.3 |
| Poverty Income Ratio (<125%) | 32.9 | 51.5 | 35.3 | 37.7 | 28.1 | 42.4 |
| Carotid intima-media thickness[3] | 0.066 ± 0.01[4] | 0.068 ± 0.01[5] | 0.067 ± 0.01[6] | 0.075 ± 0.01[7] | 0.072 ± 0.01[8] | 0.072 ± 0.01[9] |

[1] Results listed as $\bar{x}$ ± SEM, unless otherwise noted. WF-Pre = premenopausal white females, AAF-Pre = premenopausal African American females, WF-Post = postmenopausal white females, AAF-Post = postmenopausal African American females, WM = white males, AAM = African American males.
[3] $\bar{x}$ ± SD; [4] n = 188; [5] n = 266; [6] n = 123; [7] n = 109; [8] n = 318; [9] n = 401

**TABLE 2**

Differences in iron status between races, by sex, and menopausal status of women[1]

| | Iron[2] (mcg/dL) | Ferritin[3] (ng/mL) | Iron[4] Saturation % | Total Iron Binding[5] Capacity (mcg/dL) |
|---|---|---|---|---|
| WF-Pre (n = 222) | 80.7 ± 2.6 | 40.2 ± 3.1 | 22.4 ± 0.8 | 369.9 ± 4.0 |
| AAF-Pre (n = 371) | 70.0 ± 2.1 | 55.0 ± 4.9 | 19.4 ± 0.6 | 377.4 ± 3.7 |
| *P* | 0.0008 | 0.2645 | 0.0019 | 0.1264 |
| WF-Post (n = 156) | 81.8 ± 2.4 | 83.7 ± 5.5 | 23.0 ± 0.7 | 361.2 ± 3.9 |
| AAF-Post (n = 175) | 78.9 ± 2.1 | 134.1 ± 13.8 | 22.7 ± 0.6 | 354.7 ± 4.6 |
| *P* | 0.4847 | 0.0036 | 0.7703 | 0.3068 |
| WM (n = 391) | 99.3 ± 2.0 | 182.4 ± 9.7 | 29.9 ± 0.6 | 337.3 ± 2.4 |
| AAM (n = 559) | 89.1 ± 1.6 | 189.9 ± 8.3 | 27.0 ± 0.5 | 335.1 ± 2.3 |
| *P* | <0.0001 | 0.4663 | <0.0001 | 0.5590 |

[1] Results listed as $\bar{x}$ ± SEM. WF-Pre = premenopausal white females, AAF-Pre = premenopausal African American females, WF-Post = postmenopausal white females, AAF-Post = postmenopausal African American females, WM = white males, AAM = African American males.

[2]Serum iron – Reference value: 50 -170ug/dL

[3]Serum ferritin – Reference value: ≤ 300ng/mL (men), ≤ 200ng/mL (women)

[4]Iron Saturation % - Reference value: 25-35%

[5]Total Iron Binding Capacity – Reference value: 240 – 450 ug/dL

Iron test reference values adapted from: http://www.irondisorders.org/iron-tests(31)

**TABLE 3**

Comparison of average 10-year risk and relative risk utilizing the Framingham Heart Study coronary heart disease risk equations, across race by sex and menopausal status of women and within race for women by menopausal status[1]

| | WF-Pre (n = 222) | AAF-Pre (n = 371) | WF-Post (n = 156) | AAF-Post (n = 175) | WM (n = 391) | AAM (n = 559) |
|---|---|---|---|---|---|---|
| 10-year Risk | 2.9 ± 0.2[2] | 2.9 ± 0.2[3] | 7.6 ± 0.4[2] | 7.7 ± 0.4[3] | 8.9 ± 0.3 | 7.2 ± 0.2 |
| ***P***[4] | 0.9913 | | 0.8758 | | <0.0001 | |
| Relative Risk | 1.4 ± 0.06 | 1.2 ± 0.04 | 1.3 ± 0.08 | 1.3 ± 0.06 | 1.7 ± 0.06 | 1.4 ± 0.04 |
| ***P***[4] | 0.0535 | | 0.7112 | | <0.0001 | |

[1] Results listed as $\bar{x}$ ± SEM. WF-Pre = premenopausal white females, AAF-Pre = premenopausal African American females, WF-Post = postmenopausal white females, AAF-Post = postmenopausal African American females, WM = white males, AAM = African American males.

[2, 3]Values within a row with same superscript indicate significant difference ($P$ <0.0001)

[4]Comparison by race for premenopausal women, postmenopausal women, and men.

**TABLE 4**

Comparative 10-year risk between Framingham and the HANDLS study sample[1]

| Average 10-year risk for developing Coronary Heart Disease | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ----------Male---------- | | | | ----------Female---------- | | | |
| Age (years) | Overall Male Framingham | Overall HANDLS | White HANDLS | AA[2] HANDLS | Overall Female Framingham | Overall HANDLS | White HANDLS | AA[2] HANDLS |
| 30-34 | 3% | 4% | 3% | 4% | <1% | 1% | 1% | 1% |
| 35-39 | 5% | 4% | 4% | 4% | 1% | 1% | 1% | 1% |
| 40-44 | 7% | 5% | 7% | 5% | 2% | 2% | 2% | 2% |
| 45-49 | 11% | 7% | 9% | 6% | 5% | 4% | 5% | 4% |
| 50-54 | 14% | 9% | 11% | 10% | 8% | 7% | 7% | 7% |
| 55-59 | 16% | 11% | 11% | 11% | 12% | 8% | 7% | 8% |
| 60-64 | 21% | 14% | 15% | 13% | 12% | 11% | 9% | 12% |

[1]Source: Modified based on Coronary Disease Risk Prediction Score Sheet for Men Based on Total Cholesterol Level (www.nhlbi.nih.gov/about/framingham/risktmen.pdf) and the Coronary Disease Risk Prediction Score Sheet for Women Based on Total Cholesterol Level (www.nhlbi.nih.gov/about/framingham/risktwomen.pdf) in comparison with HANDLS data

[2]African Americans

**TABLE 5**
Summary table for General Linear Model for 10-year coronary heart disease risk in the HANDLS sample[1]

| Covariates | DF | F Value | *P* Value |
|---|---|---|---|
| Age | 1 | 581.3 | <0.0001 |
| Sex | 2 | 85.4 | <0.0001 |
| BMI | 1 | 36.8 | <0.0001 |
| Serum Ferritin | 1 | 17.6 | <0.0001 |
| Education | 1 | 13.8 | 0.0002 |
| Employment | 1 | 10.1 | 0.0015 |
| Race | 1 | 9.5 | 0.0021 |
| Tsat%[2] | 1 | 9.0 | 0.0028 |
| Race/Sex Interaction | 2 | 8.1 | 0.0003 |
| Race/Sex/Education Interaction | 5 | 3.8 | 0.0021 |

[1] $R^2$ = 0.42, Coefficient variable = 69.2, Root MSE = 4.3, 10-year CHD risk mean = 6.3
[2] Tsat% = transferrin saturation percentage

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Appendix A

# DATA TABLES

**TABLE 1**
Summary table for General Linear Model
for carotid intima-media thickness (IMT) in the HANDLS sample[1]

| Covariates | DF | F Value | *P* Value |
|---|---|---|---|
| Age | 1 | 171.9 | <0.0001 |
| BMI | 1 | 65.0 | <0.0001 |
| Sex | 2 | 21.5 | <0.0001 |
| Race | 1 | 13.0 | 0.0003 |
| Race/Sex Interaction | 2 | 6.4 | 0.0018 |
| Tsat%[2] | 1 | 2.9 | 0.0898 |
| Race/Education Interaction | 2 | 2.8 | 0.0606 |
| Serum Ferritin | 1 | 1.9 | 0.1712 |
| Employment | 1 | 1.5 | 0.2245 |

[1] $R^2$ = 0.21, Coefficient variable = 16.4, Root MSE = 0.01, Mean IMT mean = 0.07
[2] Tsat% = transferrin saturation percentage

**TABLE 2**
Differences in iron status between races[1]

| | AA[2] (n = 1105) Mean ± SEM | White (n = 769) Mean ± SEM | *P* Value |
|---|---|---|---|
| Iron (mcg/dL) | 81.2 ± 1.2 | 90.4 ± 1.4 | <0.0001 |
| Ferritin (ng/mL) | 135.8 ± 5.3 | 121.3 ± 5.6 | 0.0676 |
| Iron Saturation % | 23.7 ± 0.3 | 26.4 ± 0.4 | <0.0001 |
| Total Iron Binding Capacity (mcg/dL) | 352.4 ± 1.9 | 351.6 ± 1.9 | 0.7677 |

[1] Results listed as $\bar{x}$ ± SEM
[2]African Americans

**TABLE 3**
Differences in iron status by race and sex[1]

| | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|
| Sex | | | | |
| Female (n=924) | 76.2 ± 1.2 | 71.3 ± 3.6 | 21.4 ± 0.3 | 368.6 ± 2.1 |
| Male (n=950) | 93.3 ± 1.3 | 186.8 ± 6.3 | 28.2 ± 0.4 | 336.0 ± 1.7 |
| *P* | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Sex by Race | | | | |
| White Female (n=378) | 81.1 ± 1.8 | 58.1 ± 3.1 | 22.7 ± 0.5 | 366.3 ± 2.9 |
| AA[a] Female (n=546) | 72.9 ± 1.6 | 80.4 ± 5.7 | 20.5 ± 0.4 | 370.1 ± 3.0 |
| *P* | 0.0010 | 0.0361 | 0.0037 | 0.3254 |
| White Male (n=391) | 99.3 ± 2.0 | 182.4 ± 9.7 | 29.9 ± 0.6 | 337.3 ± 2.4 |
| AA[a] Male (n=559) | 89.1 ± 1.6 | 189.9 ± 8.3 | 27.0 ± 0.5 | 335.1 ± 2.3 |
| *P* | <0.0001 | 0.4704 | <0.0001 | 0.5609 |

[1] = All values are $\bar{x}$ ± SEM
[a]African American

**TABLE 4**
Differences in iron status between races, including menopausal status of the HANDLS cohort[1]

| | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|
| Pre – Female | | | | |
| White (n=222) | 80.7 ± 2.6 | 40.2 ± 3.1 | 22.4 ± 0.8 | 369.9 ± 4.0 |
| AA[a] (n=371) | 70.0 ± 2.1 | 55.0 ± 4.9 | 19.4 ± 0.6 | 377.4 ± 3.7 |
| *P* | 0.0008 | 0.2645 | 0.0019 | 0.1264 |
| Post - Female | | | | |
| White (n=156) | 81.8 ± 2.4 | 83.7 ± 5.5 | 23.0 ± 0.7 | 361.2 ± 3.9 |
| AA (n=175) | 78.9 ± 2.1 | 134.1 ± 13.8 | 22.7 ± 0.6 | 354.7 ± 4.6 |
| *P* | 0.4847 | 0.0036 | 0.7703 | 0.3068 |
| Males | | | | |
| White (n=391) | 99.3 ± 2.0 | 182.4 ± 9.7 | 29.9 ± 0.6 | 337.3 ± 2.4 |
| AA (n=559) | 89.1 ± 1.6 | 189.9 ± 8.3 | 27.0 ± 0.5 | 335.1 ± 2.3 |
| *P* | <0.0001 | 0.4704 | <0.0001 | 0.5609 |

[1] = All values are $\bar{x}$ ± SEM
[a]African American

**TABLE 5**
Differences in iron status by BMI classification of entire cohort[1]

| BMI (n) | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|
| Normal[a] (n = 542) | 92.3 ± 1.9[bcd] | 134.6 ± 7.4[d] | 27.5 ± 2.6[bcd] | 345.2 ± 2.6[cd] |
| Overweight[b] (n = 563) | 86.9 ± 1.5[acd] | 138.2 ± 7.4[d] | 25.3 ± 0.5[acd] | 352.0 ± 2.5 |
| Obese[c] (n = 571) | 81.7 ± 1.6[abd] | 128.7 ± 7.2[d] | 23.7 ± 0.5[abd] | 356.3 ± 2.6[a] |
| Morbidly Obese[d] (n = 198) | 68.0 ± 1.8[abc] | 96.4 ± 8.5[abc] | 19.3 ± 0.5[abc] | 358.8 ± 3.9[a] |

[1] = All values are $\bar{x}$ ± SEM
Within a group, superscript letters indicate significant difference
Normal[a] = Underweight + Normal weight (<18.5 - 24.9), Overweight[b] = 25.0 - 29.9, Obese[c] = 30.0 – 39.9, Morbidly Obese[d] = 40+
Significance $P < 0.05$

**TABLE 6**

Differences in iron status by gender and pre/postmenopausal classification for BMI categorization[1]

| | BMI | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Pre - Female** | Normal[a] (n=154) | 87.3 ± 3.7[bc] | 57.0 ± 7.8 | 24.5 ±1.1[bc] | 370.0 ± 5.3 |
| | Overweight[b] (n=144) | 73.2 ± 3.0[a] | 54.9 ± 9.0 | 20.1 ± 0.9[a] | 382.4 ± 6.1 |
| | Obese[c] (n=295) | 67.5 ± 2.2[a] | 42.9 ± 2.7 | 18.7 ± 0.6[a] | 373.3 ± 3.8 |
| **Post – Female** | Normal[a] (n=70) | 88.2 ± 3.8[c] | 99.5 ± 9.9 | 25.5 ± 1.2[c] | 356.3 ± 7.0 |
| | Overweight[b] (n=87) | 83.3 ± 2.6 | 112.8 ± 13.1 | 23.5 ± 0.8 | 359.6 ± 5.8 |
| | Obese[c] (n=174) | 75.5 ± 2.2[a] | 113.5 ± 12.9 | 21.4 ± 0.6[a] | 357.4 ± 4.2 |
| **Male** | Normal[a] (n = 318) | 95.6 ± 2.5 | 179.9 ± 11.1 | 29.4 ± 0.8[c] | 330.7 ± 3.0[c] |
| | Overweight[b] (n=332) | 93.8 ± 2.0 | 181.0 ± 10.7 | 28.1 ± 0.6 | 336.8 ± 2.7 |
| | Obese[c] (n=300) | 90.3 ± 2.0 | 200.6 ± 10.9 | 27.0 ± 0.7[a] | 340.6 ± 2.9[a] |

[1] = All values are $\bar{x}$ ± SEM

Within a gender/menopausal status group, superscript letters indicate significant difference

Normal[a] = Underweight + Normal weight (<18.5 - 24.9), Overweight[b] = 25.0 - 29.9, Obese[c] = 30.0+

Pre - Female = Premenopausal Females, Post - Female = Postmenopausal Female

Significance $P < 0.05$

**TABLE 7**

Differences in iron status by race and classification for BMI categorization[1]

| | BMI (n) | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **White** | Normal[a] (n = 206) | $96.7 \pm 3.0^{cxyz}$ | $110.9 \pm 9.7^{xy}$ | $28.7 \pm 0.9^{cyz}$ | $345.7 \pm 3.9^{z}$ |
| | Overweight[b] (n = 240) | $93.0 \pm 2.3^{cxz}$ | $120.2 \pm 10.8^{xy}$ | $27.0 \pm 0.7^{cyz}$ | $351.8 \pm 3.5$ |
| | Obese[c] (n = 323) | $84.2 \pm 2.1^{abz}$ | $128.8 \pm 8.8$ | $24.4 \pm 0.7^{abxz}$ | $355.1 \pm 2.8^{x}$ |
| **Black** | Normal[x] (n = 336) | $89.5 \pm 2.3^{ayz}$ | $149.1 \pm 10.2^{abz}$ | $26.8 \pm 0.7^{cyz}$ | $344.9 \pm 3.4^{cz}$ |
| | Overweight[y] (n = 323) | $82.3 \pm 2.0^{abxz}$ | $151.6 \pm 10.0^{abz}$ | $24.1 \pm 0.6^{abxz}$ | $352.1 \pm 3.5$ |
| | Obese[z] (n = 446) | $73.8 \pm 1.7^{abcxy}$ | $114.3 \pm 7.7^{xy}$ | $21.2 \pm 0.5^{abcxy}$ | $358.2 \pm 3.1^{ax}$ |

[1] = All values are $\bar{x} \pm$ SEM

Within a group, superscript letters indicate significant difference

Significance $P < 0.05$

**TABLE 8**
Differences in iron status by socioeconomic identifiers[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Poverty** | Above 125% | 86.3 | 133.7 | 25.3 | 350.5 |
| | Below 125% | 82.7 | 123.8 | 24.1 | 354.4 |
| | ***P*** | **0.05** | **0.22** | **0.03** | **0.17** |
| **Education** | <High School | 85.4 | 134.5 | 25.0 | 352.2 |
| | High School + | 84.7 | 127.7 | 24.7 | 352.0 |
| | ***P*** | **0.72** | **0.42** | **0.70** | **0.94** |
| **Employment** | Yes | 88.1 | 123.0 | 25.7 | 350.8 |
| | No | 80.1 | 140.2 | 23.5 | 354.0 |
| | ***P*** | **<0.0001** | **0.03** | **<0.0001** | **0.25** |

**TABLE 9**

Differences in iron status by gender and pre/postmenopausal classification for employment[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Pre – Female** | Employed (n=356) | 78.7 ± 2.2 | 41.8 ± 2.9 | 21.6 ± 0.6 | 374.4 ± 3.3 |
| | Unemployed (n=237) | 66.9 ± 2.3 | 60.9 ± 6.8 | 19.0 ± 0.7 | 374.9 ± 4.8 |
| ***P* value** | | **0.0002** | **0.1471** | **0.0063** | **0.9124** |
| **Post – Female** | Employed (n=181) | 82.6 ± 2.1 | 103.5 ± 8.1 | 23.4 ± 0.6 | 358.3 ± 4.2 |
| | Unemployed (n=150) | 77.5 ± 2.3 | 118.7 ± 14.3 | 22.1 ± 0.7 | 357.1 ± 4.4 |
| ***P* value** | | **0.2203** | **0.3820** | **0.2758** | **0.8393** |
| **Male** | Employed (n=592) | 95.3 ± 1.6 | 177.9 ± 7.2 | 28.9 ± 0.5 | 334.2 ± 1.9 |
| | Unemployed (n=358) | 89.9 ± 2.0 | 201.6 ± 11.7 | 27.0 ± 0.6 | 338.9 ± 3.0 |
| ***P* value** | | **0.0301** | **0.0241** | **0.0131** | **0.2247** |

[1] = All values are $\bar{x}$ ± SEM

Pre - Female = Premenopausal Females, Post - Female = Postmenopausal Female

**TABLE 10**
Differences in iron status by race, and employment status, for females, including pre- and post-menopausal distinction[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Pre-menopausal** | AA‡ – Employed (n=215)[a] | 73.0 ± 3.0[c] | 43.0 ± 4.3 | 19.9 ± 0.7[c] | 378.3 ± 4.7 |
| | Unemployed (n=156)[b] | 65.8 ± 2.9[c] | 71.6 ± 9.9 | 18.7 ± 0.9[c] | 376.1 ± 6.1 |
| | White – Employed (n=141)[c] | 87.4 ± 3.3[abd] | 40.0 ± 3.6 | 24.0 ± 0.9[abd] | 368.4 ± 4.5 |
| | Unemployed (n=81)[d] | 69.0 ± 4.0[c] | 40.4 ± 5.6 | 19.5 ± 1.3[c] | 372.6 ± 7.7 |
| **Post-menopausal** | AA – Employed (n=98)[a] | 82.0 ± 2.8 | 126.9 ± 13.9[c] | 23.2 ± 0.8 | 359.2 ± 6.2 |
| | Unemployed (n=77)[b] | 74.9 ± 3.1 | 143.3 ± 26.0[cd] | 21.9 ± 0.9 | 349.0 ± 6.9 |
| | White – Employed (n=83)[c] | 83.2 ± 3.3 | 75.8 ± 5.4[ab] | 23.7 ± 1.0 | 357.4 ± 5.6 |
| | Unemployed (n=73)[d] | 80.2 ± 3.4 | 92.7 ± 9.9[b] | 22.2 ± 1.0 | 365.5 ± 5.3 |

[1] = All values are $\bar{x}$ ± SEM
‡African American
Within a menopausal group, superscript letters indicate significant difference
Pre = Premenopausal Females, Post = Postmenopausal Female
Significance $P < 0.05$

**TABLE 11**

Differences in iron status by race, and employment status, for males[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **AA‡** | Employed (n=326)[a] | 89.2 ± 2.1[c] | 179.5 ± 10.1 | 27.0 ± 0.6[c] | 333.5 ± 2.8 |
| | Unemployed (n=233)[b] | 89.0 ± 2.5[c] | 204.6 ± 13.9[c] | 26.8 ± 0.8[c] | 337.2 ± 3.8 |
| **White** | Employed (n=266)[c] | 102.9 ± 2.5[abd] | 175.9 ± 10.2[b] | 31.1 ± 0.8[abd] | 355.0 ± 2.7 |
| | Unemployed (n=125)[d] | 91.5 ± 3.3[c] | 196.1 ± 21.4 | 27.3 ± 1.1[c] | 342.1 ± 4.8 |

[1] = All values are $\bar{x}$ ± SEM

‡African American

Within a group, superscript letters indicate significant difference

Significance $P < 0.05$

**TABLE 12**

Differences in iron status by sex with pre/post-menopausal classification for poverty[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Pre – Female** | +125% Poverty (n=329) | 76.5 ± 2.1 | 46.7 ± 3.8 | 21.4 ± 0.6 | 371.2 ± 3.5 |
| | -125% Poverty (n=264) | 70.9 ± 2.6 | 52.9 ± 5.6 | 19.5 ± 0.7 | 378.9 ± 4.4 |
| ***P* value** | | **0.0750** | **0.6303** | **0.0400** | **0.1028** |
| **Post – Female** | +125% Poverty (n=210) | 80.4 ± 1.9 | 105.3 ± 7.1 | 22.9 ± 0.6 | 358.5 ± 3.7 |
| | -125% Poverty (n=121) | 80.0 ± 2.8 | 119.1 ± 17.7 | 22.8 ± 0.8 | 356.5 ± 5.5 |
| ***P* value** | | **0.9310** | **0.4437** | **0.9402** | **0.7607** |
| **Male** | +125% Poverty (n = 603) | 93.7 ± 1.6 | 191.1 ± 8.3 | 28.3 ± 0.5 | 336.5 ± 2.0 |
| | -125% Poverty (n=347) | 92.6 ± 2.2 | 179.4 ± 9.4 | 28.0 ± 0.6 | 335.0 ± 3.0 |
| ***P* value** | | **0.6595** | **0.2721** | **0.7020** | **0.6994** |

[1] = All values are $\bar{x}$ ± SEM

Pre - Female = Premenopausal Females, Post - Female = Postmenopausal Female

**TABLE 13**

Differences in iron status by race, and poverty status, for females, including pre/post-menopausal distinction[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Pre-Menopausal** | AA‡ +125% (n=180)[a] | 70.9 ± 2.8[c] | 52.3 ± 6.3 | 20.2 ± 0.9[c] | 371.7 ± 5.1 |
| | -125% (n=191)[b] | 69.1 ± 3.2[c] | 57.6 ± 7.4 | 18.7 ± 0.8[c] | 382.8 ± 5.3 |
| | White +125% (n=149)[c] | 83.2 ± 3.2[ab] | 39.9 ± 3.6 | 22.9 ± 0.9[ab] | 370.6 ± 4.6 |
| | -125% (n=73)[d] | 75.6 ± 4.5 | 40.7 ± 5.7 | 21.5 ± 1.4 | 368.7 ± 7.8 |
| **Post-Menopausal** | AA +125% (n=109)[a] | 77.5 ± 2.3 | 127.5 ± 12.2[c] | 22.0 ± 0.7 | 357.1 ± 5.3 |
| | -125% (n=66)[b] | 81.1 ± 4.0 | 145.0 ± 30.7[cd] | 23.7 ± 1.1 | 350.8 ± 8.6 |
| | White +125% (n=101)[c] | 83.5 ± 3.0 | 81.4 ± 5.8[ab] | 23.7 ± 1.0 | 360.0 ± 5.0 |
| | -125% (n=55)[d] | 78.7 ± 3.8 | 88.0 ± 11.5[b] | 21.7 ± 1.0 | 363.3 ± 6.2 |

[1] = All values are $\bar{x}$ ± SEM
‡African American
Within a menopausal group, superscript letters indicate significant difference
Pre = Premenopausal Females, Post = Postmenopausal Females
Significance $P < 0.05$

**TABLE 14**

Differences in iron status by race, and poverty status, for males[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **AA‡** | +125% Poverty (n=322)[a] | 88.3 ± 2.1[cd] | 199.3 ± 11.8 | 26.5 ± 0.6[c] | 337.5 ± 2.8 |
| | -125% Poverty (n=237)[b] | 90.2 ± 2.5[c] | 177.2 ± 11.1 | 27.6 ± 0.8[c] | 331.8 ± 3.8 |
| **White** | +125% Poverty (n=281)[c] | 99.9 ± 2.3[ab] | 181.6 ± 11.7 | 30.3 ± 0.8[ab] | 335.4 ± 2.8 |
| | -125% Poverty (n=110)[d] | 97.7 ± 4.1[a] | 184.3 ± 17.6 | 28.9 ± 1.2 | 342.0 ± 4.7 |

[1] = All values are $\bar{x}$ ± SEM

‡African American

Within a group, superscript letters indicate significant difference

Significance $P < 0.05$

**TABLE 15**

Differences in iron status by gender and pre/postmenopausal classification for education[1]

| | Education | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Pre – Female** | <HS (n=163) | 73.8 ± 3.0 | 48.2 ± 5.6 | 20.2 ± 0.9 | 381.1 ± 5.3 |
| | HS+ (n=430) | 74.1 ± 2.0 | 49.9 ± 4.0 | 20.7 ± 0.5 | 372.2 ± 3.3 |
| ***P* value** | | **0.9354** | **0.9034** | **0.6160** | **0.0917** |
| **Post – Female** | <HS (n=104) | 78.5 ± 2.7 | 122.1 ± 19.6 | 22.3 ± 0.8 | 357.0 ± 5.7 |
| | HS+ (n=227) | 81.1 ± 1.9 | 105.0 ± 7.2 | 23.0 ± 0.6 | 358.1 ± 3.6 |
| ***P* value** | | **0.5598** | **0.3609** | **0.5902** | **0.8765** |
| **Male** | <HS (n = 314) | 93.7 ± 2.3 | 183.5 ± 9.2 | 28.3 ± 0.7 | 335.6 ± 3.2 |
| | HS+ (n=636) | 93.1 ± 1.5 | 188.5 ± 8.2 | 28.1 ± 0.5 | 336.1 ± 2.0 |
| ***P* value** | | **0.8282** | **0.6481** | **0.7521** | **0.8915** |

[1] = All values are $\bar{x}$ ± SEM
Within a gender/menopausal status group, superscript letters indicate significant difference
Pre - Female = Premenopausal Females, Post - Female = Postmenopausal Female
<HS = Less than a high school education, HS+ = Equal to or greater than a high school education
Significance $P < 0.05$

**TABLE 16**

Differences in iron status by race, and education, for females, including pre/post-menopausal distinction[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **Pre-menopausal** | AA‡ <HS (n=107)[a] | 73.3 ± 4.0[d] | 51.3 ± 7.6 | 20.1 ± 1.1[d] | 383.5 ± 6.9[d] |
| | HS+ (n=264)[b] | 68.7 ± 2.5[d] | 56.6 ± 6.1 | 19.2 ± 0.7[d] | 374.9 ± 4.4 |
| | White <HS (n=56)[c] | 74.7 ± 4.6 | 42.3 ± 7.4 | 20.4 ± 1.3 | 376.4 ± 8.0 |
| | HS+ (n=166)[d] | 82.7 ± 3.1[ab] | 39.4 ± 3.2 | 23.1 ± 0.9[ab] | 367.8 ± 4.6[a] |
| **Post-menopausal** | AA <HS (n=52)[a] | 71.5 ± 3.1 | 155.2 ± 37.7[cd] | 20.9 ± 1.0 | 351.1 ± 9.3 |
| | HS+ (n=123)[b] | 82.0 ± 2.6 | 125.2 ± 11.6[d] | 23.4 ± 0.8 | 356.2 ± 5.3 |
| | White <HS (n=52)[c] | 85.4 ± 4.4 | 88.9 ± 8.9[a] | 23.8 ± 1.3 | 363.0 ± 6.7 |
| | HS+ (n=104)[d] | 80.0 ± 2.8 | 81.1 ± 6.9[ab] | 22.6 ± 0.9 | 360.3 ± 4.8 |

[1] = All values are $\bar{x}$ ± SEM
‡African American
Within a group, superscript letters indicate significant difference
Pre - Female = Premenopausal Females, Post - Female = Postmenopausal Female
<HS = Less than a high school education, HS+ = Equal to or greater than a high school education
Significance $P < 0.05$

**TABLE 17**

Differences in iron status by race, and education, for males[1]

| | | Iron (mcg/dL) | Ferritin (ng/mL) | Iron Saturation % | Total Iron Binding Capacity (mcg/dL) |
|---|---|---|---|---|---|
| **AA‡** | <HS (n=192)[a] | 89.0 ± 2.8[cd] | 177.6 ± 11.4 | 27.1 ± 0.9[cd] | 333.9 ± 4.4 |
| | HS+ (n=367)[b] | 89.2 ± 1.9[cd] | 196.4 ± 11.1 | 26.9 ± 0.6[cd] | 335.7 ± 2.6 |
| **White** | <HS (n=122)[c] | 101.1 ± 4.0[ab] | 192.8 ± 15.5 | 30.4 ± 1.2[ab] | 338.3 ± 4.5 |
| | HS+ (n=269)[d] | 98.5 ± 2.3[ab] | 177.6 ± 12.3 | 29.7 ± 0.8[ab] | 336.8 ± 2.8 |

[1] = All values are $\bar{x}$ ± SEM
‡African American
Within a group, superscript letters indicate significant difference
<HS = Less than a high school education, HS+ = Equal to or greater than a high school education
Significance $P < 0.05$

**TABLE 18**
Average 10-year cardiovascular risk and relative risk overall by sex and race[1]

| | Women (n=924) | Men (n=950) | *P* | AA‡ (n=1105) | *P* | White (n=769) |
|---|---|---|---|---|---|---|
| **10 Year Risk** | 4.6 ± 0.2 | 7.9 ± 0.2 | <0.0001 | 5.8 ± 0.2 | <0.0001 | 6.9 ± 0.2 |
| **Relative Risk** | 1.3 ± 0.03 | 1.6 ± 0.03 | <0.0001 | 1.3 ± 0.02 | <0.0001 | 1.6 ± 0.04 |

[1] = All values are $\bar{x}$ ± SEM
‡African American
10 Year Risk = Average risk for developing coronary heart disease based on the Framingham Heart Study's 10-year risk equations

**TABLE 19**

Average 10-year cardiovascular risk and relative risk overall including sex and race[1]

| | | 10 Year Risk | Relative Risk |
|---|---|---|---|
| **Female** | White (n=378)[a] | $4.8 \pm 0.3^{cd}$ | $1.4 \pm 0.05^{c}$ |
| | AA‡ (n=546)[b] | $4.4 \pm 0.2^{cd}$ | $1.3 \pm 0.03^{cd}$ |
| **Male** | White (n=391)[c] | $8.9 \pm 0.3^{abd}$ | $1.7 \pm 0.06^{abd}$ |
| | AA (n=559)[d] | $7.2 \pm 0.2^{abc}$ | $1.4 \pm 0.04^{bc}$ |

[1] = All values are $\bar{x} \pm$ SEM

‡African Americans

10 Year Risk = Average risk for developing coronary heart disease based on the Framingham Heart Study's 10-year risk equations

Superscript letters indicate significance

Significance $P < 0.05$

**TABLE 20**
Comparative 10-year risk between Framingham and HANDLS – Males

| | Average 10-year risk for developing coronary heart disease | | | |
|---|---|---|---|---|
| Age (years) | **Overall Framingham** | **Overall HANDLS** | **Whites HANDLS** | **AA[a] HANDLS** |
| **30-34** | 3% | 4% | 3% | 4% |
| **35-39** | 5% | 4% | 4% | 4% |
| **40-44** | 7% | 5% | 7% | 5% |
| **45-49** | 11% | 7% | 9% | 6% |
| **50-54** | 14% | 9% | 11% | 10% |
| **55-59** | 16% | 11% | 11% | 11% |
| **60-64** | 21% | 14% | 15% | 13% |

Source: Modified based on Coronary Disease Risk Prediction Score Sheet for Men Based on Total Cholesterol Level (www.nhlbi.nih.gov/about/framingham/risktmen.pdf) in comparison with HANDLS data
[a]African Americans

**TABLE 21**
Comparative 10-year risk between Framingham and HANDLS – Females

| | Average 10-year risk for developing coronary heart disease | | | |
|---|---|---|---|---|
| Age (years) | **Overall Framingham** | **Overall HANDLS** | **Whites HANDLS** | **AA[a] HANDLS** |
| **30-34** | <1% | 1% | 1% | 1% |
| **35-39** | 1% | 1% | 1% | 1% |
| **40-44** | 2% | 2% | 2% | 2% |
| **45-49** | 5% | 4% | 5% | 4% |
| **50-54** | 8% | 7% | 7% | 7% |
| **55-59** | 12% | 8% | 7% | 8% |
| **60-64** | 12% | 11% | 9% | 12% |

Source: Modified based on Coronary Disease Risk Prediction Score Sheet for Women Based on Total Cholesterol Level (www.nhlbi.nih.gov/about/framingham/risktwomen.pdf) in comparison with HANDLS data
[a]African Americans

**TABLE 22**
Average 10-year coronary heart disease risk and relative risk overall by sex with menopausal distinction[1]

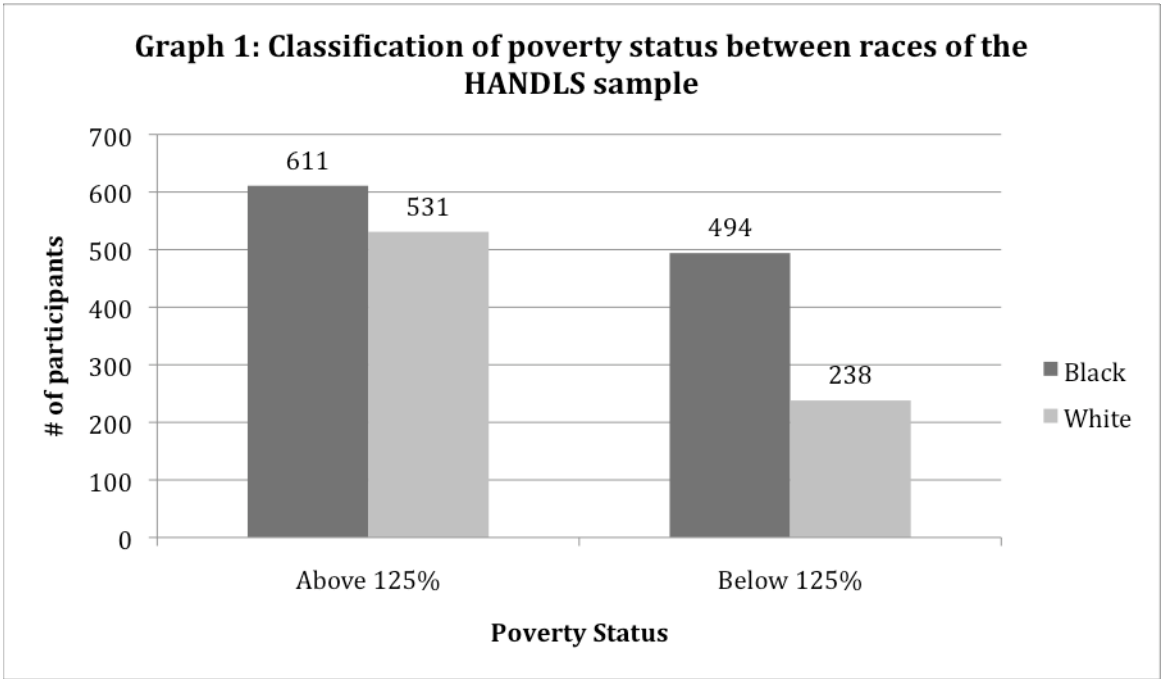
| | 10 Year Risk | Relative Risk |
|---|---|---|
| **Pre-Female[a] (n=593)** | 2.9 ± 0.1[bc] | 1.3 ± 0.03[c] |
| **Post-Female[b] (n=331)** | 7.6 ± 0.3[a] | 1.3 ± 0.05[c] |
| **Male[c] (n=950)** | 7.9 ± 0.2[a] | 1.6 ± 0.03[ab] |

[1]10-year Risk = Risk for developing coronary heart disease based on Framingham Heart Study's risk equations.
Relative Risk = Absolute risk divided by that of a low risk group.

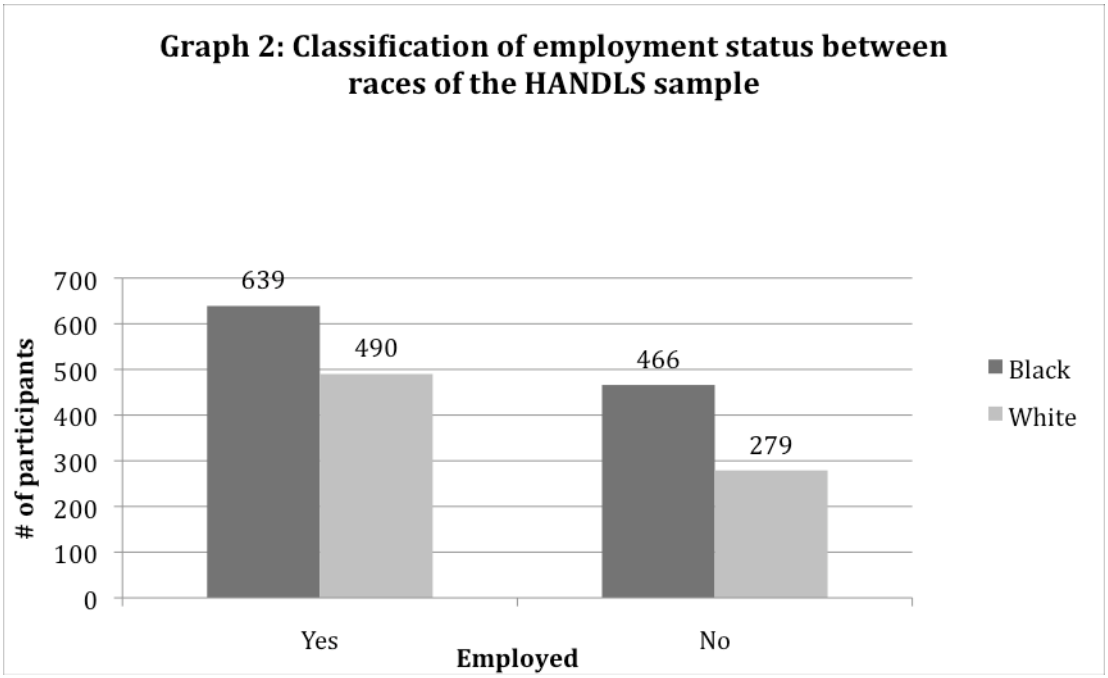
**TABLE 23**
Average 10-year risk for the development of coronary heart disease and relative risk with race, sex,
and menopausal distinction[1]

| | 10 Year Risk | Relative Risk |
|---|---|---|
| **Pre – Female** | | |
| White (n=222) | 2.9 ± 0.2 | 1.4 ± 0.06 |
| AA[2] (n=371) | 2.9 ± 0.2 | 1.2 ± 0.04 |
| ***P*** | **0.9913** | **0.0535** |
| **Post - Female** | | |
| White (n=156) | 7.6 ± 0.4 | 1.3 ± 0.08 |
| AA[2] (n=175) | 7.7 ± 0.4 | 1.3 ± 0.06 |
| ***P*** | **0.8758** | **0.7112** |
| **Male** | | |
| White (n=391) | 8.9 ± 0.3 | 1.7 ± 0.06 |
| AA[2] (n=559) | 7.2 ± 0.2 | 1.4 ± 0.04 |
| ***P*** | **<0.0001** | **<0.0001** |

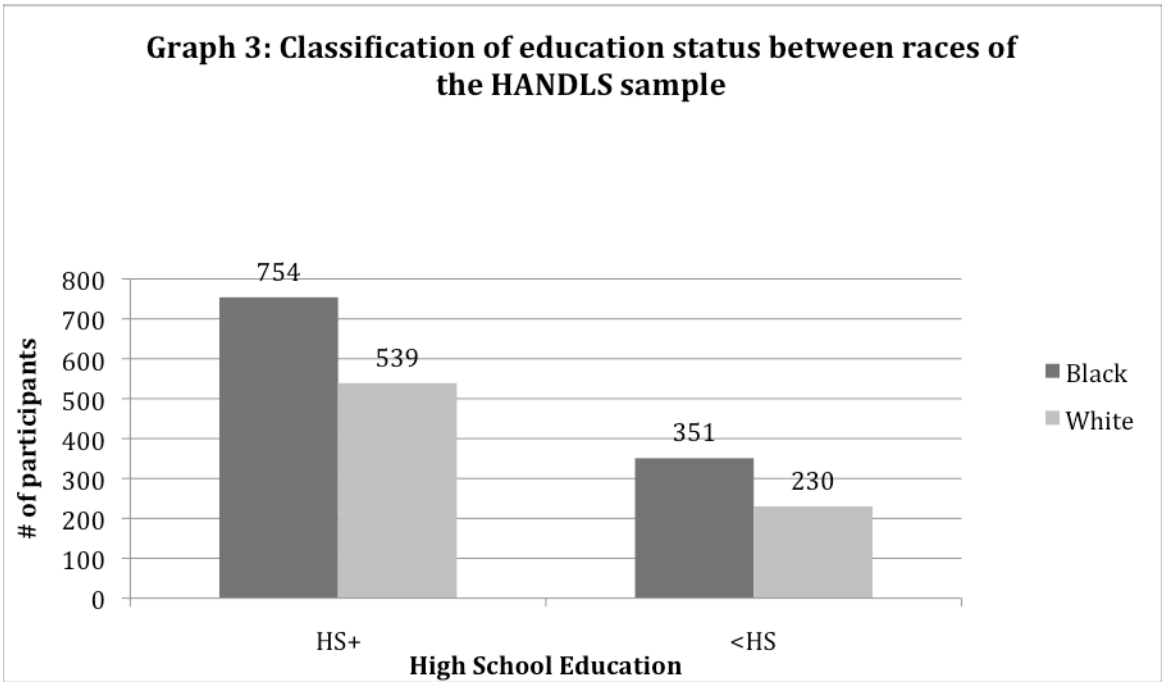
[1] = All values are $\bar{x}$ ± SEM
[2]African American



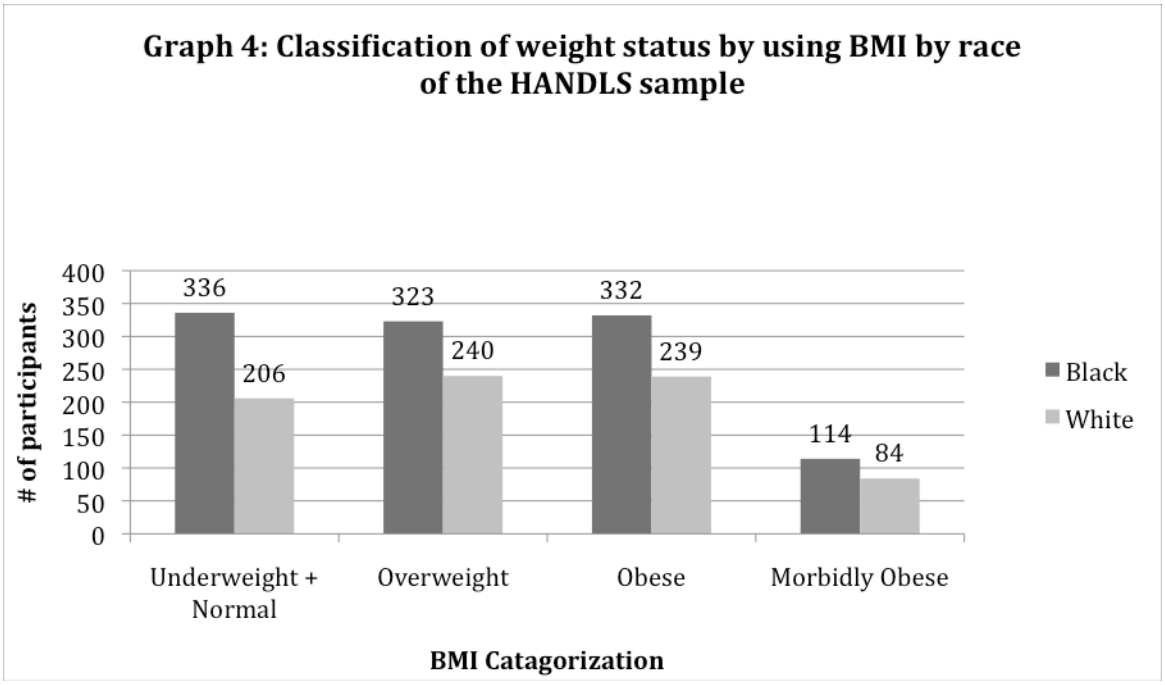

Chi Square Value = 36.0 ***P*** = <0.0001 DF = 1




Chi Square Value = 6.6 ***P*** = 0.0104 DF = 1

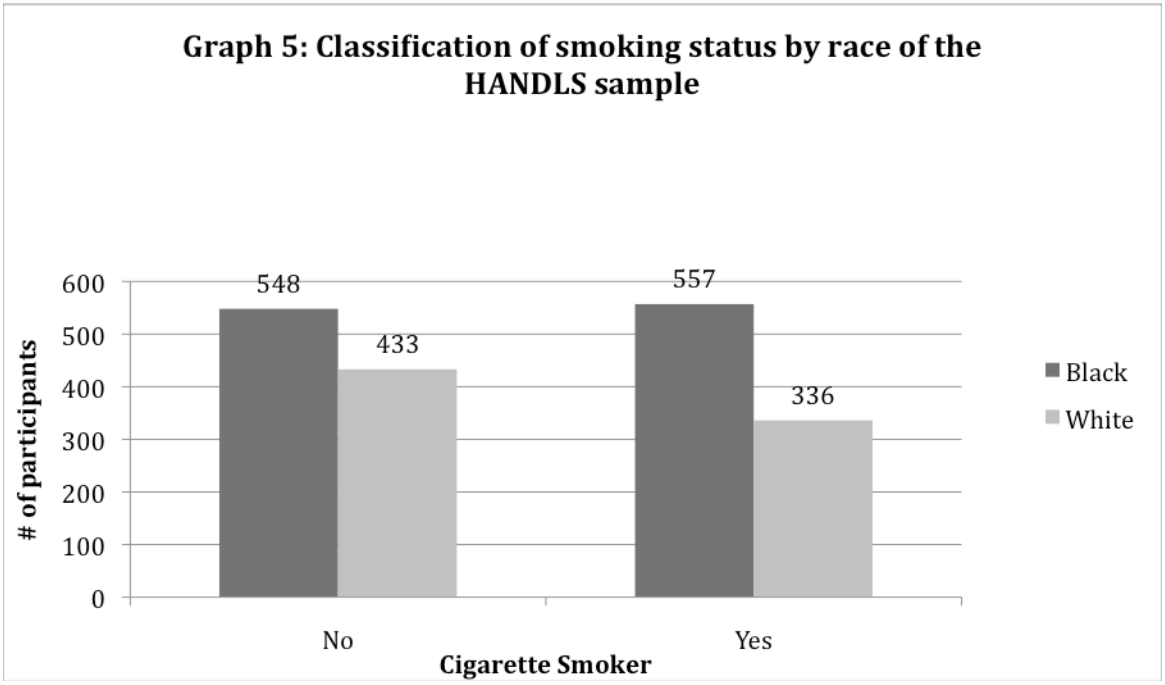



Chi Square Value = 0.7 ***P*** = 0.3929 DF = 1

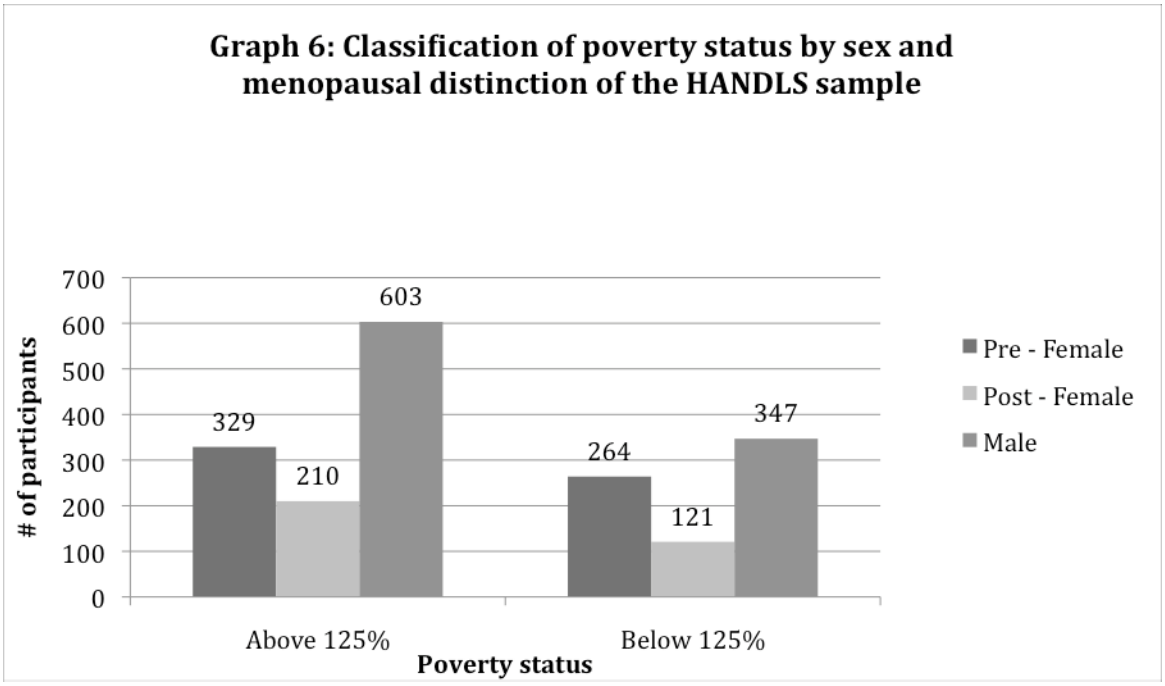



Chi Square Value = 3.0 ***P*** = 0.3976 DF = 3

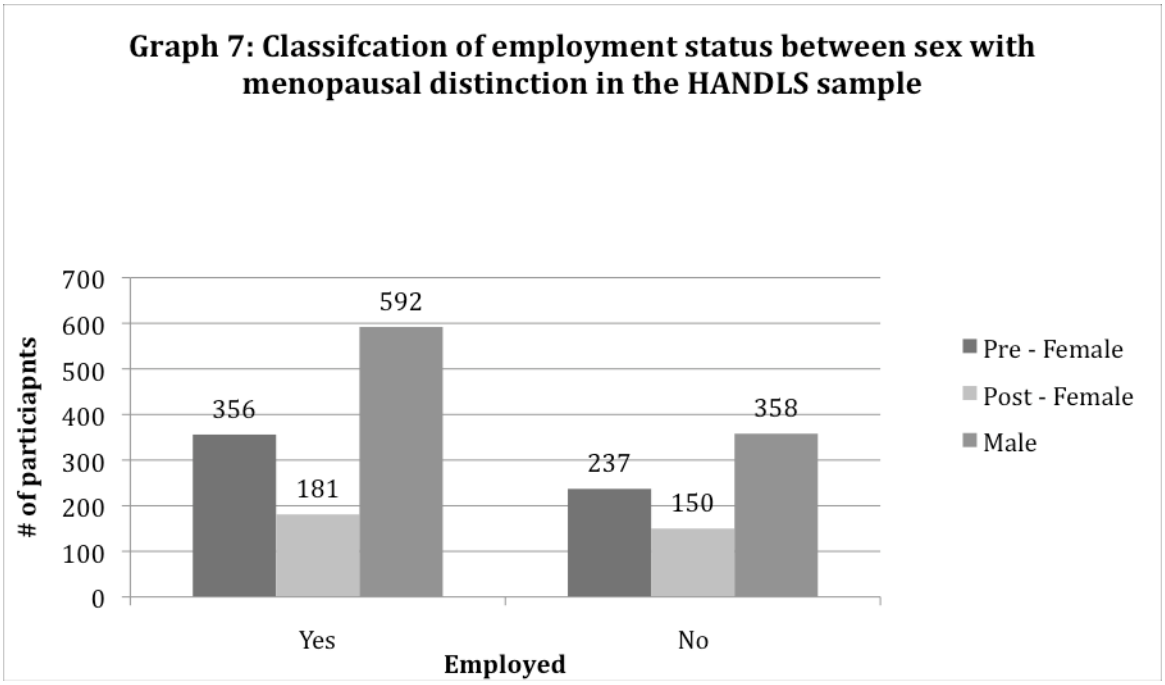
BMI Categories: Underweight = <18.5 – 24.9, Overweight = 25.0 – 29.9, Obese = 30.0 - <40.0, Morbidly Obese = 40+



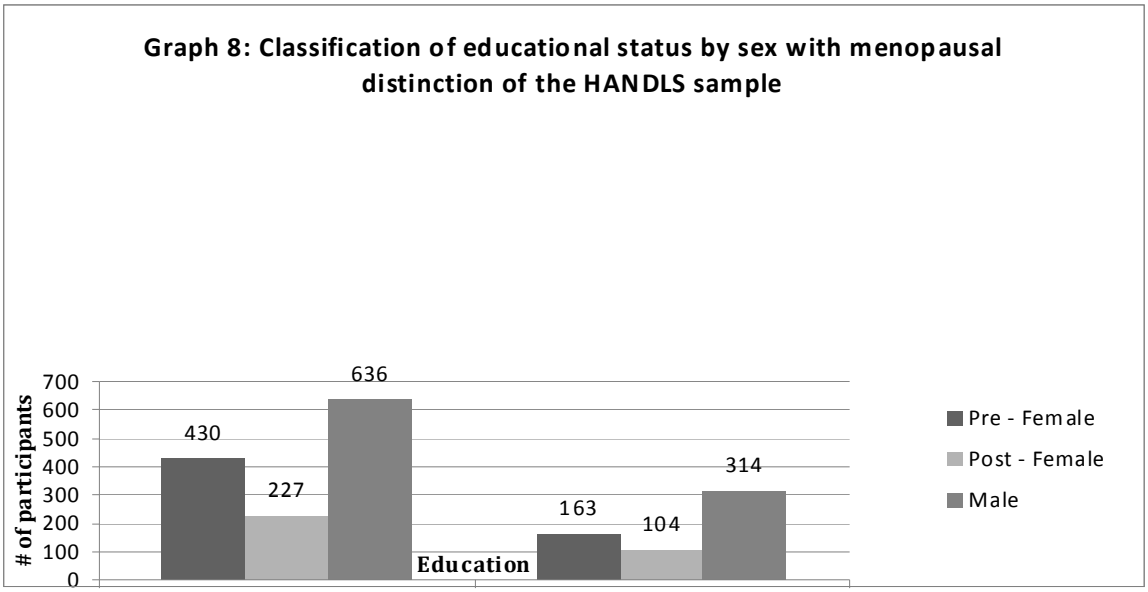

Chi Square = 8.2 ***P*** = 0.0042 DF = 1



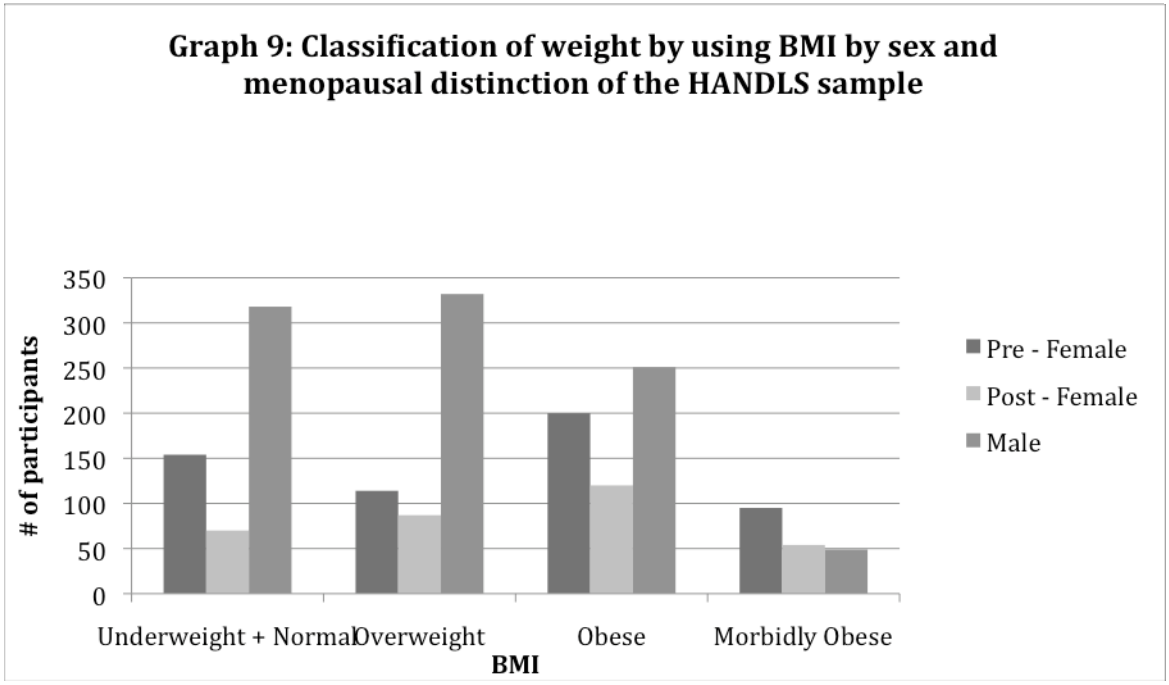

Chi Square = 10.9 ***P*** = 0.0044 DF = 2



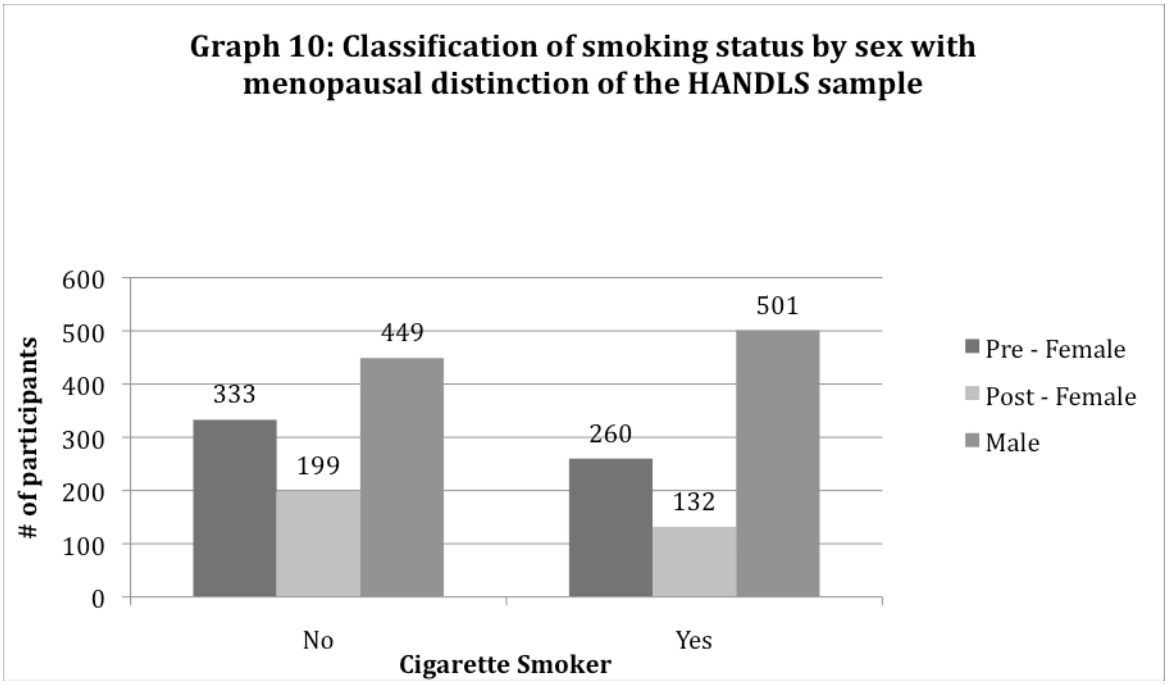

Chi square = 6.0 ***P*** = 0.0501 DF = 2



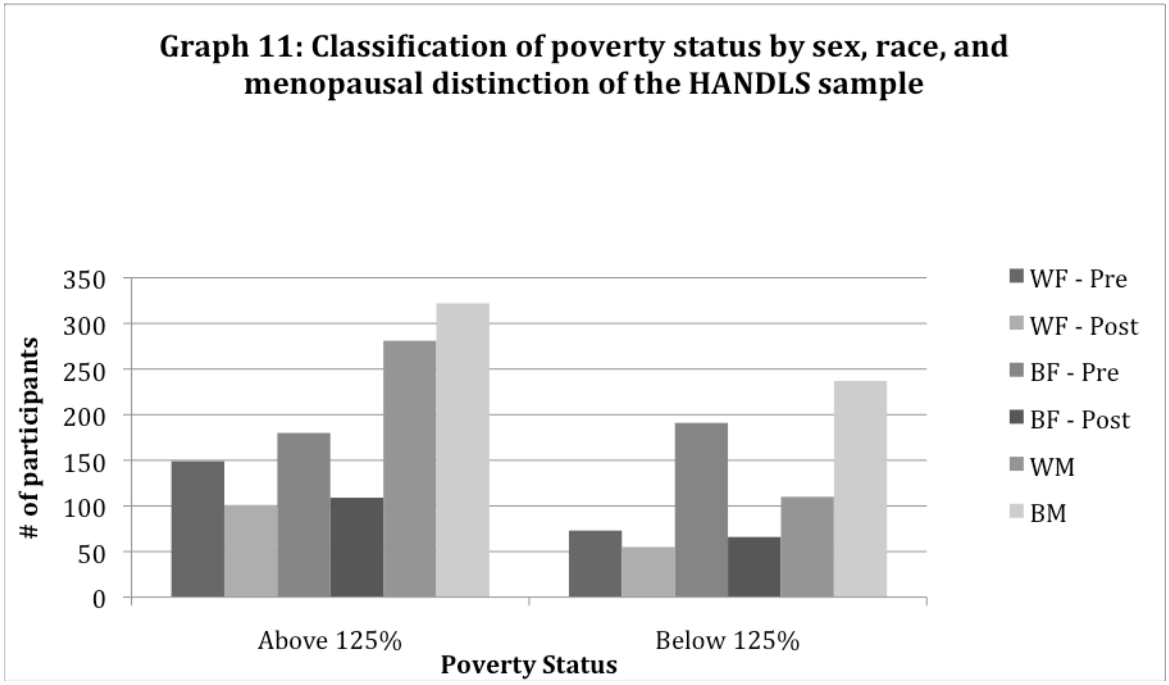

Chi square = 5.3 ***P*** = 0.0700 DF = 2



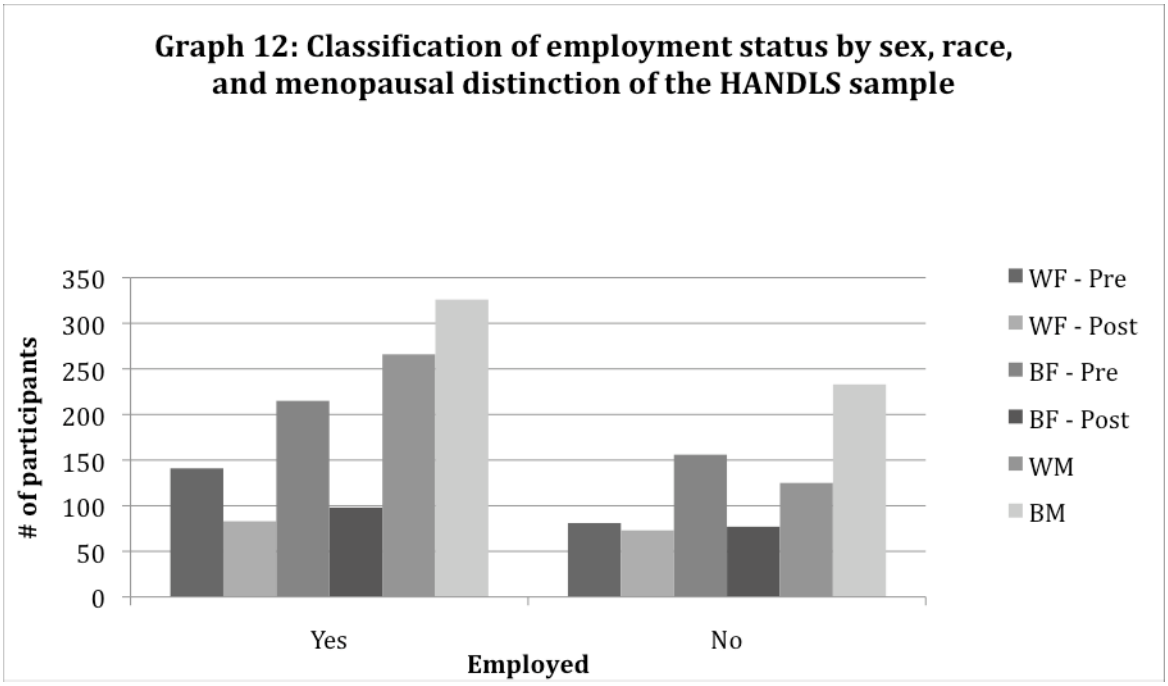

Chi square = 95.4 ***P*** = <0.001 DF = 6



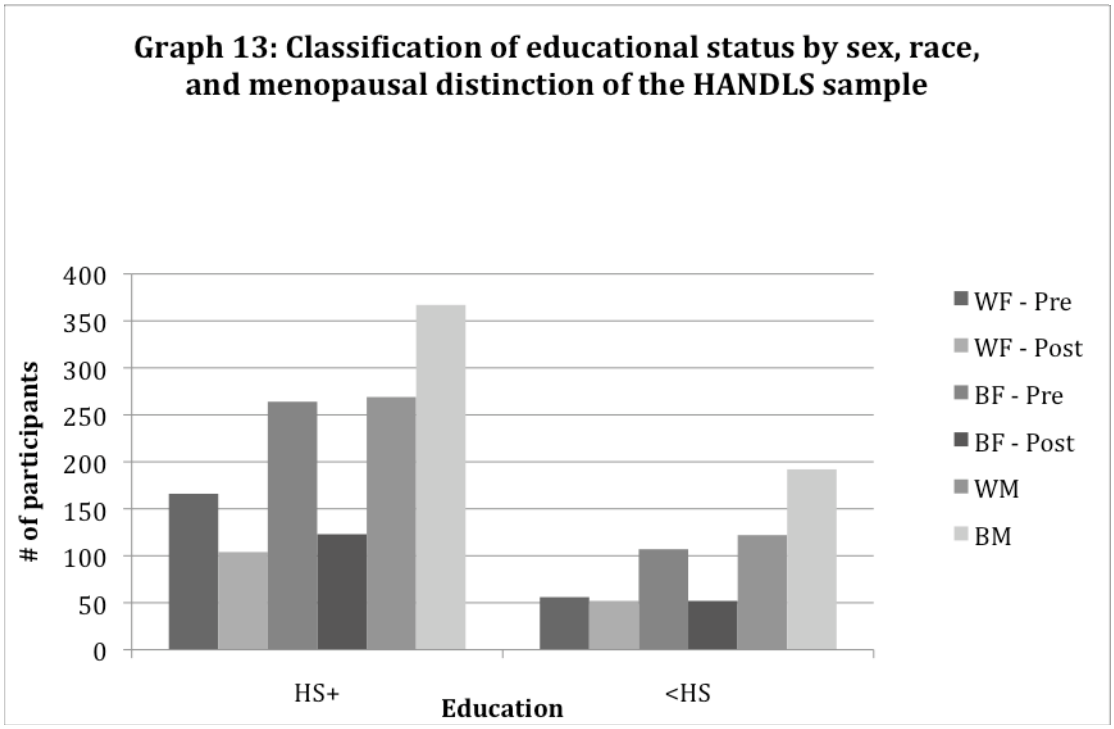

Chi square = 21.3 ***P*** = <0.0001 DF = 2



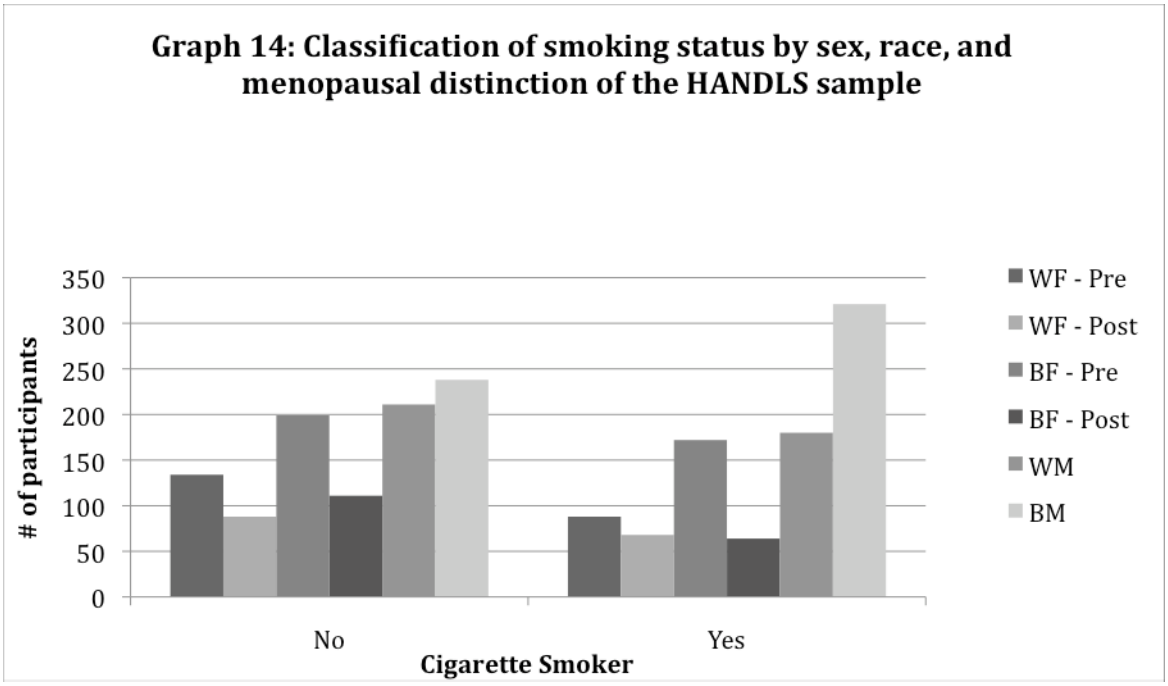

Chi square = 50.9 ***P*** = <0.0001 DF = 5



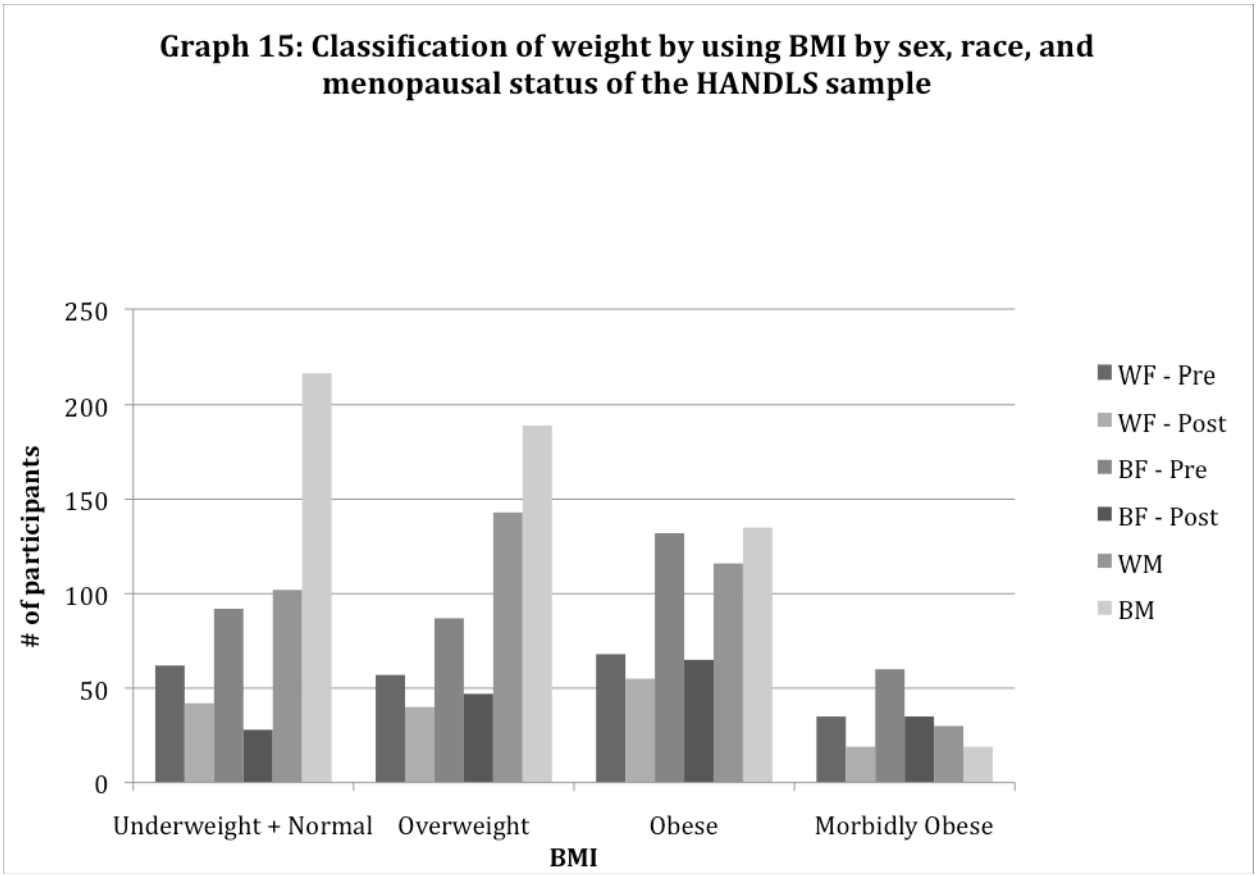

Chi square = 17.1 ***P*** = 0.0043 DF = 5




Chi square = 7.7 ***P*** = 0.1714 DF = 5




Chi square = 37.4 ***P*** = <0.0001 DF = 5




Chi square = 124.9 ***P*** = <0.0001 DF = 15

## Appendix B

## AUTHOR GUIDELINES

**Information for Authors**
**THE AMERICAN JOURNAL OF CLINICAL NUTRITION**

The most up to date guidelines can be found at: http://www.ajcn.org/misc/ifa.shtml.

## INTRODUCTION

The purpose of The American Journal of Clinical Nutrition (AJCN) is to publish original research studies relevant to human and clinical nutrition. Well-controlled clinical studies that describe scientific mechanisms, efficacy, and safety of dietary interventions in the context of disease prevention or a health benefit will be considered. Public health and epidemiologic studies relevant to human nutrition, and innovative investigations of nutritional questions that employ epigenetic, genomic, proteomic, and metabolomic approaches are encouraged. Solicited editorials, book reviews, solicited or unsolicited review articles, invited controversy position papers, and letters to the Editor that relate to prior AJCN articles are essential components of the AJCN. All submitted material with scientific content will undergo peer review by the Editors or their designees before acceptance for publication. Symposia or workshop articles may be published as supplements to the AJCN and are funded by their sponsors at a special page charge. The AJCN welcomes queries about the publication of supplements. The AJCN uses a 2-part acceptance process for supplements. The first step involves editorial acceptance of the topic and content as provided by the symposium organizer; the second step involves anonymous peer review of the individual articles. To be considered for publication, supplement articles must be received within 3 mo of each symposium or workshop. Each manuscript should not exceed 15 text pages, exclusive of tables, figures, and references; must adhere to AJCN style and format; and will be reviewed according to the same scientific standards used to evaluate original research articles. All material to be considered for publication in a regular issue should be submitted electronically at the following website: https://www.rapidreview.com/ASCN2/CALogon.jsp. See "Tips for authors submitting manuscripts to the AJCN" for helpful advice regarding electronic submission. All material to be considered for publication in a supplement issue should be sent to the following address:

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4) participated in a consulting/financial arrangement with an author in the past 3 years;

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6) any other personal, financial or other relationship with one or more of the authors that might influence an unbiased assessment of the current work.

## SUMMARY OF REQUIREMENTS

Each manuscript component should begin on a new page in the following sequence:

Title page
Abstract
Text
Acknowledgments
References
Tables: each table on a separate page, complete with title and footnotes
Legends for figures
Figures

Identify on the title page the author who will be responsible for correspondence regarding the manuscript. The signed Authors' Agreement form and copies of any documents granting permission needed to reproduce material in print and electronic form or to use illustrations of identifiable subjects should be scanned and e-mailed to ajcn@nutrition.org. If scanning is not possible, then the Authors' Agreement form and any necessary documents may be faxed to (301) 634-7892. Authors should keep copies of all submitted material. The AJCN encourages authors to provide the names, fields of interest, addresses, telephone and fax numbers, and e-mail addresses of 4–6 unbiased and qualified potential expert reviewers from outside the authors' institutions.

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The AJCN can publish only about 25% of the more than 1500 original submissions received per year. Submitted manuscripts may be rejected without detailed comments after initial review by at least 2 AJCN editors if the manuscripts are considered inappropriate or of insufficient scientific priority for publication in the AJCN. All other manuscripts undergo a complete review by at least 2 consulting editors or other selected experts. Criteria for acceptance by the AJCN include originality, validity of data, clarity of writing, strength of the conclusions, and potential importance of the work to the field of clinical nutrition. Submitted manuscripts will not be reviewed if they do not conform to standard English usage and to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Internet: http://www.icmje.org/), which is also available free of charge from the Secretariat Office, Annals of Internal Medicine, American College of Physicians, Independence Mall West, Sixth Street at Race, Philadelphia, PA 19106-1572.

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Articles are copyedited according to AJCN style policy, the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," and the style manual of the Council of Science Editors (Scientific style and format: the CSE manual for authors, editors, and publishers. 7th ed. Reston, VA: The Council, 2006). Please refer to the following references for recommendations on reporting the details of randomized trials:

Moher D, Schulz KF, Altman DG; CONSORT GROUP (Consolidated Standards of Reporting Trials). The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. Ann Intern Med 2001;134(8):657–62 or at www.consort-statement.org.

Altman DG, Schulz KF, Moher D, Egger M, Davidoff F, Elbourne D, Gotzsche PC, Lang T; CONSORT GROUP (Consolidated Standards of Reporting Trials). The revised CONSORT statement for reporting randomized trials: explanation and elaboration. Ann Intern Med 2001;134(8):663–94 or at www.consort-statement.org.

Gagnier JJ, Boon H, Rochon P, Moher D, Barnes J, Bombardier C; CONSORT Group. Reporting randomized, controlled trials of herbal interventions: an elaborated CONSORT statement. Ann Intern Med 2006;144(5):364–7 or at www.consort-statement.org.

Please refer to the following references for recommendations on reporting the details of epidemiological and genetics studies:

von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008;61: 344–9. doi:10.1016/j.jclinepi.2007.11.008.

Little J, Higgins JPT, Ioannidis JPA, et al. STrengthening the REporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. Ann Intern Med 2009;150:206–15.

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Scientific conduct

Each author must have participated sufficiently, intellectually or practically, in the work to take public responsibility for the content of the article, including the conception, design, and conduct of the experiment, and for the data interpretation. An article with corporate (collective) authorship must specify the key persons responsible for the article; others contributing to the work should be recognized separately.
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Instructions for manuscript preparation

The manuscript should be formatted as follows: 216 × 279 mm (8½ × 11 in) or ISO A4 (212 × 297 mm), with margins of at least 2.5 cm; use double-spacing and 12-point type throughout. Do not justify the right margin. The abstract and text pages should have line numbers in the left margin. Number pages consecutively in the upper right-hand corner of each page, beginning with the title page. Foreign authors are advised to have their manuscripts reviewed by a scientific colleague who is fluent in English so that the manuscripts will conform to US English usage and grammar.

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The title page should contain: 1) the title of the article, beginning with a key word if possible, with only the first letter of the first word capitalized; 2) The names of all authors (first name, middle initial, last name) including their departmental and institutional addresses. Indicate which authors are associated with which institutions by numbered footnotes. Identify a corresponding author and provide a complete mailing address, telephone number, fax number, and email address. Please note that all authors' names should appear on the manuscript exactly as they should appear in PubMed if the paper is published. ASN will not replace files to correct author names once published. 3) The last name of each author for the purpose of PubMed indexing; 4) the affiliation of each author at the time the work was done, with the use of author initials in parentheses to designate which affiliation corresponds to which author; 5) disclaimers, if any; 6) the name, mailing address, telephone and fax numbers, and e-mail address of the author responsible for correspondence about the manuscript; 7) the name and mailing and e-mail addresses of the author to whom requests for reprints should be addressed or a statement that reprints will not be available from the author; 8) sources of support, including grants, fellowships, and gifts of materials (eg, chemicals, experimental diets); and 9) a short running head of not more than 50 characters (count letters and spaces).

Abstract

A properly constructed and informative abstract is helpful for the initial editorial review of the submitted manuscript. Original research articles must include a structured abstract that contains no more than 250 words, is written in complete sentences, and includes the following headings:

Background: Provide 1 or 2 sentences that explain the context of the study.
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Design: Describe the study design, including the use of cells, animal models, or human subjects. Identify the control group. Identify specific methods and procedures. Describe interventions, if used.
Results: Report the most important findings, including results of statistical analyses.
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Review articles, special articles, and reports should include an unstructured abstract (no more than 250 words) that states the purpose of the article and emphasizes the major concepts and conclusions.

## Text

Use active voice whenever possible. Use past tense when describing and discussing the experimental work on which the article is based. Reserve present tense for reference to existing knowledge or prevailing concepts and for stating conclusions from the experimental work. Clearly differentiate previous knowledge and new contributions. Do not use level when referring to a concentration. Use metric units of measure; SI units are no longer required. The text of observational and experimental articles should be divided into sections with the following headings: Introduction, Subjects (or Materials, for cell or animal studies) and Methods, Results, and Discussion. Long articles may require subheadings within some sections. Authors should consult recent issues of the AJCN for guidance on the formatting of other types of articles, book reviews, and editorials.

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Clearly state the purpose of the article. Summarize the rationale and background for the study or observation, giving only strictly pertinent references. Do not include methods, data, results, or conclusions from the work being reported. The Introduction should be limited to 1.5 manuscript pages.

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Describe clearly your selection of the experimental and control subjects and provide eligibility and exclusion criteria and details of randomization. Describe the methods for, and success of, any masking (blinding) of observations. Report any complications of experimental treatments. Identify the methods, apparatus (manufacturer's name and location in parentheses), and procedures in sufficient detail to allow other researchers to reproduce the results. Do not use trademark names, such as Teflon, as generic terms. Give references for established methods, including statistical methods; provide references and brief descriptions of methods that have been published but are not well known; and describe new or substantially modified methods, giving reasons for using them and evaluating their limitations. Identify precisely all drugs and chemicals used, including generic names, dosages, and routes of administration. If trade names for drugs and chemicals are included, give the manufacturer's name and location.

Ethics. When reporting experiments on human subjects, indicate that the procedures followed were in accordance with the ethical standards of the responsible institutional or regional committee on human experimentation or in accordance with the Helsinki Declaration of 1975 as revised in 1983. Do not use patients' names, initials, or hospital identification numbers. When reporting experiments on animals, indicate approval by the institution's animal welfare committee and state whether the National Research Council's guide for the care and use of laboratory animals was followed.

Clinical Trials. The AJCN requires registration of all clinical trials that begin after July 1,

2008 in the appropriate public trials registry. Such registries include those maintained by the US National Library of Medicine (http://www.clinicaltrials.gov) and Current Controlled Trials (http://controlled-trials.com). Prior to July 1, 2008, the AJCN strongly recommends that such trials be registered.

Statistics. Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (eg, CIs, SDs, or SEs), even for differences that were not significant. Report the numbers of observations. Specify any general-use computer programs used, including the version number and the manufacturer's name and location. Include general descriptions of statistical methods in the

Subjects (or Materials) and Methods section and specific descriptions in each table and figure legend. Indicate whether variables were transformed for analysis. Provide details about what hypotheses were tested, what statistical tests were used, and what the outcome and explanatory variables were (where appropriate). Indicate the level of significance used in tests if different from the conventional 2-sided 5% alpha error and whether or what type of adjustment is made for multiple comparisons. When data are summarized in the Results section, specify the statistical methods used to analyze them. Avoid nontechnical uses of technical statistical terms, such as random (which implies a randomizing device), normal, significant, correlation, sample, and parameter. Define statistical terms, abbreviations, and symbols not listed under "Commonly used approved abbreviations" below. Detailed statistical analyses, mathematical derivations, and the like may sometimes be suitably presented as one or more appendixes.

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Present your results in a logical sequence in the text, tables, and figures. Do not present specifics of data more than once and do not duplicate data from tables or figures in the text; emphasize or summarize only important observations. Do not present data from individual subjects except for very compelling reasons. Report losses to observation (such as dropouts from a clinical trial). Use boldface for the first mention of each table or figure.

Discussion

The Discussion should not exceed 4 typewritten pages except in unusual circumstances as approved by the Editor. Emphasize concisely the novel and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results. Include the implications of the findings and their limitations and relate the observations to other relevant studies. Link conclusions with the goals of the study and avoid unqualified statements and conclusions that are not completely supported by the data. Avoid claiming priority and alluding to work that has

not been completed. State new hypotheses and recommendations when warranted by the results and label them clearly as such.

Acknowledgments

Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name and for providing to the Editor a copy of the permission, if requested. Each author is required to list his or her contribution to the work (such as design of the experiment, collection of data, analysis of data, writing of the manuscript, or provision of significant advice or consultation) and to disclose any financial or personal relationships with the company or organization sponsoring the research at the time the research was done. Such relationships may include employment, sharing in a patent, serving on an advisory board or speakers' panel, or owning shares in the company.
The source of support for the research reported in the paper should be listed on the title page, not as an acknowledgement.

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Number references consecutively in the order in which they are first mentioned in the text. Identify references by Arabic numerals in parentheses. References cited in tables or in legends to figures should be numbered according to the first citation of the table or figure in the text. Appendixes should have a separate reference section. It is rarely necessary to cite more than 50 references in an original research article. Try to avoid citing published abstracts as references [if a published abstract is cited, include "(abstr)" at the end of the reference]. Abstracts from scientific meetings not published in peer-reviewed journals may not be used as references. Unpublished observations and personal communications (written, not oral) may not be used as references but may be inserted in parentheses with the names of the responsible researchers and the year of the observation or communication. Authors are responsible for obtaining written permission from everyone so cited and for providing to the Editor a copy of the permission, if requested. Doctoral dissertations may be used as references. Include manuscripts accepted but not yet published; designate journal name followed by "(in press)." Report foreign titles in the original language, identify the language, and provide the English translation in parentheses. The references must be verified by the author against the original documents.

Journals

1) Journal article with DOI: If an article has a DOI number ("digital object identifier" number unique to the publication), it may be included at the end of the reference.

Hamer M, Steptoe A. Prospective study of physical fitness, adiposity, and inflammatory markers in healthy middle-aged men and women. Am J Clin Nutr 2009;89:85-89. doi:

10.3945/ajcn.2008.26779.

2) Standard journal article: list all authors when 6 or fewer; when 7 or more, list only the first 3 and add “et al.” Abbreviate journal titles according to Index Medicus style, which is used in MEDLINE citations.
Jeffery RW, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? Am J Clin Nutr 2003;78:684–9.

3) Corporate author

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.

Books and other monographs

4) Personal authors

Shils M, Shike M, Olson J, Ross AC. Modern nutrition in health and disease. 9th ed. Baltimore: Lippincott Williams & Wilkins, 1998.

5) Committee report or corporate author

National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. Washington, DC: National Academy Press, 2000.

6) Chapter in book

Young VR, Tharakan JF. Nutritional essentiality of amino acids and amino acid requirements in healthy adults. 2nd. ed. In: Cynober LA, ed. Metabolic and therapeutic aspects of amino acids in clinical nutrition. Boca Raton, FL: CRC Press, 2004:439–70.

7) Agency publication

US Department of Agriculture, US Department of Health and Human Services. Nutrition and your health: dietary guidelines for Americans. Washington, DC: US Government Printing Office, 2000. [USDA Home and Garden Bulletin no. 232.]

Internet references

8) Website

National Center for Health Statistics. National Health and Nutrition Examination Survey. Version current 1 October 2003. Internet: http://www.cdc.gov/nchs/nhanes.htm (accessed 13 October 2003).

9) Online journal article

Sinha A, Madden J, Ross-Degnan D, Soumerai S, Platt R. Reduced risk of neonatal respiratory infections among breastfed girls but not boys. Pediatrics [serial online] 2003;112:e303. Internet: http://pediatrics.aappublications.org/cgi/content/full/112/4/e303 (accessed 14 October 2003).

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Tables must be included in the text file, and each should appear one per page. Remember to use double-spacing. Number tables consecutively with Arabic numerals (do not use 1A, 1B, etc) and supply a brief descriptive title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in the heading or table title. Each table should contain enough detail (including statistics) that the table is intelligible without reference to the text. Explain in footnotes all nonstandard abbreviations that are used in the table. Commonly used approved abbreviations (see the section of the same name below) may be used without explanation. Additionally, explanations are not needed for ANOVA, BMI, F (females), and M (males). For footnotes, use superscript Arabic numerals. For reporting results of statistical analyses, superscript letters can be used if explaining the results in the usual manner would be too complicated (see a recent issue of the AJCN for examples). The first appearance in a horizontal row determines the order of the footnotes. Identify statistical measures of variation, such as SD and SE. Omit internal horizontal and vertical rules. Cite each table in the text in consecutive order. Use boldface for the first mention of each table. If you use data from another published source, acknowledge the source fully. Number references in tables according to the location of the first citation of each table in the text.

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Cite each figure in consecutive order in the text. Use boldface for the first mention of each figure. Spell out the word “Figure”; do not use “Fig.” If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce or adapt the material in print and electronic format. Except for documents in the public domain, permission is required from the copyright holder, regardless of authorship or publisher. Legends for all figures should be typed with double-spacing on a

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Supplemental material may be included with manuscript submissions. All supplemental data should be clearly labeled either as "Supplemental Data for Reviewers Only" or as "Online Supplemental Material" if it is submitted for online publication only in The AJCN. Supplemental files for upload may include articles published/in press elsewhere, reports or technical briefs related to manuscript submission, figure source files, questionnaires, permissions, videos, etc. Online
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Submit manuscript text, including tables, in a Word 6.0.doc file (please note: the Word 2007.docx file format is not accepted); tables must be included in the text file; do not submit tables in separate files. Submit each figure in a separate file. Preferred formats for image (figure) files are PDF, TIFF, or EPS. Files must conform to the minimum-

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Microsoft PowerPoint (PPT) and Word (DOC) files can be acceptable if properly prepared and submitted in their native format. When creating print-quality files in MS Office applications, follow these general guidelines:

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3) When inserting pictures or images into files, be sure to select "insert" and not "insert link," which will not properly embed the hi-res image into the MS Office file.

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Image resolution

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It is recommended to use standard fonts in order to avoid potential problems with font substitution or embedding problems. Acceptable fonts include Arial, Helvetica, Times Roman, Symbol, Mathematical PI, and European PI. All other fonts, if not embedded, may be replaced, resulting in data loss or realignment.

Color space

All digital art submitted, including black and white figures, must be bitmap (Monochrome), grayscale, RGP, or CMYK. Color files should be supplied in RGB color whenever possible and should have an ICC profile applied. RGB best utilizes the color projection capabilities of computer display devices and has become the standard color space for displaying images for the online journal. Authors are strongly encouraged to submit color figures in RGB format. Note that the RGB color space is significantly larger than the process CMYK color space. Therefore, depending upon the content of the image, color shifts may occur when converting to CMYK and appear in print if colors in the original image are outside the process CMYK gamut.

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2) If a figure is very small in the system-generated PDF file, the resolution of the figure file was not high enough. A higher resolution figure should be uploaded before the PDF is approved.

3) Tables must be included in the text file; do not submit tables in separate files.

4) To check/preflight your digital art files before submission, see http://dx.sheridan.com/onl/rgb/. Authors are requested to create and keep high-resolution print copies of the figures, in the event that they are needed for publication purposes.

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Use only standard abbreviations. Consult the following sources for standard abbreviations: Scientific Style and Format and Standard for Use of the International System of Units (SI): the Modern Metric System (American Society for Testing and Materials. IEEE/ASTM SI 10-1997.

West Conshohocken, PA: ASTM, 1997) or www.ieee.org/web/publications/PSPB/index.html. Avoid abbreviations in the title, and avoid the use of abbreviations for single words. The complete phrase or expression should precede the first use of an abbreviation in the text unless it is a standard unit of measurement, chemical compound preceded by a digit, or one of the following standard abbreviations: ADP, AIDS, AMP, ATP, DMEM, DNA, EDTA, eg, EGTA, GDP, GTP, HCl, HDL, HEPES, HIV, HPLC, ie, LDL, ln, MEM, MOPS, NAD, NADH, NADP, NADPH, RNA, RPMI, tris, and VLDL.

Abbreviations for statistical terms

coefficient of correlation, sample, r
coefficient of multiple correlation, R
coefficient of variation, CV
confidence interval, CI
degrees of freedom, df
mean, $\bar{x}$
not significant, NS
number of observations, n
probability, P
standard deviation, SD
standard error of the estimate, SEE
standard error of the mean, SEM
variance ratio, F

Metric units are required and the use of the International System of Units (SI units) is optional. For a comprehensive listing of SI conversion factors, consult SI Units for Clinical Measurement (Young DS, Huth EJ. Philadelphia: American College of Physicians, 1998), Am J Clin Nutr 1998;67:166–81 or J Nutr 1990;120:20-35. Dosage forms and dietary ingredients may be expressed in gram or mole quantities. Energy may be expressed in kilocalories or joules; the conversion factor for converting kilocalories to kilojoules is 4.184. Do not report energy in Calories with a capital C; use kcal, MJ, or kJ instead. Temperatures should be reported in degrees Celsius. Blood pressures should be reported in millimeters of mercury. Use of katals to report enzyme activity is optional.

Commonly used approved abbreviations

Standard units of measurement
ampere, A  liter, L
becquerel, Bq  meter, m
coulomb, C  minute, min
curie, Ci  mole, mol
day, d  month, mo
degree Celsius, °C  ohm, Ω
farad, F  pascal, Pa
gram, g  second, s
hertz, Hz  sievert, Sv
hour, h  volt, V
joule, J  watt, W
katal, kat  week, wk
kelvin, K  year, y
kilocalorie, kcal

Acceptable standard units
length: m, mm, μm
area: m2, mm2, μm2
volume: L, mL, μL, pL
mass: kg, g, mg, μg, ng, pg
mass concentration: kg/L, g/L, mg/L, μg/L
substance concentration: mol/L, mmol/L, μmol/L, nmol/L

Unacceptable units
length: not acceptable: in, ft, yd, Å, mμ
area: not acceptable: sq in, in2, μ2
volume: not acceptable: pint, gallon, cc, ccm, λ, μμL
mass: not acceptable: oz, lb, gr, gm, gms, mgm, mgms, mgs
mass concentration: not acceptable: mg %
substance concentration: not acceptable: M, N

Combining prefixes
tera- (1012), T  micro- (10−6), μ
giga- (109), G  nano- (10−9), n
mega- (106), M  pico- (10−12), p
kilo- (103), k  femto- (10−15), f
milli- (10-3), m  atto- (10−18), a

## NOMENCLATURE

In general, the AJCN follows the nomenclature policies of the IUPAC-IUB Joint Commission on Biochemical Nomenclature. The vitamin nomenclature is summarized at J Nutr 1990:120:12-19, and the amino acid nomenclature is summarized at J Nutr. 1987:117:15. Both articles can be accessed at http://jn.nutrition.org. Authors are responsible for ensuring that their terminology conforms with these policies. For guidelines on gene and protein nomenclature, authors should consult the following websites:

http://www.informatics.jax.org/ (mouse, rat, and chicken),
http://rgd.mcw.edu/ (rat),
http://www.genenames.org/ (human and other species), and
http://au.expasy.org/ (proteins).

As recommended by the American Society for Microbiology, the spelling of bacterial names should follow the Approved Lists of Bacterial Names (Amended) & Index of the Bacterial and Yeast Nomenclatural Changes (V. B. D. Skerman et al. ed., ASM Press, Washington, DC, 1989) and the validation lists and notification lists published in the International Journal of Systematic and Evolutionary Microbiology (formerly the International Journal of Systematic Bacteriology). Further information on currently approved bacterial names can be found at: Bacterial Nomenclature Up-to-Date (http://www.dsmz.de/microorganisms/main.php?contentleft_id=14) and at List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.cict.fr). If authors must use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title, when appropriate, and at its first use in the abstract and the text. Correspondingly, an appropriate statement concerning the nomenclatural status of the name should be made in the text.

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Revised 09/29/2009

## Appendix C

## INFORMED CONSENT

**Healthy Aging in Neighborhoods of Diversity across the Life Span Informed Consent**

Introduction

We must have your written informed consent before we perform research tests or examinations. We follow federal regulations for research with human subjects. These regulations require us to make sure that you understand what examinations we will perform and the risks that are involved, if there are any.

This booklet reviews the tests that we will perform in this research. We perform these tests free of charge. You should understand the purpose of this study before you agree to participate in this research. We welcome any questions that you might have about what to expect in this study. You may participate in any of the tests, but you need not participate in all of the tests. You may stop any time after a test starts. You may ask questions any time during a test.

We want to make sure that you understand the tests in this study. We must witness your signature on the consent form. Please do not sign the consent form until you arrive at the Mobile Medical Research Vehicles.

Purpose of the Study

The purpose of this study is to learn about changes in health over time. We want to study as many people in different communities as we can by using our Mobile Medical Research Vehicles (MRVs).

Our goal is to study the rate of 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 people's bodies change over time. We will invite you to participate in our study every three years when we visit your neighborhood with our Mobile Research Vehicles. The study data will be collected in two parts. The first part of the study consists of a household interview. This interview includes questions about your background, educational experience, occupational history, health and health care experiences, physical activities, and a few questions about your neighborhood. For the second part of the study you will visit our Mobile Medical Research Vehicles (MRV-I and MRV-II). The MRV-I

will be used for the medical history and physical examination, body composition, test of the heart's function, strength testing, bone density, and laboratory samples. MRV-II will be used for consenting, questionnaires, cognitive and memory testing, and the emotions and heart rate test. We plan to administer the same tests every three years for the next 20 years.

We also want to study why some people are healthier as they get older than others. 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. We call our study Healthy Aging in Neighborhoods of Diversity across the Life Span.

List of Tests and Statements of Risk

You may participate in some or all of our tests. You may stop any test anytime you want even after you agree to do it. We want you to understand the risks in taking some of these tests. We welcome your questions about the tests and any risks even after the test starts. Risks, if any, are stated and discussed with the description of the test, or in the section on Assessment of Risks in this booklet.

Household Survey

The household survey is designed to take place in your home. You will be asked to answer several questions about your background, household characteristics, educational experience, occupational history, ethnic identity and discrimination experiences, health and health care experiences, physical activities, stress and coping, and a few questions about your neighborhood.

Nutritional Dietary Recall

During this interview we will ask you to remember all the foods and beverages you have consumed during the last 24 hours. We will have some cups and measures to help you remember the amounts. A trained interviewer will record your answers and ask questions designed to help you remember using a method developed by the United States Department of Agriculture (USDA). The risks for the household survey and dietary recall interview are very minimal. The only risk of this part of the study is that you may become tired. All examiners who are involved in asking these questions are experienced in using these procedures and they will minimize any discomfort that you might feel.

Body Composition

We will weigh you and measure various parts of your body. There are no risks from this test.

Bone Density

We will measure the size and thickness of the bones in your arm, lower back, and hipbone. We will also measure how much lean tissue you have and how much fat tissue you have. These measures will tell us if you are likely to have bone fractures or osteoporosis. We will ask you to lie down on a device called a DEXA scanner. The scanner uses small amounts of X-ray radiation to make measurements as a detector examines your body. The risk to you, if any, is estimated to be slight. The risks are discussed in the section on Assessment of Risks in this booklet.

Muscle Strength Testing

Grip Strength Test

Handgrip strength in both hands will be measured using an adjustable, hand-held, hydraulic grip strength dynamometer. The hydraulic grip strength dynamometer is a device you hold in your hand and squeeze. It measures the strength of your handgrip. You will be asked to sit with the arm to be tested resting on the table. The dynamometer is held in the hand to be tested and is resting on a mouse pad. We will ask you to grip the two bars of the dynamometer in your hand, and to slowly squeeze the bars as hard as you can. The test is repeated on the other hand. Exclusions. You will not be tested on the affected hand if you have had arm or hand surgery like fusion,
arthroplasty, tendon repair, synovectomy, or other related surgery in the past 3 months.

Chair Stand

Using a standard armless chair placed securely against a wall, you be asked to rise from the chair without using your arms and return to a seated position. If this is done successfully, you will be asked to repeat that movement 10 times. Exclusions. There are no formal exclusions from attempting the single chair stand; inability to rise from a chair without using arms excludes participants from doing repeated chair stands.

Balance Test

We will ask you to stand with your feet together and with your feet in a heel-to-toe position for 30 seconds each. We will also ask you to try to stand on one leg for
30 seconds. You may stand on whichever leg is more comfortable. The examiner will demonstrate exactly what is expected. We will ask you to try to hold your foot up for thirty seconds. We will ask you to repeat this test 2 times.
The information we collect will help us to understand how strength changes as people get older. We want you to know that there are very minimal risks associated with these tests. The only risks are that there is a slight risk of falling and you may feel tired after these tests.

Medical History and Physical Examination

Medical History

We will ask you questions about your medical history. The examiner will add information to the form when you have your physical examination.

Smoking, Drug and Alcohol History

We will ask you about your smoking habits and use of drugs and alcohol. We will also ask about information about your parents' smoking habits.

Physical Examination

Our physician or nurse practitioner will give you a physical exam in our private exam room. They will check your blood pressure and pulse in both arms.
They will listen to your heart and lungs, examine your eyes, joints, and check your reflexes and other parts of your nervous system. The physician or nurse practitioner will also examine your abdomen. Our physician or nurse practitioner will not do a complete physical exam. You should still see your personal physician for regular check-ups.

Tests of your Heart Functions

We will do tests to find out about changes in your heart and blood vessels. We will discuss the results with you after we finish the tests. If we find a heart problem, we will discuss the problem with you and we will send the results to your personal doctor if you want us to.

Resting Electrocardiogram (EKG)

We will place wires called electrodes on your skin to record your heartbeats. By looking at the electrical pulse of your heart we will examine you heart rate and rhythm, and check if you have had a heart attack. There are no risks from this procedure.

Carotid Doppler Ultrasonography

We will ask you to lie down and rest for 10 minutes. We will place a small ultrasonic probe on your neck to take pictures of the artery in your neck and measure the thickness of this blood vessel. There are no risks from this test. There is no radiation in this test. Ultrasound is not the same as an x-ray and does not involve any radiation.

Pulse Wave Velocity

The measurement of the stiffness of your blood vessels is performed entirely non-invasively (no needles or sticking involved). You will be asked to lie flat on your back and we will place a sensor on your wrist, one over the artery in your neck (carotid artery), and another sensor over the artery in your groin (femoral artery). We will then record the arterial waveform tracings. There are no risks associated with this procedure.

Problem Solving and Memory Testing

We will ask you to do some tasks that exercise your thinking and memory. These tasks ask you to remember words, numbers, and pictures. These tasks also ask you to find similar words or to think of words beginning with certain letters or belonging to certain categories. They will also ask you to imagine how objects look in different positions.

The tests for remembering are called the Benton Visual Retention Test, the California Verbal Learning Test, and the Digit Span Test. The tests for words are called the Wide Range Achievement Test and the Category Fluency Test. The test for comparing objects is called the Identical Pictures Test. The test for switching letters and numbers is called the Trailmaking Test. The test for imagining objects in different positions is called the Card Rotations Test. Other tests, called Mental Status Tests, measure several types of memory abilities. These tests are given in a private, quiet room with a tester who will help you understand how to do the best you can.

We want you to know that some people find these tests tiring. Sometimes, people feel nervous when they do these tests. All examiners who are involved in giving these tests are experienced in using these procedures and they will minimize any discomfort that you might feel. If the tests are disturbing you, then you may stop testing any time you want.

Questionnaires

We will ask you to complete several questionnaires about your social support, racial and cultural identification, family income, your feelings and interests, coping, and mental health. These questionnaires will be filled out on the Mobile Research Vehicles by using a computer and headphones. We will help you do the questionnaires if you want us to. If you have trouble seeing or reading the questions you may ask one of our testers to help you. These tests are given in a private, quiet room.

Emotions and Heart Rate

This interview is to see how recalling emotions and standing up affects heart rate and blood pressure. You will be asked to recall past experiences while we record your heart rate and blood pressure. There are no risks with this test.

Buccal Mucosa Smear

As part of the medical evaluation, a buccal mucosa smear will be collected from you, if you agree, using the Whatman FTA collection system. This system collects buccal cells from inside your mouth using foam tipped applicator which is placed into the mouth and rubbed on the inside of both cheeks for 30 seconds by you. The sample obtained is then transferred to the Indicating FTA cards. The extracted DNA will be used for epigenetic analysis. Blood, Tissue, and Urine Sampling

If you agree, we will ask you to give us a blood sample and a urine sample. To prepare you for the blood tests we will ask you not to eat or drink anything after midnight the night before your visit to the MRVs. The blood draw will be performed right before you are served breakfast. We will use these samples to measure your health and so that we can measure changes in your health if we test you again. We will measure your white and red blood cells, your cholesterol, salt and sugar, how well your blood carries oxygen through your body, and how fast you heal from minor cuts. We will also measure blood chemistry that may tell us how well your body organs work, such as your heart, liver, and kidneys.

Women between the ages of 30 and 55 years will get a pregnancy test. We will be testing for communicable diseases including Hepatitis B, Hepatitis C, and Syphilis. 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.

Doctors often make new discoveries by testing blood and urine. We would like to freeze a portion of your blood and urine samples to save them in our frozen tissue bank. We are not sure what new discoveries will appear in the future. We want to set aside your samples until there are new tests that will help us understand health and aging.

More and more, we are discovering that our genes are important for understanding our health. Your genes are the parts of each cell inherited from your mother and father. Your genes are what make you a unique individual. Genes are made from DNA. We want to use some of your donated blood to freeze your DNA. We are not sure what studies will use your DNA. New studies may look at how your genes affect age-related diseases.

The samples saved in our bank will be stored at very low temperatures. Unlike household freezers, these freezers can preserve samples for many years, perhaps many decades. We will label your samples with code numbers. Only the principal investigators in this study will know your code number. Only researchers in this study will know the results of tests using your genes. We will not reveal your results to anyone who is not associated with this research.

We will ask you if you want the results of the tests that we perform on your blood and urine. We will also ask you if you want us to send your results to your personal physician. We do not plan to report the results of the studies we do on your genes because these tests do not diagnose or predict the development of specific diseases at this time. In the future, we may offer you some of the results if the Food and Drug Administration approve some of the tests.

We will ask you to donate about 62 milliliters of blood (about 4½ tablespoons). For comparison, the Red Cross usually asks for a donation of about 500 milliliters of blood (about two cups).

Compensation

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 do not perform 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.

You are participating in a research study and our physicians and technicians are not your primary health-care providers. We will provide medical feedback to you and, with your permission, to your personal physician about your health based on the tests in which you participate. If you need a referral to a physician, we will provide a list of local physicians.

Assessment of Risks

Buccal Mucosa Smear

The possible risks for this procedure include irritation of the inside of the cheek and/or gum line by the foam tipped swab used to collect cells and saliva.

Blood Sampling

We want you to know that there are some risks in donating a blood sample. The trained HANDLS staff member will insert a needle in a vein in your arm. There is a risk of an infection from the needle puncture. There is also a risk of a black and blue mark, and you may feel faint. These risks are very small. Our staff is well trained and has drawn blood many times. It is common to have a small black and blue mark, but it disappears after a day or so. Some people have begun perspiring, or they felt nauseated and their pulse slowed. None of them had any after effects.

Radiation

Each day everyone receives a certain amount of natural radiation from various sources in the environment. The exact amount of radiation is measured in units called millirems. The National Council on Radiation Protection and Measurements measures average radiation exposure. They estimate that people in our country receive 300 millirems of annual exposure.

The radiation you will receive from participating in this study is equivalent to an exposure of less than 1 millirem to your whole body. This whole body dose is called the effective dose. The average annual background radiation in the United States is an effective dose of 300 millirems per year. The amount of radiation in this study is equal to the background radiation in about one day. Using the standard way of describing radiation dose, you will receive 1.5 millirems to the skin over your lower spine and hip area, and .24 millirems to the skin over your forearm. Thus, your body will receive a small dose of radiation. Please be aware that this radiation exposure is necessary for this research study only, and is not essential for your medical care. The NIH Radiation Safety Committee, a group of experts on radiation matters, has reviewed the use of radiation in this research study and has approved this use as being necessary to obtain the research information desired.

The radiation dose you will receive is within the NIH Radiation Safety Guidelines for research subjects, that is, the effective dose is less than 5000 millirems in one year. The potential long-term risk from the radiation in this study is uncertain, but these doses have never been associated with any definite adverse effects. Thus the risk to you, if any, is estimated to be slight. Please advise your doctor if you have participated in research studies at the NIH or other institutions that involved the use of radiation so that it may be determined that the total radiation from all studies is not excessive. Examples of such studies include x-ray studies conducted in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine studies, for example technetium and PET scans.

If you are female, you may participate in this study only if you are certain you are not pregnant. If you become pregnant (or suspect pregnancy) before the study is completed, you must inform the investigator.

## Appendix D

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