

ABSTRACT

Title of Dissertation: WHY DO AFRICAN AMERICANS HAVE HIGHER SERUM FERRITIN THAN EUROPEAN AMERICANS, DESPITE LOWER HEMOGLOBIN?

Yang Pan, Ph.D., 2006

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Blacks have been consistently observed to have higher serum ferritin (SF) concentrations than whites; however, few studies have attempted to explore why this difference exists. 3,554 non-Hispanic white (NHW) and non-Hispanic black (NHB) male subjects, aged 20-65 years, were selected from the third National Health and Nutrition Examination Survey (NHANES III) to determine the possible factors that may contribute to the observed black-white SF difference. Results of multiple regression analyses showed that age, body mass index (BMI), % energy from carbohydrate and fat, calcium intake, serum total protein, serum α -carotene, mean cell volume (MCV), iron binding capacity (TIBC) and γ glutamyl transferase (GGT) were significantly associated with SF concentration. When the final regression model was run after excluding subjects with abnormal serum total protein, TIBC and GGT levels, the SF difference dropped to 3.95 μ g/L (initial difference=37.06 μ g/L) between NHWs and NHBs. The results suggest that the noted black-white SF difference is a result of factors including overall nutrition and health, iron status and hepatic well-being. Higher SF, low Hb and reduced TIBC level observed in blacks are consistent with the definition of anemia of chronic disease

(ACD). Future investigations are needed to confirm the role of ACD in the black-white SF difference.

We also examined different sub-populations to investigate whether the likelihood and magnitude of SF elevation in regard to acute inflammation and hepatitis C are different between NHBs and NHWs by using logistic regression analyses. Compared to NHWs, NHBs had a 2.239-fold (95% CI, 1.333 to 3.760) greater risk to have elevated SF in regard to acute inflammation, and had significantly higher odds ratios of having elevated SF per unit change in C-reactive protein (CRP), white blood cell count (WBC), serum albumin, lymphocyte and platelet count. However, no significant results were found for hepatitis C. The results indicated that NHWs and NHBs had different patterns of iron accumulation in regard to acute inflammation. Further elucidation is needed about how ferritin regulation is perturbed in diseases, and whether increased stored iron plays a role in different clinical outcomes and drug response. Adequately powered studies are needed to further investigate the relationship between stored iron and hepatitis C host response.

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EUROPEAN AMERICANS, DESPITE LOWER HEMOGLOBIN?

By

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TABLE OF CONTENTS

	LIST OF FIGURES.....	iv
	LIST OF TABLES.....	v
Chapter 1.	INTRODUCTION.....	1
	TERM SPECIFICATION.....	6
	OBJECTIVES.....	7
	RESEARCH QUESTIONS.....	8
Chapter 2.	BACKGROUND.....	11
	I. Iron and Iron metabolism.....	11
	II. Iron Transport.....	12
	III. Iron Storage.....	13
	IV. Iron Reutilization.....	14
	V. Iron Loss.....	14
	VI. Serum ferritin.....	14
	III. Other Iron status Indicators.....	17
	1. Hemoglobin.....	17
	2. Erythrocyte protoporphyrin.....	19
	3. Transferrin saturation.....	20
	4. Transferrin receptors.....	21
Chapter 3.	REVIEW OF SELECTED TOPICS IN THE LITERATURE.....	22
	I. Interpretation of an elevated serum ferritin.....	22
	1. Iron overload.....	22
	A. Primary iron overload.....	22
	1) Hereditary hemochromatosis.....	22
	2) Hereditary aceruloplasminemia.....	24
	B. Secondary iron overload.....	25
	1) Iron loading anemia with or without blood transfusion.....	26
	2) Porphyria cutanea tarda.....	28
	3) Excessive dietary iron.....	28
	2. Liver disease.....	29
	A. Nonalcoholic steatohepatitis.....	29
	B. Viral hepatitis.....	31
	1) Hepatitis B.....	31
	2) Hepatitis C.....	33
	3) Iron and viral hepatitis.....	35
	3. Inflammatory conditions.....	36
	C-reactive protein.....	38
	Erythrocyte sedimentation rate.....	39
	Other infection parameters.....	39
	4. Alcohol excess.....	40

II. Investigation of iron store difference between African Americans and European Americans.....	43
III. Investigations of differences in elevation of serum ferritin between African Americans and European Americans in regard to selected conditions.....	48
Chapter 4. METHODS.....	51
I. Survey description and sample design.....	51
II. Data preparation and use of sample weights.....	51
III. Statistical Software.....	53
IV. Primary Analysis	54
1. Study Sample.....	54
2. Variables.....	54
3. Statistical Analysis.....	60
VI. Supplemental Analyses.....	62
1. Study Sample.....	62
2. Variables.....	63
a. acute inflammation.....	63
b. hepatitis C.....	64
3. Statistical Analysis.....	65
a. acute inflammation.....	65
b. hepatitis C.....	66
Chapter 5. RESULTS.....	67
I. Primary Analysis.....	67
Descriptive statistics.....	67
Inferential statistics.....	69
Proof test.....	70
II. Supplemental Analyses.....	71
a. Acute inflammation.....	71
Descriptive statistics.....	71
Likelihood test.....	72
Magnitude test.....	73
b. Hepatitis C.....	73
Descriptive statistics.....	73
Inferential statistics.....	74
Chapter 6. DISCUSSION.....	76
I. Primary Analysis.....	76
II. Supplemental Analysis.....	82
a. Acute inflammation.....	82
b. Hepatitis C.....	86
APPENDICES.....	89
REFERENCES.....	100

LIST OF FIGURES

1. Geometric means of serum ferritin ($\mu\text{g/L}$) among NHANES III non-Hispanic white and non-Hispanic black male subjects stratified by age.
2. The proportion of subjects who had elevated serum ferritin concentration ($>300\mu\text{g/L}$) in response to acute inflammation stratified by age and ethnicity.
3. Distribution of hepatitis C genotypes between non-Hispanic white and non-Hispanic black NHANES III male subjects aged ≥ 20 yrs.

LIST OF TABLES

1. Subject characteristics in NHANES III non-Hispanic white and non-Hispanic black males, aged 20-65 yrs.
2. Dietary intake and serum nutrient status among NHANES III non-Hispanic white and non-Hispanic black males, aged 20-65 yrs.
3. Biochemical markers among NHANES III non-Hispanic white and non-Hispanic black males, aged 20-65 yrs.
4. Multiple regression sub-models with serum ferritin concentration as dependent variable.
5. Final regression models and revised model with serum ferritin concentration as dependent variable.
6. Comparisons of least square means (LS means) of serum ferritin concentration among final regression and revised models.
7. Subject characteristics of NHANES III acute inflammation present non-Hispanic black and non-Hispanic white males aged ≥ 20 yrs.
8. Risk of having elevated serum ferritin concentrations between non-Hispanic white and non-Hispanic black acute inflammation present NHANES III male subjects aged ≥ 20 yrs.
9. Risk of having elevated serum ferritin concentrations per unit change in inflammation markers between NHW and NHB acute inflammation present NHANES III male subjects aged ≥ 20 yrs.
10. Subject characteristics of NHANES III hepatitis C-infected non-Hispanic white and non-Hispanic black males aged ≥ 20 yrs.
11. Distribution of hepatitis C genotypes between non-Hispanic white and non-Hispanic black NHANES III male subjects aged ≥ 20 yrs.
12. Risk of having elevated serum ferritin concentrations between non-Hispanic white and non-Hispanic black hepatitis C infected NHANES III male subjects aged ≥ 20 yrs.
13. Risk of having elevated serum ferritin concentrations per unit change in serological markers between non-Hispanic white and non-Hispanic black hepatitis C infected NHANES III male subjects aged ≥ 20 yrs.

INTRODUCTION

There has been growing research interest in the relationship between excessive iron stores and diseases since increased iron store has been proposed to be associated with impaired glucose intolerance (1), higher risk of diabetes (2,3), vascular diseases, and cancer (4,5). Previous studies have consistently found differences in the hemoglobin and serum ferritin concentrations between African Americans (AA) and European Americans (EA) (6-8). African Americans have been shown to have higher serum ferritin concentrations than European Americans although their hemoglobin concentrations are lower. Research attention has been typically focused on finding the explanation to the difference in the hemoglobin levels between these two groups whereas the disparities in serum ferritin concentrations have been mostly ignored.

Recently, Zacharski et al. reported that different patterns of iron accumulation exist according to age, sex, and ethnicity based on analysis of NHANES III data (9). Serum ferritin levels were compared with the percent transferrin saturation in 20,040 individuals > 17 years of age from the third NHANES database. Ferritin levels in non-Hispanic black males exceeded those in non-Hispanic whites and Hispanics for each decade of life, and differences increased with age. Ferritin levels were comparable for black, white and Hispanic women during the premenopausal years. However, after menopause, ferritin levels in black women rose more rapidly and exceeded those of white and Hispanic women. Overall, they found ferritin levels were approximately 7% to 8% greater for blacks than for whites and Hispanics, and differences remained throughout the second half of life.

In recent years, the issue of the health effects caused by excessive iron stores has gained considerable visibility. Since one of the two overarching goals of Healthy People 2010 is to eliminate health disparities, scientists are now trying to explore the underlying biological mechanisms contributing to the higher disease and mortality rates among minorities, especially African Americans, Native Americans and Hispanics. Given the fact that the greatest health disparities have been consistently observed between blacks and whites, most research attention has been focused on finding the explanation for the noted differences in disease prevalence, complications, clinical outcomes and treatment response between these two groups.

Serving as a powerful pro-oxidant, iron plays its role in disease progression by catalyzing in oxidation-reduction reaction and causing organ-specific (liver, muscle, and pancreas) oxidative damage. Interest in the relationship between different iron accumulation patterns with disease distribution has increased in both the scientific and public health communities. Several studies have reported that some diseases affect blacks disproportionately (10-16). Actually, the death rate from all causes in blacks is approximately 1.5 times that of whites. Compared with whites, blacks have worse survival after myocardial infarction (10), a 2-fold-higher incidence of and morbidity from stroke (11,12), and a higher risk of infrarenal peripheral vascular disease (13). Morbidity and mortality rates from diabetes (14), AIDS (15), and cancer (16) are also significantly higher for blacks than for whites. Although the higher disease rates in blacks is most likely the result of a complex interplay of individual attributes, minority and socioeconomic stress and environmental characteristics, several researchers proposed the hypothesis that the differences in iron stores may contribute to the disease differences

between blacks and whites, especially for type 2 diabetes mellitus, cardiovascular diseases and cancer (17-21). The implication of a relationship between excessive iron stores and higher disease frequencies among blacks may be one of the reasons for the continued findings of health disparities between blacks and whites. But in order to further test this “iron hypothesis,” the question of why there is a difference in iron stores between blacks and whites, and what are the potential factors contributing to this difference needs to be investigated first. Serum ferritin, the most commonly used indicator of total body iron stores, hence becomes the focal point for the investigation of iron store disparities. Therefore, the primary goal of our study is to determine the possible factors which contribute to serum ferritin difference between blacks and whites by using national representative data.

Genetic variance is often cited as a possible reason for differences in diseases and iron status between blacks and whites. Consistently found higher disease rates and lower hemoglobin concentrations among blacks than among whites are often misleadingly explained by “race.” The chief argument against the notion is that biological race sometimes can be medically meaningless because genetic polymorphisms are more variable within-ethnicity than between-ethnicity. Although genetic factors may play a role in the development and progression of diseases, scientists believe it should be our ultimate goal to have evidence on individual genotypes to guide medicine, a development that would make racial identity biologically irrelevant, but that is decades away (22).

An increasing number of research studies have also suggested the possibility that environmental or nutritional factors could play important roles in disease progression and outcome. Our current study was therefore designed to assess whether environmental and

nutritional factors may contribute to the differences in serum ferritin concentrations between blacks and whites by testing the hypothesis that the presence of infection, viral liver diseases, alcohol consumption, nutrition and socioeconomic status are related to the observed difference in serum ferritin.

Previous studies have found that hepatitis C infected blacks were more likely to have increased iron stores than hepatitis C infected non-blacks (23), and their response to antiviral treatment was lower compared to non-blacks (24). Therefore, we were inspired to further explore whether there is a difference in the likelihood and magnitude on elevation of serum ferritin in regard to two conditions: acute inflammation and hepatitis C between blacks and whites. The selection of these two conditions was based on their significant effect on raising the serum ferritin concentrations and documented or assumed higher prevalence among blacks than whites. As it was discussed intensively in later section, the prevalence of hepatitis C has been documented to be significantly higher in blacks than whites. The epidemiology information for inflammation, however, is very limited due to lacking of the well-established model defining inflammation, especially in a large population. Studies have reported that African Americans have a greater propensity for inflammation than European Americans because African Americans are more likely to carry genetic variants known to stimulate the inflammatory response (26-29). Therefore, we assume that the prevalence of acute inflammation is also higher in blacks than whites.

Although there have been different underlying mechanisms proposed for these clinical observations, such as different cellular immune response in the determination of the outcome of hepatitis viral infection and antiviral treatment response, specific variants in

genes that encode proteins regulating inflammation response, the further exploration and confirmation of these hypotheses is beyond the scope of our research. Our current study focused primarily on detecting the differences in the likelihood and magnitude on elevated serum ferritin between blacks and whites in response to two conditions. And the results of this investigation will not only contribute to the understanding of the ethnic differences in clinical outcome and treatment responsiveness, but also serve as a probe for future studies.

To our knowledge, this is the first study that attempted to assess the determinants of the long-observed black-white serum ferritin difference by using nationally representative data. The study contributed to the understanding of the ethnic differences in iron stores between blacks and whites, and its implications in disease disparities. We hope our present study can initiate more future studies to further discover the underlying mechanisms responsible for the observed black-white serum ferritin difference and clarify the relevant contribution of certain environmental factors to the exaggerated iron accumulation in blacks.

Term Specification

There are several categories of race/ethnicity used in the current proposal. These categories are sociopolitical constructs and should not be interpreted as being scientific or anthropological in nature.

European American: a term for Americans of European descent, who are usually referred to as White or Caucasian. The term is more specific than White or Caucasian in that these terms in their official usage include European, Latin American, Southwest Asian, and Middle Eastern.

non-Hispanic White is an official term that is more specific, but is still not as specific as European American. The reason for this is that 1) the term "Non-Hispanic White" excludes Hispanics who are of European (especially Spanish) descent, and 2) includes non-Europeans such as Arabs and Iranians; however, even so, when examining demographic data, the "Non-Hispanic White" category is that which corresponds most closely to European American.

African Americans, also known as Afro-Americans, Black Americans, or simply blacks, are an ethnic group in the United States of America whose ancestors, usually in predominant part, were indigenous to West and sub-Saharan Africa. Many African Americans also have European and/or Native American ancestors.

non-Hispanic Black is also an official term that is more specific, it refers to a person having origins in any of the black racial groups of Africa (except those of Hispanic origin). But it may include many African Americans also have European and/or Native American ancestors.

Note: Unless otherwise mentioned, the term “black” and “white” used in this dissertation refers to non-Hispanic black and non-Hispanic white.

OBJECTIVES

1. To determine the possible factors that may contribute to the observed difference in serum ferritin concentration between NHANES III non-Hispanic white and non-Hispanic black, male aged 20-65 years.
2. To investigate whether there are differences in the likelihood and magnitude on elevated serum ferritin concentration in response to acute inflammation and hepatitis C, respectively, between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged ≥ 20 years.

RESEARCH QUESTIONS

1. Is there a statistically significant difference in the serum ferritin concentration between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
2. Is there a statistically significant difference in any of the iron-relevant dietary variables (total kilocalories, carbohydrate, total fat, protein, fiber, vitamin A, vitamin C, carotenes, iron, zinc, calcium, phosphorus and copper) between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
3. Is there a statistically significant difference in any of the iron-related serum nutrient indices (serum value of vitamin A, carotenes (alpha & beta carotenes, beta cryptoxanthin, lutein/zeaxanthin, lycopene), vitamin C, calcium, phosphorus, serum total protein, lead and selenium) between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
4. Is there a statistically significant difference in any of the iron status indices including hemoglobin (Hb), serum iron, total iron binding capacity (TIBC), serum transferrin saturation (TS), serum protoporphyrin (EPP), hematocrit (Hct), red blood cell count (RBC), red cell distribution width (RDW), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
5. Is there a statistically significant difference in any of the infection markers including C-reactive protein (CRP), white blood cell count (WBC), lymphocyte number, mononuclear count, platelet count, plasma fibrinogen, and serum albumin between

- non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
6. Is there a statistically significant difference in the following serological variables [aspartate aminotransferase (AST), alanine Aminotransferase (ALT), alkaline phosphatase (ALP), gamma Glutamyl transferase (GGT), serum total bilirubin, serum lactate dehydrogenase (LDH)] between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
 7. Is there a statistically significant difference in the socioeconomic variables including mean education, poverty income ratio, or marital status between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
 8. Is there a statistically significant difference in the life-style factors including alcohol intake, cigarette smoking, physical activity between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?
 9. Is there a statistically significant difference in prevalence of acute inflammation, hepatitis C, respectively, between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged ≥ 20 years?
 10. What proportion of the variation in serum ferritin concentration between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years is attributed to presence of infection, liver disease, nutrient and alcohol intake, socioeconomic status respectively?
 11. Is there a statistically significant difference in the above mentioned proportions between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years?

12. Is there a statistically significant difference in the serum ferritin concentration between non-Hispanic black and non-Hispanic white, male, NHANES III subjects, aged 20-65 years after removal of subjects with abnormal values of serum total protein, TIBC and GGT?
13. Is there a statistically significant difference in the percentage of individuals who had elevated serum ferritin concentration ($>300\mu\text{g/L}$) between non-Hispanic black and non-Hispanic white, males, NHANES III subjects with acute inflammation and hepatitis C present, aged ≥ 20 years, respectively?
14. Is there a statistically significant difference in the odds ratio of having increased serum ferritin concentration between acute-inflammation present non-Hispanic black and white, male, NHANES III subjects, aged ≥ 20 years?
15. Is there a statistically significant difference in the odds ratio of having increased serum ferritin concentration between hepatitis C infected non-Hispanic black and white, male, NHANES III subjects, aged ≥ 20 years?
16. Is there a statistically significant difference in the odds ratio of having elevated serum ferritin concentrations per unit increment of CRP, WBC, lymphocyte number, mononuclear count, platelet count, and serum albumin between acute-inflammation present non-Hispanic black and white, male, NHANES III subjects, aged ≥ 20 years?
17. Is there a statistically significant difference in the odds ratio of having elevated serum ferritin concentrations per unit increment of ALT, AST, ALP, LDH and serum total bilirubin between hepatitis C infected non-Hispanic black and white, male, NHANES III subjects, aged ≥ 20 years?

BACKGROUND

Iron and Iron metabolism

Iron, one of the most abundant metals on Earth, is essential to most life forms and to normal human physiology. Iron is an integral part of many proteins and enzymes that maintain good health. It has been found that the total body iron typically averages 4.0g for a man and 2.5g for a woman. The iron-containing compounds in the body can be grouped into 2 categories: functional (known to serve a metabolic or enzymatic function, for example the hemoglobin, myoglobin, cytochromes, cytochrome oxidase, and catalase) and storage (used for storage and transport of iron). Approximately 2/3 of total body iron is functional iron, most of which is in the form of hemoglobin within circulating erythrocytes. Other iron-containing enzymes and myoglobin make up $\approx 15\%$ of functional iron. In men $\approx 1/3$ of total body iron is in the form of iron stores, whereas in women only $\approx 1/8$ of the total is in that form. Since iron has an important role in the formation of hemoglobin, myoglobin and other compounds, it is necessary to understand how this trace element is utilized and metabolized in our body.

Three major factors affect iron balance and metabolism: intake, stores, and loss. The two determinants of iron intake are the quantity and bioavailability of iron in the diet and the capacity to absorb iron. Iron metabolism is unusual in that iron absorption from the gastrointestinal tract is the primary regulatory mechanism of iron balance (30,31). Iron absorption is influenced by the dietary iron content, bioavailability of dietary iron, amount of storage iron and rate of erythrocyte production (31). Iron availability from foods also varies widely. It is greatest from meat of mammalian origin, less from poultry or fish, and least from eggs, milk and foods of plant origin such as legumes (32,33). This

difference in absorbability of iron is related to the difference between heme and non-heme iron. Non-heme iron consists mostly of iron salts, it is found mainly in plants, dairy products, and iron-fortified foods. Heme iron is mostly found in hemoglobin in meat, poultry, and fish. Although non-heme iron accounts for more than 85% of iron in the American diet, heme iron is 2-3 times better absorbed than the former. Absorption of dietary iron can occur in all parts of the small intestine, and nonheme and heme iron are absorbed by different mechanisms. The exact mechanism by which iron absorption is regulated remains uncertain, but it appears that different pathways are responsible for the regulation of nonheme and heme iron uptake. In case of iron deficiency, absorption is enhanced for both heme and nonheme iron, and enhancement of absorption is more pronounced for nonheme iron.

Iron Transport

In blood or other body fluids, iron is transported by a protein called transferrin. Formation of transferrin depends on the combination of iron with apotransferrin. Iron must first be oxidized in its ferric state before it can bind to apotransferrin. Ceruloplasmin, a copper-containing plasma protein with ferroxidase activity, catalyzes the oxidation of ferrous iron to its ferric form so that it can bind tightly to apotransferrin in the plasma. This role of copper as part of ceruloplasmin is crucial to iron metabolism. Copper deficiency results in accumulation of iron in sites such as the intestine and liver, and reduces iron transport to tissues.

Transferrin transports not only newly absorbed dietary iron that has been transported across the basolateral membrane of the mucosal cell but also transports iron that has been released following the degradation of hemoglobin. In fact, most of the iron entering

the plasma for distribution by transferrin is contributed from hemoglobin destruction and release from storage. Thus, transferrin ferries iron throughout the body delivering both new and recycled iron to tissues for use or for storage. Most of the iron is transported to the bone marrow where hemoglobin is formed, and excess iron is stored in liver hepatocytes and reticuloendothelial cells.

Cell membrane contains a protein called transferrin receptor. Transferrin receptors are found on almost all cell surfaces and consist of two subunits that each binds one transferrin molecule. Once the transferrin molecule is bound to the receptor, the transferrin-transferrin-receptor complex is internalized by endocytosis. Binding to transferrin receptors occurs in pits on the surface of the cell. Upon endocytosis, the pits become coated vesicles (also called an endosome), within which iron is released from transferrin.

Following endocytosis of the iron-transferrin-transferrin receptor complex in the coated vesicles, iron is released into the cytosol, and apotransferrin still attached to the membrane receptor is returned to extracellular fluid. Within the cytosol, iron is either transported to mitochondria where iron is inserted into protoporphyrin to form heme or is taken up by ferritin within siderosomes.

Iron Storage

Iron in excess of need is stored intracellularly as ferritin and hemosiderin, principally in the macrophage system of liver, spleen and bone marrow. The hepatocytes of the liver contain about 60% of body iron; the remaining 40% is found in reticuloendothelial cells that are found in the spleen, liver and bone marrow.

Once iron is released into the cytosol, it combined with apoferritin to form ferritin, the

primary storage form of iron in cells. Ferritin is not a stable compound, it is constantly being degraded and resynthesized, thereby providing an available intracellular iron pool. Hemosiderin, the other iron storage protein, is thought to be a degradation product of ferritin, representing, for example, aggregated ferritin or a deposit of degraded apoferritin and coalesced iron atoms. Iron in hemosiderin can also be labilized to supply free iron, however the rate at which iron is released is slower than that from ferritin.

Iron Reutilization

The avid manner in which the body converts and reutilizes iron is an important characteristic of iron metabolism. A normal adult catabolizes enough hemoglobin each day to release 20 to 25mg of iron, most of which is promptly recycled by formation of new hemoglobin molecules. More than 90% of hemoglobin iron is repeatedly recycled by phagocytosis of old erythrocytes, which occurs chiefly in macrophages of the liver and spleen.

The iron released by the action of the erythrocytic digestion is either bound to transferrin and ultimately redistributed for cell use or enters the storage pool as ferritin or hemosiderin.

Iron Loss

Daily external losses of iron are very limited in healthy individuals [gastrointestinal blood (hemoglobin), 0.35mg; gastrointestinal mucosal (ferritin), 0.10mg; biliary, 0.20mg; urinary, 0.08mg; and skin, 0.20mg]. Additional losses of iron may occur through bleeding and in women this increases to 2mg a day during menstruation.

Serum Ferritin (SF)

Ferritin and hemosiderin are the foremost iron storage compounds present primarily in

the liver, reticuloendothelial cells, and bone marrow (34,35). Ferritin, an iron-containing protein complex, found principally in the intestinal mucosa, spleen, and liver, functions as the primary form of iron storage in the body. In healthy persons, most iron is stored as ferritin (an estimated 70% in men and 80% in women) and smaller amounts are stored as hemosiderin. Ferritin is a large protein shell (MW 450,000) comprised of 24 subunits, covering an iron core containing up to 4000 atoms of iron. Functions of ferritin range from short and long term storage of iron to intracellular housekeeping (delivering iron to enzymes and proteins that need iron to function) inside the cells and is very important in iron homeostasis.

Hemosiderin is aggregated ferritin partially stripped of its protein component. Unlike ferritin, hemosiderin is insoluble in aqueous media. Hemosiderin contains slightly more iron (~30% by weight) than does ferritin. In the liver and spleen of normal animals, there is a slight preponderance of ferritin iron over hemosiderin iron. With increasing concentrations of tissue iron, this ratio is reversed, and at high levels, the additional storage iron is deposited as hemosiderin. Both forms can be mobilized for hemoglobin synthesis when the need for iron exists. Reducing substances such as ascorbate, dithionite, and reduced flavin mononucleotide cause rapid release of iron from ferritin.

Ferritin is found in most cells of the body, especially macrophages, hepatocytes and erythrocytes. Synthesis occurs in the liver and the rate correlates directly with the cellular iron content. Control of ferritin synthesis occurs post-transcriptionally (at the mRNA level). There are iron- and cytokine-responsive elements in ferritin mRNA. Increased iron or cytokines (such as IL-1, IL-6) promotes ferritin translation, resulting in increased iron storage. This is one of the causes of iron "sequestration" that occurs in animals in

terms of cutting off the supply to the invading organism with chronic or inflammatory disease and will reduce serum iron values. The concentration of serum ferritin correlates well with the amount of stored iron in normal (and most diseased) subjects. Serum ferritin concentrations are also quite stable from day-to-day, in contrast to serum iron.

Because of its relatively high stability and solubility, and its direct proportionality to body iron stores in normal individuals (36), serum ferritin is the most commonly used indicator of total body iron stores for normal individuals. The common cutoff for serum ferritin to identify low iron stores is $<12\mu\text{g/L}$ for younger children and $<15\mu\text{g/L}$ for adults (37). A serum ferritin value $>200\mu\text{g/L}$ for premenopausal female and $>300\mu\text{g/L}$ for male usually indicates increased body iron stores and warrant further evaluation for possible iron overload or other diseases. Table 1. depicts normal ranges of serum ferritin for adult males and females.

Table 1. Normal ranges of serum ferritin for adult males and females.

SF Concentrations ($\mu\text{g/L}$)	Adult Males	Adult Females
Normal range	25–300 $\mu\text{g/L}$	25-200 $\mu\text{g/L}$
Elevated	$>300\mu\text{g/L}$	$>200\mu\text{g/L}$ (premenopausal) $>300\mu\text{g/L}$ (postmenopausal)

* Adapted from reference 37.

The strong point of serum ferritin test is that a decrease in the amount of stored iron is the only known cause for a low serum ferritin result. However, an elevated serum ferritin concentration doesn't necessarily indicate genuine increased stored iron. In cases of significant or chronic inflammatory conditions, or disease processes that cause tissue destruction, serum ferritin is also an acute phase reactant and can be significantly elevated.

There are several separate disease categories that can elevate serum ferritin

concentrations, such as iron overload, inflammation, liver disease, alcohol excess. Each of these categories, and their individual impact on serum ferritin concentrations, will be briefly discussed in the following section.

Other Iron Status Indicators

Hemoglobin (Hb)

Hemoglobin is the iron-containing, oxygen-carrying pigment in red blood cells. The hemoglobin molecule has a molecular weight of 68,000 and is composed of 4 heme subunits, each with a polypeptide chain or globin attached (38). Hemoglobin plays a critical role in transferring oxygen from lung to tissues. Its structure of 4 hemes and 4 globin chains provides an efficient mechanism for combining with oxygen without being oxidized. A remarkable feature of hemoglobin is the ability to become fully oxygenated during the short erythrocyte transit time in the pulmonary circulation and then to become largely deoxygenated as erythrocytes traverse tissue capillaries.

Assessment of hemoglobin status has been a commonly used parameter to assess nutritional status. Hemoglobin test is usually used to help diagnosing and monitoring anemia and polycythemia vera (a clonal disorder involving a multipotent hematopoietic progenitor cell in which there is accumulation of phenotypically normal red cells, granulocytes, and platelets in the absence of a recognizable physiologic stimulus). Hemoglobin is the test most commonly used to screen for iron deficiency because changes in Hb concentration occur at the late stages of iron deficiency. Anemia occurs when hemoglobin production is sufficiently depressed to result in a hemoglobin concentration or hematocrit (Hct) below the central 90% or 95% of range for a healthy reference sample of the same age and sex (39). Guidelines (Table 2.) have been

established for hemoglobin assessment based on sex and age of individual (40,41).

Normal range of hemoglobin is usually defined as 130-180g/L for adult male and 120-160g/L for adult females.

Table 2. Maximum hemoglobin concentration and hematocrit values for anemia (40,41.)

	Hemoglobin concentration (<g/dL)	Hematocrit (<%)
Children (age in years)		
1-<2*	11.0	32.9
2-<5	11.1	33.0
5-<8	11.5	34.5
8-<12	11.9	35.4
Men (age in years)		
12-<15	12.5	37.3
15-<18	13.3	39.7
>=18	13.5	39.9
Nonpregnant and lactating women (age in years)	11.8	35.7
12-<15	12.0	35.9
15-<18	12.0	35.7
>=18		
Pregnant women (week's gestation)		
12	11.0	33.0
16	10.6	32.0
20	10.5	32.0
24	10.5	32.0
28	10.7	32.0
32	11.0	33.0
36	11.4	34.0
40	11.9	36.0

* the values listed for children aged 1-<2 years can be used for infants aged 6-12 months.

When an individual's Hb concentration falls below the determined standard for his sex and age, he is said to be anemic. Because smoking and altitude also have impact on hemoglobin and hematocrit values, additional adjustments were made when hemoglobin concentration and hematocrit values were used in diagnosing anemia (table 3.)

In the United States, hemoglobin values are significantly lower among blacks than whites: $\approx 8\text{g/L}$ lower for adults and $\approx 4\text{g/L}$ lower for children aged <5 years. The question of observed difference in hemoglobin concentration may be a function of complex

biological, environmental and social interactions, and more sophisticated systematic research are required to further address the issue.

Table 3

Adjustment of Maximum Hemoglobin and Hematocrit Values for Anemia

Factors	Comment	Adjustment		
Smoking status		Cigarette Smoking Packs per day	Hgb <g/dL	Hct %
		0.5-<1.0 1.0-<2.0 >=2.0	+0.3 +0.5 +0.7	+1.0 +1.5 +2.0
Adapted from CDC website				
Altitude	Long-term residency at high altitude (greater than or equal to 3,000 feet) ...causes a generalized upward shift in Hb concentration and Hct. The cutoff values should be adjusted for this factor.	Altitude (feet)	Hgb <g/dL	Hct %
		3,000-3,999 4,000-4,999 5,000-5,999 6,000-6,999 7,000-7,999 8,000-8,999 9,000-9,999 10,000-11,000	+0.2 +0.3 +0.5 +0.7 +1.0 +1.3 +1.6 +2.0	+0.5 +1.0 +1.5 +2.0 +3.0 +4.0 +5.0 +6.0
Adapted from CDC website				

Erythrocyte protoporphyrin (EP)

Another measure of iron status is Erythrocyte protoporphyrin, which is the immediate precursor of Hb (42). Protoporphyrin, is a metal-free porphyrin, CHN(COOH), that combines with iron to form the heme of hemoglobin, myoglobin, cytochrome, and other iron-containing proteins. EP can be easily measured using a hematofluorometer which requires only a couple of drops of blood and minimal technical experience (43). EP is elevated in the more advanced stage of iron deficient erythropoiesis as its levels increase slowly after the onset of iron deficiency. Raised levels of EP can also be found in subjects with lead poisoning and acquired defects in hemoglobin synthesis. In addition to

lead poisoning and iron deficiency, inflammatory conditions can also elevate erythrocyte protoporphyrin concentration (44,45).

This measure of iron status has several advantages and disadvantages relative to other laboratory measures. For example, the day-to-day variation within persons for erythrocyte protoporphyrin concentration is less than that for serum iron concentration and transferrin saturation (46). A high free erythrocyte protoporphyrin concentration is an earlier indicator of iron-deficient erythropoiesis than is anemia, but it is not as early an indicator of low iron stores as is low serum ferritin concentration (47). Inexpensive, clinic-based methods have been developed for measuring erythrocyte protoporphyrin concentration, but these methods can be less reliable than laboratory methods (44).

Transferrin Saturation (TS)

Transferrin saturation is the ratio of serum iron to iron-binding capacity (TIBC) and is the most accurate indicator of iron supply to the bone marrow. A reduction in transferrin saturation below 16% is a reliable index of an undersupply of iron to the developing erythrocyte (48). Very elevated transferrin saturation (>50% for women and >60% for men) is a good screening indicator for hereditary hemochromatosis (49). As with serum ferritin, conditions other than abnormal iron status can cause depressed or elevated transferrin saturation. Infections and inflammatory conditions often cause depression of serum iron concentrations, thereby lowering the transferrin saturation value (50).

The factors that affect serum iron concentration and TIBC, such as iron status, diurnal variation (46,51), and day-to-day variation within persons, can affect the measured transferrin saturation as well. The diurnal variation is larger for transferrin saturation than it is for Hb concentration or Hct (46,51). Transferrin saturation is an indicator of iron-

deficient erythropoiesis rather than iron depletion; hence, it is less sensitive to changes in iron stores than is serum ferritin concentration (47,52).

Transferrin Receptors (TfR)

Serum transferrin receptor assay is the newest measure of iron status detecting iron deficiency at the cellular level. Transferrin receptors are found on cell membranes and allow iron-bound transferrin to enter the cell. When the iron supply is inadequate, there is an up-regulation of transferrin receptors to enable the cell to compete more effectively for iron. The number of membrane receptors is in proportion to the receptors found in plasma. An increase in serum receptor levels is seen in patients with iron deficient erythropoiesis or iron deficiency anemia. Therefore, an elevated concentration of serum transferrin receptors is good evidence of tissue iron deficiency. Besides iron deficiency, any other condition that results in a high rate of erythrocyte turnover or increased erythropoiesis, such as hemolytic anemia, can also cause the transferrin receptor level to be elevated because the iron supply can not keep up with the increased demand of heme synthesis (53). The cutoff value in defining an elevated transferrin receptor level based on normal values for adults is $>8.5\mu\text{g/L}$ (54).

Unlike serum ferritin, transferrin receptor remains normal in patients with acute or chronic inflammation or liver disease (55) and appears to be effective in distinguishing iron deficiency anemia from anemia of chronic disease (56). However, because transferrin receptor concentration does not become depressed in iron overload, it is not useful in screening for or diagnosing iron overload or hereditary hemochromatosis.

REVIEW OF SELECTED TOPICS IN THE LITERATURE

I. Interpretation of an elevated serum ferritin.

Because the decreased amount of stored iron is the only known cause for a low serum ferritin value, whereas an elevation of serum ferritin is multifactorial, the interpretation of an elevated serum ferritin concentration becomes more important and is therefore crucial in the research design and result interpretation. The following section will review the factors come under the broad headings of: iron overload, acute inflammatory conditions, liver disease and alcohol excess, which can cause an elevation in serum ferritin.

1. Iron overload

From a general point of view, iron overload can be classified as primary or secondary depending on whether it results from a primary defect in the regulation of iron balance or is secondary to other genetic or acquired disorders.

A. Primary iron overload.

1) Hereditary Hemochromatosis

In the United States most iron overload conditions are primary and the most common cause of primary iron overload is hereditary hemochromatosis. Hereditary hemochromatosis (HHC) is an autosomal recessive disorder of iron metabolism characterized by increased iron absorption and deposition in the liver, pancreas, heart, joints, and pituitary gland. Without treatment, death may occur from cirrhosis, primary liver cancer, diabetes, or cardiomyopathy (57).

The prevalence of hemochromatosis varies among different ethnic groups, but the disease is most common in populations of northern European extraction. On the basis of screening studies using iron indices, hemochromatosis is estimated to occur in 2-5 per

1000 persons in white populations in the USA. (58,59) 0.3% of Whites, 0.06% of blacks, and 0.03% of Mexican Americans are homozygous for the C282Y mutation (the most prevalent genotype in the US); that is, 1 in 385 individuals, approximately 718,000 homozygous individuals are in the United States (60).

It was the identification of the *HFE* gene in the 1996 that brought a major breakthrough in the understanding of hereditary hemochromatosis. The *HFE* gene encodes for a novel 343-amino acid major histocompatibility complex (MHC) class I molecule. The exact role of HFE in iron metabolism and the means whereby mutations result in the increased iron absorption found in hereditary hemochromatosis are still unknown, but a number of potentially relevant observations have been made (61).

Missense mutations (the DNA sequence change occurs in a coding region and alters an amino acid) in the *HFE* gene, which is located on the short arm of chromosome 6 (62), are responsible for about 85% of cases of hereditary hemochromatosis in the United States (63); elsewhere the proportion ranges from about 60% to 100%. The most prevalent (homozygous in 83% of patients in the US) is a C282Y mutation, a single mutation of G to A at nucleotide 845 results in the substitution of tyrosine for cysteine at amino acid 282. Amongst subjects of Northern European descent, 80-100% of those with clinical features of hereditary hemochromatosis are homozygous for the C282Y mutation (64-66). Heterozygotes are rarely to be found and are unlikely to develop the disease in the absence of other risk factors for iron overload but can transmit the gene mutation to their children.

Fifteen to 20 percent of the patient population is heterozygous for a different mutation resulting in the substitution of aspartate for histidine at amino acid 63 termed His63Asp

or H63D mutation. This mutation alters the binding affinity for the transferrin receptor and does not usually contribute to increased iron overload in the absence of the C282Y mutation. Patients heterozygous for both C282Y and H63D mutations are termed heterozygotes and only a minority of compound heterozygotes develop clinical symptoms of hemochromatosis (11% of cases) (64,67,68). Patients heterozygous for either C282Y or H63D mutations can also develop iron overload in the setting of alcohol excess, non-alcoholic hepatic steatosis or porphyria cutanea tarda. Whilst the C282Y mutation is largely confined to subjects of European extraction, the H63D mutation is much more widespread (69).

The third mutation resulting in a serine to cysteine substitution at amino acid 65 (Ser65Cys or S65C) has recently been suggested to be associated with a mild form of hemochromatosis (70). There are at least 38 other allelic variants of the *HFE* gene (71-73). Most do not appear to be clinically significant at this time. Other forms of hemochromatosis, such as juvenile hemochromatosis, African iron overload disorder, are known to have a genetic basis but are yet to be more clearly defined (73, 74).

Given the fact that the prevalence of hemochromatosis in the United States is higher among European Americans than African Americans and its salient feature of significantly elevated serum ferritin concentration, it is reasonable for us to assume that the presence of hemochromatosis doesn't contribute to the observed higher serum ferritin concentrations in blacks. Therefore, we decided not to include the presence of hemochromatosis as a factor in our primary analysis.

2) Hereditary aceruloplasminemia

Hereditary aceruloplasminemia is a very rare autosomal recessive disease

characterized by iron overload and progressive neurodegeneration of the retina and basal ganglia associated with specific inherited mutations in the ceruloplasmin gene located on chromosome 3. The disease is caused by the absence of ceruloplasmin (Cp), a copper-containing α_2 -glycoprotein found in the plasma of all vertebrate species (75). The epidemiology information about this disease has not been well-established so far due to its rareness.

Acting as a ferroxidase, which catalyses the oxidation of ferrous to ferric iron, a change required for release of iron to plasma transferrin, ceruloplasmin plays an essential role in the mobilization and oxidation of iron from tissue stores with subsequent incorporation of ferric iron into transferrin (76). A deficiency of Cu will result in iron deposition in the liver, pancreas, basal ganglia, and other organs. A mild-to-moderate degree of anemia with low serum iron and elevated serum ferritin is a constant feature of this disease (77-80). Patients with aceruloplasminemia have hepatic iron and serum ferritin concentrations equivalent to those observed in persons with hemochromatosis, but are only mildly anemic because the nonenzymatic oxidation of iron permits some transfer of iron into transferrin.

Since aceruloplasminemia is a very rare disease in US, it won't be included in our current analysis.

B. Secondary iron overload.

This group includes iron overload either due to or associated with ineffective erythropoiesis, parenteral administration or ingestion of excessive amounts of iron, and disorders which interact with chronic liver disease and those causes of primary iron overload.

1) Iron loading anemia with or without blood transfusion.

Thalassemia major and sideroblastic anemia are the two best studied examples of iron overload secondary to blood transfusions and ineffective erythropoiesis. Because abnormalities in hemoglobin can decrease erythrocyte life span, the pool of erythrocyte precursors is markedly expanded in certain hemoglobinopathies, leading to increased enteral absorption of dietary iron (81-83).

Thalassemia encompasses a group of hemolytic anemias caused by inherited abnormalities of hemoglobin production. The disease is prevalent in the Mediterranean region, Middle East and Southeast Asia, and among ethnic groups originating from these areas. In US, approximately 1000 people are affected currently (84). There are several types of thalassemia that are distinguished on the basis of specific hemoglobin chain affected and the particular synthesis defect present. Thalassemia major (also known as Mediterranean anemia or Cooley's anemia) is an inherited form of hemolytic anemia, characterized by hemoglobin production abnormalities. It occurs when the patient is homozygous for abnormal hemoglobin production, manifested as complete failure of beta-chain synthesis and associated with persistent production of fetal hemoglobin. Patients with thalassemia major present with severe anemia, hepatosplenomegaly, skeletal deformities, growth arrest, jaundice, and increased gastrointestinal absorption of iron. Transfusion is the mainstay of the care of individuals with thalassemia major. The purpose of transfusion is twofold; to improve the anemia and to suppress the ineffective erythropoiesis. Chronic transfusions prevent most of the serious growth, skeletal, and neurological complications of thalassemia major. However, once started, the transfusion-related complications become a major source of morbidity, the worst of which is iron

overload (85).

Sideroblastic anemias (SA) are a heterogeneous group of inherited and acquired hematopoietic disorders characterized by the association of anemia with the presence of non-heme non-ferritin iron deposits within the mitochondria of erythroid precursors in the bone marrow (ringed sideroblasts). It has been generally assumed that mitochondrial iron deposits are secondary to the failure of heme synthesis that would lead to ineffective erythropoiesis and increased iron absorption (82,83,86). Sideroblastic anemia is a complicated disorder and therefore difficult to treat. Often SA acts like iron deficiency anemia (IDA), but unlike IDA, iron tests are normal or increased (e.g. increased serum iron and ferritin) with SA and the exact cause of iron overload in sideroblastic anemia patients is unclear. Treatment depends on the cause; if acquired, remove the offending agent and anemia may disappear. In cases of severe anemia, whole red blood cell transfusion may be required.

The repeated whole blood transfusion will contribute significantly to the existing iron burden and worsen the iron overload situation on transfusion-dependent patients. Transfused blood is disposed through the macrophages of the reticuloendothelial (RE) system, which breaks down the hemoglobin of ingested erythrocytes (87). Thus, large amounts of iron accumulate as clumps of hemosiderin within the RE cells and excess iron, by some means, makes its way into the extracellular fluid and in the plasma until the capacity of apotransferrin to take up iron becomes saturated. At this point excess iron is delivered to the hepatocytes and other parenchymas leading to the development of organ damage (81,88). One unit of transfused blood (500mL) contains ~200-250mg iron from hemoglobin, equivalent to the amount of dietary iron absorbed over 150-200 days.

Transfusion of 6-12 units of blood per year can very easily lead to clinically evident iron overload within a few years.

2) Porphyria cutanea tarda

Porphyria cutanea tarda (PCT) is a term that encompasses a group of related disorders, all of which arise from deficient activity of the heme synthetic enzyme uroporphyrinogen decarboxylase (URO-D) in the liver. PCT is a form of hepatic porphyria associated with a blistering skin eruption, hypertrichosis, and hepatic overproduction of uroporphyrins and heptacarboxyl porphyrins. It includes types that are clearly inherited (familial PCT) and acquired types that may occur in the context of genetic predisposition (sporadic PCT). The absence of a porphyria registry in the United States impedes calculation of its accurate frequencies (89).

Clinical expression of both the familial type and the acquired type of PCT often requires exposure to inducing agents or conditions that adversely affect hepatocytes, particularly ethanol, estrogens, hepatitis, and human immunodeficiency viruses (HIV), and excess iron in the tissue associated with the presence of hemochromatosis genes or other causes (90). Excess iron in the tissue is frequently found in patients with PCT and appears to play a major role in its pathophysiology.

3) Excessive dietary iron

A less common form of iron overload is related to excessive oral iron intake. The best-known example is Bantu-type hemochromatosis, a syndrome seen among Bantu tribesmen in southern Africa who consume large quantities of maize beer, which is high in iron (40-80mg/L). The high iron content comes from the use of iron containers to brew the beer; the acidity of the beer increases iron solubility (91). Recent evidence suggests

that genetic factors may also contribute to iron overload in Africans who have very high iron intakes (92). But in US, this iron container induced excessive iron intake is highly unlikely to occur. Therefore, this reason won't be considered as a factor in our analysis.

Due to the sporadic cases of these secondary iron overload diseases, we reasonably assume that these diseases won't be the contributing factors to the observed serum ferritin difference between AAs and EAs. Therefore, they won't be included in our analysis.

Besides iron overload conditions, there is a series of non-iron overloaded situations, including liver disease, inflammatory conditions, and alcohol excess, which can also lead to increased serum ferritin concentrations.

2. Liver disease

A. Nonalcoholic steatohepatitis (NASH)

In the last few years another disease in which iron appears to have a role has become more important: this disease, called nonalcoholic steatohepatitis (NASH), is described as inflammation of the liver associated with the accumulation of fat in the liver with absence of alcoholism. NASH differs from the nonalcoholic fatty liver (NAFL), a simple accumulation of fat in the liver in that the inflammation causes damage to the liver cells while simple fatty liver probably does not. Also in contrast with NAFL, NASH is a disease that can progress to serious forms of chronic liver disease, almost 20% of patients with NASH progress to cirrhosis over a decade (93-96).

NASH is not connected with other causes of chronic liver disease, including hepatitis B and C viruses, autoimmune disorders, alcohol, drug toxicity, and the accumulation of copper (Wilson's Disease) or hemochromatosis (93).

There is no known specific cause of NASH, however, The most commonly reported risk factors associated with NASH include obesity, type 2 diabetes mellitus, and

hyperlipidemia (97-100). With the increasing prevalence of obesity in the United States, it is currently estimated that the prevalence of NASH is 2% to 3% in the general population, making it the most common liver diseases in the United States. Because obesity and type 2 diabetes in the U.S. occur at higher rates in African Americans compared with white Americans, researchers hypothesized that the prevalence of NASH is also higher in African Americans than European Americans, but data are still limited on the estimates of NASH prevalence in different ethnic groups. The following tables demonstrate current US trend of obesity and type 2 diabetes rates in different ethnic groups.

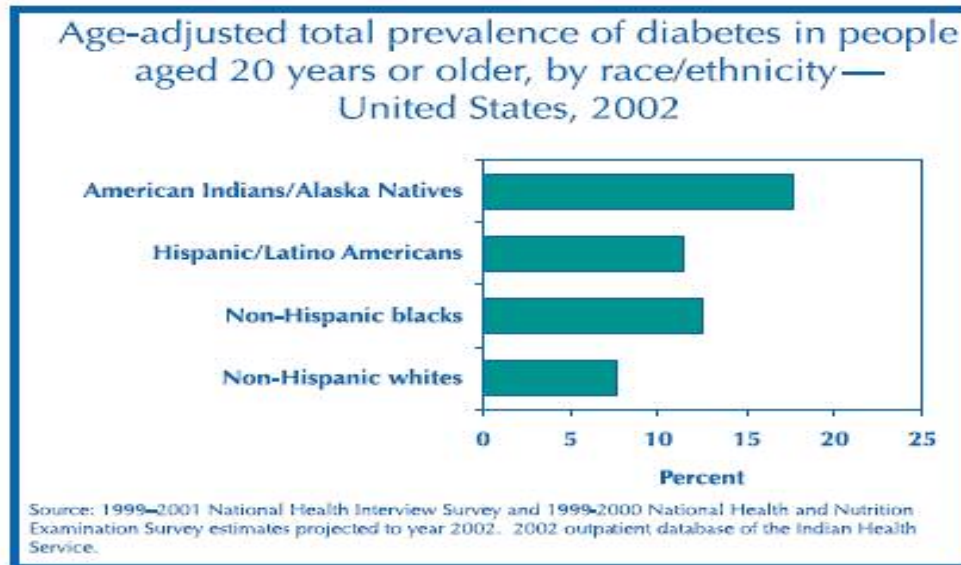
The diagnosis of NASH can be made with certainty only by examination of liver histology. The most frequent biochemical abnormality in the blood in NASH is persistent, mild to moderately elevated transaminases (ALT and AST). Increased ferritin with normal transferrin saturation is frequently found in patients with NASH. The elevated

Increase in Obesity (BMI ≥ 30) Prevalence Among U.S. Adults (Ages 20 to 74) by Racial / Ethnic Group and Gender				
Racial / Ethnic Group	Men Prevalence (%)		Women Prevalence (%)	
	1988 to 1994	1999 to 2000	1988 to 1994	1999 to 2000
Black (non-Hispanic)	21.3	28.8	39.1	50.8
Mexican American	24.4	29.4	36.1	40.1
White (non-Hispanic)	20.7	27.7	23.3	30.6

Source: CDC, National Center for Health Statistics, National Health and Nutrition Examination Survey. Health, United States (Table 70) 2002.

Increase in Severe Obesity (BMI ≥ 40) Prevalence Among U.S. Adults (Ages 20 and older) by Racial / Ethnic Group and Gender				
Racial / Ethnic Group	Men Prevalence (%)		WomenPrevalence (%)	
	1988 to 1994	1999 to 2000	1988 to 1994	1999 to 2000
Black (non-Hispanic)	2.4	3.5	7.9	15.1
Mexican American	1.1	2.4	4.8	5.5
White (non-Hispanic)	1.8	3	3.4	4.9

Source: CDC, National Center for Health Statistics, National Health and Nutrition Examination Survey. Flegal et. al. JAMA 2002;288:1723-7.



ferritin is thought to be due to the simultaneous disorder of iron and glucose and/or lipid metabolism, in most of the cases associated with insulin resistance. The elevated ferritin reflects iron overload only in those patients in whom it persists despite an appropriate (diabetic) diet (101).

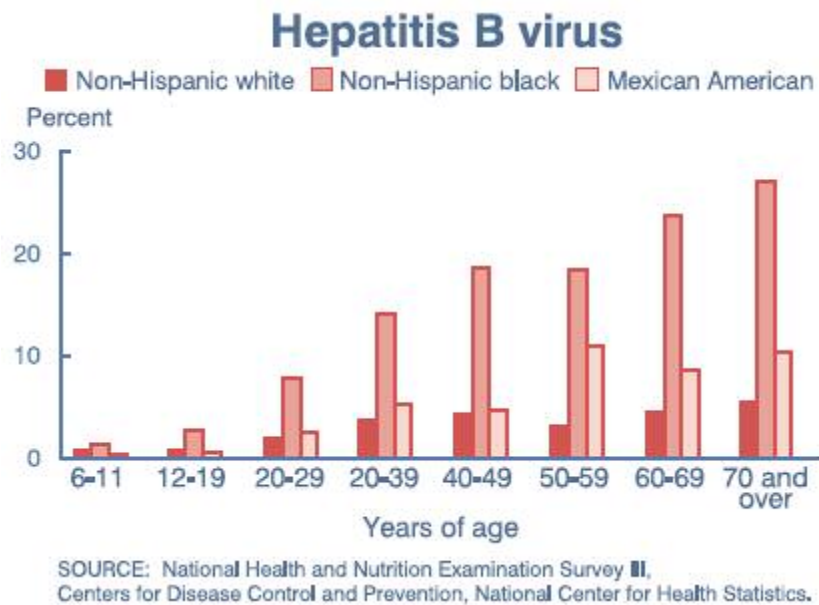
B. Viral hepatitis

Elevation of serum iron can be found in patients with chronic hepatitis secondary to viral infection with hepatitis B and C, indicative of the liver inflammation but not iron overload.

1) Hepatitis B

Hepatitis B is caused by the hepatitis B virus (HBV), a mostly double-stranded DNA virus in the *Hepadnaviridae* family. The prevalence of chronic HBV infection is high (>8%) in all socioeconomic groups in certain areas: all of Africa; Southeast Asia, and the Middle East. Data released by CDC which is based on NHANES II and NHANES III showed that the prevalence of HBV infection in Americans has remained at 5% over the past two decades. HBV infection was significantly higher in non-Hispanic blacks than

whites, and prevalence of infection increased with age in all ethnic groups. The highest prevalence of infection was in older blacks (102).



HBV causes acute and chronic hepatitis. The chances of becoming chronically-infected hepatitis patients depend upon age. About 90% of infected neonates and 50% of infected young children will become chronically infected. In contrast, only about 5% to 10% of immunocompetent adults infected with HBV develop chronic hepatitis B. The natural history of chronic HBV infection can vary dramatically between individuals. Some will remain asymptomatic however are still infected, and this condition is commonly referred to as a chronic carrier state. Some individuals with chronic hepatitis B will have clinically insignificant or minimal liver disease and never develop complications. Others will have clinically apparent chronic hepatitis and some will go on to develop cirrhosis. Individuals with chronic hepatitis B, especially those with cirrhosis but even so-called chronic carriers, are at an increased risk of developing hepatocellular carcinoma (primary liver cancer) (103).

The risk of HBV infection remains high in the United States because of abusive intravenous drug use and sexual contact. HBV is transmitted horizontally by blood and blood products and sexual transmission. It is also transmitted vertically from mother to infant in the perinatal period. The risk of HBV infection is notably high in promiscuous homosexual men but it is also transmitted sexually from men to women and women to men.

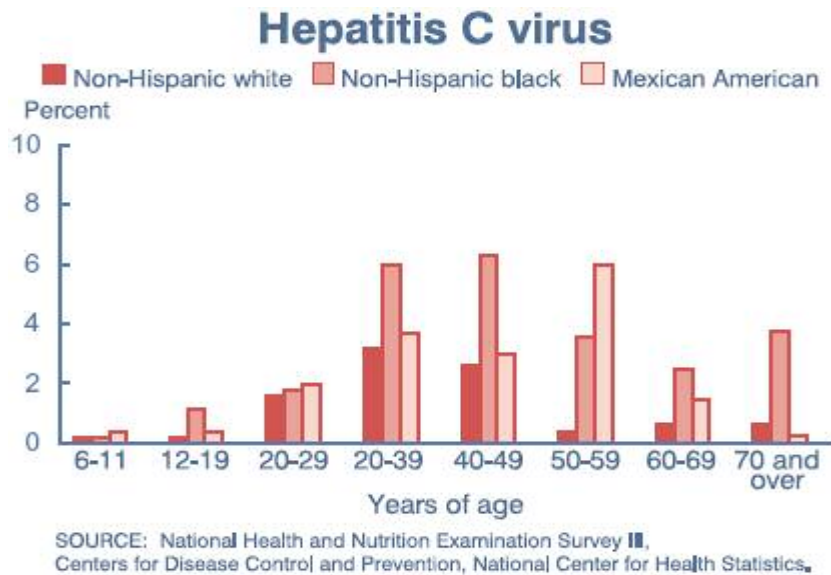
Diagnosis of hepatitis B is based on clinical syndrome, liver function and serology test. Active infection is indicated by the presence of hepatitis B surface antigen (HBsAg) in serum (data of HBsAg are provided in NHANES III Laboratory Data File). A diagnosis of acute hepatitis B is recorded for those patients who also have evidence of disturbed liver function, symptoms and a risk history suggesting recent infection. Repeatedly positive HBsAg over a 6 month period in the absence of acute symptoms or risk history to suggest recent infection indicates a chronic carrier state. A person who has positive hepatitis B antibody (HBsAb) and negative HBsAg is immune and should not be further tested for hepatitis B. Positive HBsAb with negative core antibody is usually indicative of vaccination.

2) Hepatitis C

Hepatitis C is a liver disease caused by hepatitis C virus (HCV), one of the most important causes of chronic liver disease in the United States. It accounts for about 15 percent of acute viral hepatitis, 60 to 70 percent of chronic hepatitis, and up to 50 percent of cirrhosis, end-stage liver disease, and liver cancer.

The NHANES III survey has been used as the latest source for epidemiologic data about HCV. Report based on these data estimated that 1.8% (3.9 million) of the U.S.

population had a positive HCV antibody test, however, this rate was higher in blacks than among whites (3.2% versus 1.5%). The study also found that 74% of these people had chronic infection, and again the rate of viremia was higher in blacks than whites (86% vs. 68%). Black men had higher rates of infection, and the highest prevalence rate was 9.8% among black males ages 40-49 years (102).



Hepatitis C virus is a single stranded enveloped RNA virus that belongs to the *Flaviviridae* family. It spread primarily by contact with blood and blood products. Blood transfusions and the use of shared, unsterilized, or poorly sterilized needles and syringes have been the main causes of the spread of HCV in the United States. With the introduction in 1991 of routine blood screening for HCV antibody and improvements in the test in the mid-1992, transfusion-related hepatitis C has virtually disappeared. At present, injection drug use is the most common risk factor for contracting the disease (104).

Hepatitis C is most readily diagnosed when serum aminotransferases are elevated and anti-HCV (data of anti-HCV are provided in NHANES III Laboratory Data File) is

present in serum. The diagnosis is confirmed by the finding of HCV RNA in serum.

Acute hepatitis C is diagnosed on the basis of symptoms such as jaundice, fatigue, and nausea, along with marked increases in serum ALT (usually greater than 10-fold elevation), and presence of anti-HCV or de novo development of anti-HCV. Chronic hepatitis C is diagnosed when anti-HCV is present and serum aminotransferase levels remain elevated for more than 6 months. Testing for HCV RNA (by PCR) confirms the diagnosis and documents that viremia is present; almost all patients with chronic infection will have the viral genome detectable in serum by PCR (105).

3) Iron and viral hepatitis

A link between iron and viral hepatitis was first stressed a generation ago by Blumberg and colleagues (106), who noted that the prognosis of acute hepatitis B was inversely correlated with levels of serum iron and ferritin. Specifically, patients with higher concentrations of serum iron or ferritin were found less likely to recover spontaneously from acute hepatitis B infection. Shortly after the hepatitis C virus had been cloned and methods for its unequivocal detection established, it was noted that many patients with chronic hepatitis C (CHC) had elevations in serum ferritin (107,108). These increases did not seem to be due solely to the fact that serum ferritin is an acute phase reactant.

Elevations in serum iron saturations were less frequent but also noted.

In the great majority of patients with elevated serum ferritin and/or iron saturation in whom hepatic iron concentrations (HICs) were also measured, the HICs were within the normal range or, at most, only mildly increased (<3-fold above the upper limit of normal) and thus not usually thought to be hepatotoxic (109). In several careful histopathological studies it was shown that the lobular and cellular distribution of stainable iron in the liver

was correlated with therapeutic responses to interferon. Specifically, the presence of cells in portal tracts (stromal and endothelial lining cells) that stained positive for iron was associated with reduced responses to interferon. The iron staining was an independent and significant inverse correlate of therapeutic response, on a par with viral genotype and load (110).

In the 1990s higher concentrations of serum ferritin or HICs were variably associated with decreased likelihood of responding to standard, short-acting interferons, at the time the only effective antiviral therapy for CHC (109). Unfortunately, the effectiveness of such therapy is limited, and the costs and side effects are high. Therefore, it was a natural next step to suggest that iron reduction therapy might be of benefit to increase the response rates to interferon therapy. Indeed, this hypothesis has been confirmed in at least three prospective, randomized, controlled trials (111-113).

In another United States multicenter trial, patients with CHC who previously had failed to respond to interferon were randomized to receive iron reduction alone versus iron reduction plus additional interferon. Neither group achieved significant improvements in terms of cure of CHC, but both showed evidence of histological improvements, with less severe hepatic inflammation (114). These favorable effects of iron reduction alone confirmed and extended earlier reports showing significant improvements in serum ALT levels in patients with CHC who previously had not responded to interferon when they underwent iron reduction by therapeutic venesection (115,116).

3. Inflammatory conditions

Inflammation is a natural reaction to injury or infection. It is a morbid condition of any part of the body, consisting in congestion of the blood vessels, with obstruction of the

blood current, and growth of morbid tissue. It is manifested outwardly by redness and swelling, attended with heat and pain. While bacterial infection is often the cause of internal inflammation, arthritis or allergies can also be at fault. Injury is the most common cause of external inflammation, but allergies, infection and other factors may be at the root.

Concentrations of ferritin in the plasma are increased in acute and chronic inflammation as well as in certain malignancies such as Hodgkin's Disease. Because of its dual role as acute phase reactant, the elevated serum ferritin is more likely a reflection of disease activity other than iron status, especially in the case of Systemic Lupus Erythematosus (SLE), a chronic, usually life-long, potentially fatal autoimmune disease characterized by unpredictable exacerbations and remissions with protean clinical manifestations (117). Patients with autoimmune inflammatory diseases, such as rheumatoid arthritis commonly have elevated serum ferritin too. Active infection will also be associated with an elevated serum ferritin in the absence of iron overload. An elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) should alert one to these possibilities in patients with occult inflammation. Malignancy is another important cause of an acute phase reaction but is unlikely to manifest as an isolated elevation of serum ferritin in the absence of other clinical signs or laboratory abnormalities.

Inflammation may often be accompanied by a large number of physiologic changes, distant from the site of inflammation and involving many organ systems. These systemic changes have since been referred to as the acute-phase response, even though they accompany both acute and chronic inflammation. Currently, the most widely used

indicators of the acute phase response are C-reactive protein and erythrocyte sedimentation rate (118).

C-reactive protein (CRP)

C-reactive protein is a prototypical acute phase protein whose levels rise rapidly in the body in response to trauma, burn, or inflammatory disease. The magnitude of the response reflects the extent of the injury (119). CRP is often used in clinical situations as a non-specific screening tool for inflammatory and infectious diseases. Although the rising levels of CRP often complement the levels of ESR, CRP responds more quickly as it does not depend on fibrinogen or immunoglobulin levels, and is not affected by red blood cell numbers and shape. It is also not affected by anemia, polycythemia, protein levels, age and sex, however, all these factors are found to affect ESR, thus making CRP a better and more accurate marker of infection presence and tissue injury (120).

C-reactive protein is the first protein to become elevated, usually within the first 6-10 hours of inflammation (121). It is used as a good marker of acute inflammation. CRP levels increase within a few hours and reach peak concentration as much as 1000-fold above baseline within a few days as a reaction to an infection, but they then drop back down rapidly after the acute infection passes (3-5 days after inflammatory stimulus). The half-life of CRP in serum is 6 h; thus, serum CRP concentrations decrease to normal within days once the infection or inflammation begins to subside (122,123). The usual concentration of CRP in non-infected individuals is 2mg/L or less. Mild inflammation and viral infections are generally associated with CRP levels of 10-40mg/L, while active inflammation and bacterial infection produces level of 40-200mg/L. C-reactive protein levels above 200mg/L are most often found in cases of severe bacterial infection and

burns (120).

Erythrocyte sedimentation rate (ESR)

The erythrocyte sedimentation rate (ESR), the rate at which erythrocytes settle out of unclotted blood in one hour, has been used as a common marker of infection presence. The major determinant in the sedimentation rate of erythrocytes is erythrocyte aggregation, which usually occurs along a single axis (rouleaux formation). The aggregation of erythrocytes is largely determined by electrostatic forces. Under normal circumstances, the erythrocytes have a negative charge and, therefore, repel each other. When the body is in an acute phase response to infection, many plasma proteins with positive charge are produced and then neutralize the surface charge of erythrocytes, thereby reducing repulsive forces and promoting aggregation. Therefore, infection presence is marked by an increase in ESR, as measured by millimeters per hour (124,125). An elevated C-reactive protein provides evidence of an inflammatory process despite a normal ESR, when used in conjunction with the ESR it greatly increases the sensitivity in detecting inflammatory/infectious processes, especially when variables, such as anemia, confound the ESR.

Other infection parameters

Other blood parameters such as increased fibrinogen and leucocyte count and decreased albumin have also been identified as downstream indicators of chronic inflammation (126-129). However, these parameters may be affected by several physiopathological conditions other than an acute phase reaction (specific organ failure, hematologic disorders, malnutrition). Therefore, their individual specificity as inflammatory markers is rather poor, and none of them is likely to have, by itself,

sensitive indication of inflammation. Data of C-reactive protein, fibrinogen, leucocyte count, serum albumin and other blood parameters are provided in NHANES III Laboratory Data File. ESR was not performed in NHANES III, therefore, it won't be used in our current analysis.

4. Alcohol excess

It is well known that induction of ferritin synthesis occurs during alcohol abuse, raised body iron stores are found in alcoholics and heavy drinkers. Regular consumption of alcohol is responsible for the disruption of normal iron metabolism in humans, resulting in the excess deposition of iron in the liver in approximately one-third of alcoholic subjects (130-133).

Studies have found consistently positive relationship between alcohol intake and serum ferritin values in both men and women. An increase in alcohol intake was associated with an increase in serum ferritin concentrations. The mechanisms by which alcohol affects serum ferritin are unclarified, however alcohol may influence the serum ferritin in several ways. First, alcohol has some enhanceing effect on intestinal iron absorption, especially in wine drinkers, and may in this way contribute to increased body iron stores and serum ferritin (134). Second, alcohol may exert a toxic effect on hepatocytes and cause an elevation of serum ferritin through a release of intracellular ferritin (135). Third, alcohol may have an enhancing effect on the intracellular ferritin synthesis.

Not fully characterized, is the relationship between low or safe drinking levels and indices of body iron stores, and the factors that influence the relationship between alcohol and iron stores. Alcohol (>60g/day) has been shown to exacerbate liver damage due to iron overload. The elevation of serum ferritin caused by alcohol excess can occur without

elevation of other liver enzymes and often falls dramatically with abstinence from alcohol.

In the U.S., one drink is usually considered to be 12 ounces of beer, 5 ounces of wine, or 1½ ounces of spirits (hard liquor such as gin or whiskey) (136). Each delivers about 12 to 14 grams of alcohol. The definition of moderate drinking is something of a balancing act. Moderate drinking sits at the point at which the health benefits of alcohol clearly outweigh the risks. The latest consensus places this point at no more than one to two drinks per day for men, and no more than one drink per day for women. This is the definition used by the U.S. Department of Agriculture and the Dietary Guidelines for Americans, and is widely used in the U.S.

The importance of conducting alcohol research among minorities is underscored by findings that members of many ethnic minorities in the United States report higher rates of heavy drinking and alcohol-related problems than do whites (137,138). Using data from a statewide survey of New York, Barr et al. (139) found that black men with lower incomes were significantly more likely than their white counterparts to report alcohol and other drug-related problems. Jones-Webb et al. (140) analyzed the 1984 National Alcohol survey and found that black men in the lower socioeconomic classes were significantly more likely than white men in the lower classes to report greater numbers of drinking consequences; but this was not true for black and white men in the higher socioeconomic classes. Lillie-Blanton and associates (141) reported less educated black women were more likely than their white counterparts to report heavier drinking, but the opposite was true for more educated black and white women. In 1997, Jones-Webb et al. (142) reanalyzed the longitudinal data from the 1984 and 1992 National Alcohol Surveys, and

they reported higher alcohol consumption (#drinks/month) in blacks than whites except black women and their white counterparts in 1984 (Table 4).

Table 4. Mean alcohol consumption for Blacks and Whites, 1984 and 1992.

Alcohol consumption (# drinks/month)	Men		Women	
	Black (n=223)	White (n=298)	Black (n=213)	White (n=277)
1984 (year)	63.47	48.00	17.14	19.80
1992 (year)	61.92	37.43	22.23	13.36

* Adapted from reference 142.

Based on these findings, it is reasonable to assume that alcohol consumption is higher among NHANES III black males than their white counterparts and this may play a role in the observed higher serum ferritin concentration among blacks. Therefore, the effect of alcohol consumption will be analyzed in our current analysis.

II. Investigation of iron store difference between African Americans and European Americans

Researchers as early as 1946 have noted discrepancies between the hemoglobin concentrations of North American blacks and whites (143). The study was conducted by Milam and Muench, in which they found a fairly steady difference in hemoglobin concentration of 0.5-1.0g/dL between blacks and whites, with blacks consistently posting a lower Hb concentration. During the 1970s, decreased hemoglobin concentrations among blacks were shown not to be correlated with diet (144-146). In 1973, Owen et al. reported a difference in the Hb concentrations of 0.0-1.0g/dL between 350 white and 266 black children of less than six years of age (144). The author proposed that the linear growth rate found in black children may account for the lower Hb concentration, given the children consumed the same amount of iron. Additionally, they also proposed that the possible differences in enzyme activity, specially, the enzyme 2,3-diphosphoglycerate, may also contribute to the Hb differences. In 1976 Garn looked again at the Hb differences between the two groups, this time during pregnancy (146). The study compared the Hb concentrations of white (n=180) and black (n=129) black pregnant women aged 25-40 years. The average difference in the Hb concentrations was 0.64g/dL. The authors concluded the difference to be significant enough to reexamine the manner in which Hb concentrations are used in clinical and diagnostic settings to assess presence of anemia and iron status, especially among the black population. Based on these observed differences, some researchers recommended different hemoglobin norms for blacks (147-149).

During 1980s, a few articles attributed this reduced hemoglobin values to population genetic differences (150,151), while some other studies countered such claims (152-155),

suggesting that differences resulted from hereditary hemoglobinopathies in a small percentage of the black population. In 1981, Williams did a thorough investigation of red cell indices and iron status, as well as copper and zinc status in his investigation of the reported differences in Hb concentration between blacks and whites (156). He measured white blood cell count, red blood cell count, Hb, hematocrit, MCV, MCH, MCHC, serum iron, TIBC, TS, serum ferritin, serum zinc, serum copper, ceruloplasmin, and glucose-6-phosphate dehydrogenase activity in a sample size of 111 black and white, men and women, aged 21-60 years. Significant but inconsistent differences in Hb concentrations were found between two groups for each sex. Interestingly, he also found that blacks had a significant higher ferritin concentration compared to white, yet had a lower Hb concentration which may suggest a hematological abnormality, or infection presence, however, these were not further explored in the analysis.

Another study which also found a significant higher serum ferritin concentrations in black servicemen compared to white servicemen despite significant lower Hb values is the study conducted by Jackson et al. in 1983 (155). In this study, they attempted to control for the impact of diet and income by comparing the Hb levels of black and white men of similar service rank in the military. 2,599 apparently healthy servicemen were included in the study and blood sample was used for analyzing Hb, serum iron, serum ferritin, folic acid, TIBC, TS, folic acid, and serum copper and zinc. Data analyses showed that black males (n = 331) had a 0.27 g/dL lower mean Hb than white males (n = 2268, P = 0.006). However, when only black (n = 60) and white (n = 371) subjects with known iron intakes at or above the recommended level were compared, the differences in the Hb of the two groups became even smaller. In the conclusion, Jackson stated that

although a genetic component cannot be completely ruled out at that time, nutritional factors appear to play an important role in Hb levels.

In the 1990s, some investigators again recommended that blacks might require lower hemoglobin/hematocrit standards (157,158), while counter claims pointed out that data indicating the need for dual standards were equivocal (159). In 1991, Pan and Habicht analyzed data from the NHANES I survey and found continued support for the call for separate standards, dependent on race, for assessing anemia (160). The authors concluded that the current standards for assessing anemia are not appropriate for the black population, reducing the hemoglobin cut-off by 0.5g/dL was substantial enough to justify a separate standard for blacks.

In 1992, Perry et al. attempted to explain the apparent Hb differences between blacks and whites by looking at the iron nutrition of subjects from NHANES II (161). Data from 2,515 male and female subjects aged 3-12 years, or 18-45 years was analyzed for Hb level, Hct, serum ferritin, serum iron, transferrin saturation, TIBC, erythrocyte protoporphyrin and a single 24 hour dietary recall. Results demonstrated a significant difference in Hb levels between the two groups, after controlling for sex, age, dietary iron intake and serum ferritin. Similarly, it was also found that the black subjects had higher ferritin concentrations despite lower Hb levels. Yet, the author attributed this higher ferritin concentrations in the black population to a higher presence of infection, a factor not considered in the study.

Later in 1994, Johnson-Spear and Yip reported a justification for race-specific anemia screening criteria in a study that examined the Hb differences between 388 black and 2,586 white women aged 18-44 years based on NHANES II data (162). Interestingly, it

was also Ray Yip who supported uniform standards in the assessment of anemia in a previous study (156).

Vacillation on this issue results partially from the failure to develop a convincing explanation for noted black-white differences. Despite several studies have simultaneously found a higher serum ferritin concentration but lower Hb concentrations in black populations, all the efforts were exerted to explain the differences in Hb levels, whereas the difference in ferritin was always ignored. In 1997, Susan Kent published a review article which reexamined the apparent genetic component of the observed Hb differences between the races (163). In the article, Kent proposed that this higher serum ferritin concentrations among black populations was a classic symptom of the anemia of infection or chronic disease. She also further suggested that blacks from all social levels experienced more social stress as a result of belonging to a disadvantaged group, and the cumulative negative impact of this stress on the individual, throughout a lifetime, will manifest itself in the individual's physical and mental health. However, very few studies so far had examined the extent of the impact of this proposed social stress on an individual's physical and mental health.

A recent study by Zacharski et al. examined serum ferritin concentrations by age, sex and race and compared the serum ferritin values with the percent transferrin saturation in contemporary residents of the United States (9). The sample for this study was from NHAHES III, consisted of 20,040 individuals >17 years of age including 8477 white, 5484 black, 5304 Hispanic, and 775 of "other" ethnicity. Results demonstrated that serum ferritin concentrations rose in the late teens in men and after menopause in women. And this rise was more rapid and maximum ferritin concentrations were greater for blacks

than whites and Hispanics of comparable age and sex. Ferritin concentrations in black men exceeded those in whites and Hispanics for each decade of life, and differences were amplified with age. After menopause, ferritin concentrations in black women rose more rapidly and exceeded those of white and Hispanic women. Overall, they found that ferritin concentrations were approximately 7% to 8% greater for blacks than for whites and Hispanics, and differences remained throughout the second half of life. And the distribution of ferritin values differed from the percent transferrin saturation. The authors concluded that different patterns of iron accumulation exist according to age, sex, and ethnicity, and serum ferritin concentrations reflect graded, population-based differences in body iron stores. Although they didn't further investigate why these differences exist, they pointed out the importance of testing iron hypothesis (disparities in iron stores contribute to the differences in disease among different ethnic groups) which may be pivotal in explaining the differences in disease prevalence between blacks and whites.

Despite the consistent findings of higher serum ferritin concentrations for African Americans than European Americans mentioned in several studies, there have not been any in-depth studies investigating this difference. Therefore, it is imperative, from a public health perspective, to better understand this consistently observed serum ferritin difference between black and white populations. And our current study will be the first one dedicated to detect the factors which contribute to the difference.

III. Investigations of differences in elevation of serum ferritin between African Americans and European Americans in regard to selected conditions.

There are very few studies that examined the differences in elevation of serum ferritin in response to acute inflammation, and hepatitis C between AAs and EAs. And most of them were not directly related to this topic. The only one which appeared to be relevant is the study on differences in iron stores and hepatitis C.

In 2003, Ioannou et al. investigated differences in the relationship between hepatitis C infection and iron stores between blacks and nonblacks by using data from the third NHANES (23). The incentive of this study was to test whether differences in iron stores between HCV-infected blacks and nonblacks would explain the constantly described low response of African Americans to antiviral treatment (164-169). They determined the risk of having increased iron stores, defined as elevation of both serum ferritin and transferrin-iron saturation, in HCV-RNA-positive blacks (n=100) and nonblacks (n=126) relative to HCV-RNA-negative blacks (n=4,002) and nonblacks (n=10,943). They also compared the magnitude of elevation in serum markers of iron stores in patients with abnormal liver enzymes and patients with normal liver enzymes. Subjects aged ≥ 17 years were included in the analyses, those who had advanced cirrhosis, with missing data on tested variables were excluded from the analysis. The results found that HCV-positive blacks were 5.4 times more likely to have increased iron stores than HCV-positive nonblacks. Blacks showed a larger difference in the proportion of persons with increased iron stores between HCV-positive and HCV-negative persons compared to other ethnicities. HCV-positive blacks with abnormal liver enzymes also had an increased risk of having increased iron stores. The authors concluded that blacks had a greater proportion than persons of other ethnicities respond to HCV infection with an increase in

iron stores. This striking difference in iron markers between blacks and nonblacks was unlikely related to differences in the presence of acute inflammation, since they adjusted for C-reactive protein in their analysis and CRP levels didn't affect those observations. Therefore the findings may partly explain why black HVC-infected patients are more resistant to antiviral treatment relative to whites since studies found that iron depletion by phlebotomy in patients with HCV had improved response to interferon. Although the study was limited by lack of using hepatic iron concentration, which is a better predictor of response to interferon and a more accurate marker of iron store, previous studies have shown that serum ferritin and TS had a good correlation to hepatic iron concentration (170-172). Particularly that liver biopsy is not yet a standard test performed in large population-based studies. This finding, in turn, is an evidence of iron hypothesis that differences in iron stores may contribute to the differences in diseases among ethnic groups, which may also have important clinical implications.

Unlike the study of hepatic C infection and iron stores, there have been no published reports investigating the disparities in iron stores with regard to acute inflammation among ethnic groups. It appears that most attention was focused on the relationship between ethnicity and cytokine genes (25-29). Most studies reported that African Americans have a greater predisposition to inflammation because of the more up-regulate inflammation cytokine genotypes found in them.

Ness et al. (25) recently compared genetic data on 179 African-American and 396 white women who sought prenatal care and delivered uncomplicated, single, first births at Magee-Womens Hospital of the University of Pittsburgh Medical Center between 1997 and 2001. Blood samples were analyzed for a multitude of functionally relevant allelic

variants in cytokine-regulating genes. Among these were several genes regulating the immune system proteins interleukin-1, interleukin-1 alpha, interleukin-1 beta, interleukin-6, interleukin-10, interleukin-18 and tumor necrosis factor-alpha (TNFA). They found that the proinflammatory cytokine interleukin-6 IL6-174 G/G genotype was 36.5 times (95% confidence interval (CI): 8.8, 151.9) more common among African Americans. Genotypes known to down-regulate the antiinflammatory interleukin-10 (IL10-819 T/T and IL10-1082 A/A) were elevated 3.5-fold (95% CI: 1.8, 6.6) and 2.8-fold (95% CI: 1.6, 4.9) in African Americans. Cytokine genotypes found to be more common in African-American women were consistently those that up-regulate inflammation. The results were consistent with the previous study conducted by Hoffman et al. (26), in which they also found that African Americans to be more likely to have cytokine polymorphisms with functional consequences of a predisposition to higher level of proinflammatory cytokines and lower levels of immunosuppressant. Other studies (27-29) also showed ethnic variation in allelic distribution in cytokine genes, including TNFA, IL1A, IL1B, IL2, IL6, IL12, and IFNG (interferon- γ) and increased CD80 and CD86 expression in African Americans.

METHODS

I. Survey Description and Sample Design

The National Health and Nutrition Examination Surveys (NHANES) are a series of national surveys that the National Center for Health Statistics, Center for Disease Control and Prevention (CDC), began conducting in 1966 to estimate the prevalence and risk factors for common diseases in the U.S. population. The third NHANES was the seventh in this series, conducted from 1988 to 1994, utilizing 89 survey locations that were randomly divided into two sets or phases, the first consisting of 44 locations, the second consisting of 45 locations. The NHANES survey used complex, stratified, multi-stage, clustered samples of civilian, non-institutionalized populations. A detailed description of design specifications and copies of the data collection forms can be obtained in the Plan and Operation of the Third National Health and Nutrition Examination Survey, 1988-1994 (173,174). In total, 39,695 persons were selected over the six-year period; of those, 33,994 (86%) were interviewed in their homes. All interviewed persons were invited to the Medical Examination Center (MEC) for a medical examination. 30,818 (78%) of the selected persons were examined in the MEC; an additional 493 persons were given a special limited examination in their homes.

Data Preparation and Use of Sample Weights

The NHANES III data CD-ROM (175), obtained from the National Center for Health Statistics, served as the source of data for this study. The NHANES III data files consist of five separate data sets; the Household Adult Data File, the Household Youth Data File, the Examination Data File, the Laboratory Data File, and the Dietary Recall Data File. Data from the Household Adult Data File, the Examination Data File and the Laboratory

Data File were included in this analysis (176-178). Detailed specimen collection and processing instructions were discussed in the Manual for Medical Technicians (174). The multi-level data collection and quality control system were discussed in detail in the Plan and Operation of the Third National Health and Nutrition Examination Survey, 1988-1994 (173,174). All interview, laboratory, and examination data were sent to NCHS for final processing. Details on survey instruments and forms, training manuals, and data collection procedures were documented elsewhere (179).

The variables included in this study were selected based on the impact that they exert either directly or indirectly on serum ferritin concentrations of participants. The impacts have already been discussed in detail in the literature review section. Because the data sets for the third NHANES were in a SAS format, therefore, SAS was used to sort the data sets, obtain the desired variables, and merge the data from the three data sets into one final data set. The sequence or identification number allowed for extracting the variables of interest from each of the data files (Household Adult Data File, the Examination Data File, the Laboratory Data File) and merging the extracted data to form the final data set.

Because of the complex survey design of the third NHANES, appropriate sample weights were applied in all analyses to adjust for the complex survey design effect. By weighting the sample data, analysts are able to produce estimates of statistics that would have been obtained if the entire sampling frame (for NHANES III, the entire sampling frame is the United States) had been surveyed. Sample weights can be considered as measures of the number of persons the particular sample observation represents. Weighting takes into account several aspects of the survey: the specific probabilities of

selection for the individual domains that were over-sampled, as well as non-response, and differences between the sample and the total US population. Differences between the sample and the total population may arise due to the sampling variability, differential under coverage in the survey among demographic groups, and possibly other types of response errors, such as differential response rates or misclassification errors. Since NHANES III over-sampled non-Hispanic blacks and Mexican-Americans, children under 5, and the elderly people (≥ 60 y), the population weights were used in all analyses to account for over-sampling, multi-stage sampling design, and nonresponse to the household interview and the examination. The sample weight used in this study was selected based on the recommendation from the National Center for Health Statistics, and is the appropriate sample weight when data from both phases of the survey are being analyzed as a whole.

II. Statistical Software

The newly released SAS version 9.1 was used to prepare the data source for our analysis including sorting, extracting, merging and assumption testing of the data set. The new version of SAS 9.1 incorporated some survey procedures to analyze complex survey data. SUDAAN 9.0 also has the capability to use all of the design variables from multiple stage sampling and does not need to assume sampling with replacement. In survey analysis packages, the variance estimation using SUDAAN's linearized Taylor series with unmasked all stage sampling information is considered to be the most accurate method among all softwares that take complex sampling structure into account. Therefore, our final data set was analyzed by using the SUDAAN and SAS statistical software packages.

III. Primary Analysis

1. Study Sample

A total number of 3,554 non-Hispanic white (NHW) and non-Hispanic black (NHW) male subjects, aged 20-65 years, with available serum ferritin concentration was included in this study. Among them, 1,938 were NHWs and 1,616 were NHBs. Due to the impact each has on ferritin, the subjects who were hemophiliacs and subjects who had recently undergone chemotherapy were excluded from the analysis. Subjects who had been treated for anemia (include diet, iron pills, iron shots, transfusions as treatment) within past 3 months and who had blood donation within one month before the survey were also excluded from the analysis. We only chose males to eliminate the additional, potential confounding effects such as growth, menstruation, birth control pills, pregnancy, parity, menopause or hormone replacement therapy associated with females. Adult males aged 20-65 years were selected because studies have shown that serum ferritin concentrations remain stable during adulthood but tend to rise after the age of 65 years, due to either increased iron stores or the effect of progressive development of inflammatory diseases (180,181).

2. Variables

Since our goal is to identify the possible factors which contribute to serum ferritin difference between NHWs and NHBs, variables that either directly or indirectly affect serum ferritin concentration were included in our analysis based on the existing knowledge of ferritin metabolism. Because the potential variables encompass different postulations of their impacts on serum ferritin, we divided them into different categories for sub-regression analyses in order to further reduce the number of variables to be placed

in the final regression model. Variables were grouped together and placed into one of the following six sub-categories: dietary variables (including alcohol intake), serum nutrient indices, hematological markers, infection markers, serological markers, and socioeconomic variables.

Dietary variables are the ones that we first considered as the variables influence iron nutrition and iron stores. Dietary intakes including carbohydrate, total fat, protein, fiber, vitamin C, vitamin A, carotenes, iron, zinc, calcium, phosphorus and copper were examined from the 24-h dietary recall data.

Carbohydrate, total fat and protein are the major constituents of a person's diet and are the basic indices used to assess one's nutritional status. Inadequate energy or nutrient intake will compromise the status of health and nutrition. The consequence of compromised nutrition and health status could then lead to impaired erythropoiesis and decreased iron stores. Fiber and zinc are the inhibitors of iron absorption. Fiber-associated decreased diffusion rate of nutrients result in diminished absorption of most nutrients including iron. Zinc inhibits iron absorption by competing for the same portion of a common absorptive pathway (183). Calcium and phosphorus are thought to interact with iron and inhibits its absorption through Fe:Ca:PO₄ chelate formation at the intestinal mucosa. Several studies have demonstrated that ingestion of calcium in amounts of 300 to 600 mg when given up to 18 mg iron as ferrous sulfate or when incorporated into food substantially decrease iron absorption by up to 70% (184-188). The term vitamin A is used to refer to retinol, and many but not all carotenes can be converted into retinol. Iron status is also interrelated with vitamin A. Reduced vitamin A status alters iron distribution between tissues. Studies in experimental animals showed that low plasma

retinol concentrations are associated with decreased plasma iron and blood hemoglobin and hematocrit as well as increased hepatic iron accumulation. It appears that the mechanism of interaction between vitamin A and iron is an impairment in the mobilization of iron from the liver and/or incorporation of iron into the erythrocyte. Vitamin A also may be directly acting on iron metabolism or storage or may be affecting differentiation of the red blood cell (189,190). The interaction between iron and vitamin C is related not only to the vitamin's effect on intestinal absorption of nonheme iron but also on the distribution of iron in the body. Specifically, ascorbate enhances the intestinal absorption of nonheme iron by either reducing iron to a ferrous form from a ferric form or by forming a soluble complex with the iron in the alkaline pH of the small intestine to thereby enhance iron's absorption. The effect of the vitamin C in the distribution and mobilization of storage iron has also been demonstrated, however, the underlying mechanisms are still uncertain (191,192). Copper is also believed essential for the production of red blood cells and plays a role in the transport and utilization of iron. Without the copper-dependent ferroxidase activity, iron cannot be mobilized from ferritin stores.

Alcohol intake was indicated as the total number of alcoholic drinks consumed by participants per month. In the Household Adult Questionnaire, participants were asked to report their consumption of beer ("lite" beer included), wine (wine coolers, sangria, and champagne included), and hard liquor (gin, rum, whiskey, tequila, vodka, liqueurs, etc) in times/mo. One drink of alcohol was described as 12 oz (360mL) beer, 4 oz (120mL) wine, or 1 oz (30mL) hard liquor. The total number of alcoholic drinks consumed by participants was therefore computed by adding the numbers of drinks of beer, wine, and

hard liquor. Alcohol consumption was then further divided into 4 categories: nondrinker (0 drinks/mo), light drinkers (1-30 drinks/mo), moderate drinkers (31-60 drinks/mo), heavy drinkers (>60 drinks/mo).

In addition to dietary intake, the manner in which a person utilizes these nutrients for various metabolic processes also has impact on iron status. Therefore, **serum nutrient indices** include the actual serum value of retinol, carotenes (alpha & beta carotenes, beta cryptoxanthin, lutein/zeaxanthin, lycopene), vitamin C, calcium, phosphorus, serum total protein, lead and selenium were analyzed. Here we included lead because it interacts with iron. Lead inhibits the activity of Δ -aminolevulinic acid dehydratase, an enzyme required in heme synthesis. Lead also inhibits ferrochelatase activity, the enzyme that incorporates iron into heme. In addition, increased absorption of lead occurs with iron deficiency in animals, but the mechanism through which iron deficiency improves lead absorption is unknown (193). Iron is also thought to interact with selenium, but the mechanism behind is not known. Iron deficiency is associated with decreased selenium concentrations as well as glutathione peroxidase synthesis and activity (194,195). Since NHANES didn't have data for zinc and copper serum values, we were not able to assess the serum levels for these nutrients. The dietary intake for selenium and lead was not provided in NHANES III.

As direct assessment of iron status, the **hematological markers** including hemoglobin (Hb), serum iron, total iron binding capacity (TIBC), serum transferrin saturation (TS), serum protoporphyrin (EPP), hematocrit (Hct), red blood cell count (RBC), red cell distribution width (RDW), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were included in our analysis. Each

parameter of iron status contributes to the understanding of ferritin concentrations by reflecting changes in different body iron compartments and different levels of iron depletion. Although a low serum ferritin concentration defines the onset of iron deficiency, it does not indicate the severity of the iron deficiency due to higher assay variability. Therefore, additional measurements such as TS or transferrin receptor (not available in NHANES III) will need to be taken in order to further differentiate the stages of iron deficiency. Correct interpretation of elevated serum ferritin concentrations relies on both using the appropriate gender and age specific reference range and conjunction with other iron status indicators since serum ferritin concentrations may also increase in the presence of acute inflammation, infection, anemia of chronic disease (normal to elevated), malignancy, liver disease and alcohol excess other than genuine increased iron stores. Data on these variables are provided in NHANES III Laboratory Data File.

Another major focus of this analysis is to investigate whether the presence of infection contributed to the observed serum ferritin difference between NHBs and NHWs. Therefore, a set of **infection markers**; variables that are indicative of presence of infection are included: C-reactive protein (CRP), white blood cell count (WBC), lymphocyte number, mononuclear count, platelet count, plasma fibrinogen, serum albumin. Data on these variables are provided in NHANES III Laboratory Data File.

Because serum ferritin also increases with presence of liver diseases, e.g. hepatitis C, the following **serological variables** which are indicative of the presence of liver disease were included: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), serum total bilirubin, serum lactate dehydrogenase (LDH), and hepatitis C antibody (anti-HCV), which was

used to identify the presence of hepatitis C. Data of these variables are also provided in NHANES III Laboratory Data File.

As a part of environmental effect, **socioeconomic variables** including education, poverty income ratio (PIR), and marital status are considered to have indirect impact on an individual's health and iron status. Education was measured as the highest year or grade completed by the respondent, and was further dichotomized as higher than high school or less than high school education. Poverty income ratio (PIR) is based on total household income adjusted for household size and the cost of living at the time of the study. It is used as an eligibility criterion for participation in federal and state economic assistance programs and as an index of relative socioeconomic status in NHANES III survey. We used 3 PIR strata: <1.3, 1.3-3.5, and >3.5 to represent low income level, moderate income level and high income level, respectively according to the analytic and reporting guidelines of NHANES III (196). Marital status was divided into two categories as married including living as married, and unmarried including being widowed, divorced, separated or never married.

Other variables that may have affected serum ferritin were adjusted for in the statistical analyses to reduce residual variance in the outcomes and to control for potential confounding. These variables included age, ethnicity, body mass index (BMI; in kg/m^2), cigarette smoking and physical activity. BMI was further divided into 4 categories: underweight ($\text{BMI} < 18.5$), normal ($18.5 \leq \text{BMI} < 25$), overweight ($25 \leq \text{BMI} < 30$), obese ($\text{BMI} \geq 30$). Cigarette smoking was classified as never smoked (if they had smoked <100 cigarettes in their lifetime), former smoker (≥ 100 lifetime cigarettes, not currently smoking), and current smoker (≥ 100 lifetime cigarettes, currently smoking). Physical

activity, which is a composite measure of the frequency of exercise in the last month and the intensity rating (metabolic equivalent) of each activity, was calculated by summing the activities in the last month and was divided into 3 categories: sedentary (<20th percentile), moderate (<60th percentile), and active (\geq 60th percentile).

In addition, disease prevalence including anemia, arthritis, acute inflammation, cancer (various types), congestive heart failure, diabetes mellitus, heart attack, hypertension, emphysema and stroke were also compared between NHWs and NHBs. Status of arthritis, cancer, congestive heart failure, diabetes mellitus, heart attack, emphysema and stroke was determined by the response to the question of whether a doctor has ever told the respondent that he/she had such diseases. Undiagnosed diabetes was identified by having a fasting plasma glucose concentration >126mg/dL (197) or currently talking insulin. Hypertension was defined as having systolic blood pressure \geq 140mmHg or/and diastolic blood pressure \geq 90mmHg (198), or if a doctor has told the participant that he or she had hypertension. Anemia was defined as having a hemoglobin concentration <13g/dL in men according to the World Health Organization (WHO) criteria (199). Acute inflammation was detected by having a serum CRP concentration \geq 1.0mg/dL (123,200). Presence of elevated serum ferritin was defined as having serum ferritin >300 μ g/L in men (37).

3. Statistical Analysis

Descriptive statistics were computed for all variables, including means for continuous data, frequencies for categorical variables, and standard error of the mean (SEM). Geometric mean was used to present the mean serum ferritin and protoporphyrin concentration because of their right skewed data distribution. SUDAAN's t-test and Chi-

square test were applied to compare the means of all continuous variables and frequencies of all categorical variables between NHBs and NHWs, respectively.

Statistical significance was set at a p value <0.01 for both t-test and χ^2 test.

Multiple regression analysis was used to examine the possible variables which contribute to the variation in SF concentrations. Due to the large number of regressors under each category, separate sub-regression models were carried out with SF concentration as the dependent and ethnicity as a covariate for each set of variables including dietary variables, serum nutrient indices, hematological markers, infection markers, serological markers, and socioeconomic variables. Variables that achieved $p < 0.05$ from each sub-regression model were considered significant and were retained in the final regression models. Two final regression models with serum ferritin as dependent variable were carried out with or without adjustment of additional confounders. Statistical significance was set at a p value <0.01 for the final regression models. Least square means (LS means) of SF were also computed based on each regression model to compare the SF difference between NHWs and NHBs, after controlling for different factors.

Multicollinearity among independent variables for each regression model was tested by checking the variance inflation factor (VIF). Any variable with a VIF that exceeded 4 was excluded from the model. Therefore, phosphorus intake was excluded from the regression analysis (VIF=6.72). Because of highly correlated data among dietary intakes of carbohydrate, protein and fat, the percent calorie from these nutrients were placed in the regression model instead. In the sub-model containing hematological markers, hematocrit was excluded because of its high correlation with hemoglobin value. Only MCV was retained in the model because of its high correlation with MCH and MCHC.

Log-transformation was used to correct the highly skewed data distribution of protoporphyrin. Because SF was positively skewed, the regression analyses were repeated using log-transformed ferritin values as the dependent, and the results were compared between the models with different dependent variables.

Before running the multiple regression models, those variables with $\geq 10\%$ missing data were eliminated from the analysis. Therefore, plasma fibrinogen was removed from the sub-regression model containing infection markers because of its unacceptable percent of missing value (50%). Unless otherwise mentioned, the appropriate sample weights (WTPFH6), stratum variable (SDPSTRA6) and primary sampling unit (PSU) variable (SDPPSU6) were applied to all analyses to account for the complex design effect and nonresponse.

IV. Supplemental Analyses

1. Study Sample

In our study, we are also interested in investigating whether the likelihood and magnitude of serum ferritin elevation in response to two conditions (acute inflammation, and hepatitis C) are different between NHBs and NHWs. Therefore different study populations were used for each condition.

A serum CRP concentration $\geq 1.0\text{mg/dL}$ is widely accepted as indicative of an active acute phase response (200). Therefore, for acute inflammation, 235 NHW and 164 NHB males, aged ≥ 20 years with CRP $\geq 1.0\text{mg/dL}$ were included in the final analysis.

Study population for hepatitis C included non-Hispanic black and non-Hispanic white males, aged ≥ 20 years with presence of positive serum hepatitis C antibody. Because hepatitis C could proceed to liver cirrhosis, and advanced cirrhosis secondary to any

cause is associated with iron overload (201-203), we wanted to evaluate the risk of having increased serum ferritin in the absence of advanced cirrhosis for hepatitis C. In NHANES III, no questions were asked regarding the presence of cirrhosis, and liver biopsy was not included in the survey, which is the gold standard for diagnosing cirrhosis. Therefore, we attempted to identify participants at risk of having advanced cirrhosis based on the methods used in the previous studies (204,205): presence of either a serum albumin less than 3.5g/dl, a platelet count less than 100,000/ μ L, or a total serum bilirubin greater than 2.0mg/dL. Subjects who fulfilled at least 1 criterion as well as identified as hepatitis C were excluded from the analysis. Subjects with sickle cell diseases were also excluded from analysis. Therefore, the final study population for hepatitis C consists of 62 NHW and 92 NHB male subjects.

2. Variables

a. Acute inflammation

Presence of elevated serum ferritin was defined as having serum ferritin concentration $>300\mu\text{g/L}$ in men (37). Disease prevalence including cancer (various types), congestive heart failure, diabetes mellitus, heart attack, hypertension, and stroke were also compared between NHWs and NHBs. Status of cancer, congestive heart failure, diabetes mellitus, heart attack, and stroke was determined by the response to the question of whether a doctor has ever told the respondent that he/she had such conditions. Undiagnosed diabetes was identified by having a fasting plasma glucose concentration $>126\text{mg/dL}$ (197) or currently taking insulin. Hypertension was defined as having systolic blood pressure $\geq 140\text{mmHg}$ or/and diastolic blood pressure $\geq 90\text{mmHg}$ (198), or if a doctor has told the participant that he or she had hypertension.

Several inflammation markers including plasma fibrinogen, serum albumin, white blood cell count (WBC), lymphocyte number, monocyte number and platelet count were examined. Other laboratory variables including serum iron, transferrin saturation (TS), total iron binding capacity (TIBC), total triglycerides (TG), total cholesterol, HDL and LDL cholesterol were also compared between NHWs and NHBs. Additional confounders including BMI, education, and cigarette smoking were also included in the statistical model. The description of these variables has been discussed in the previous section for primary analysis.

b. Hepatitis C

The percent of hepatitis C (HCV) infected individuals who had elevated serum ferritin concentrations were compared between NHW and NHB. Disease prevalence including cancer (various types), congestive heart failure, diabetes mellitus, heart attack, hypertension, and stroke were compared between current NHW and NHB subjects (description of variables was mentioned in above section). Several serological markers including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), serum total bilirubin, serum lactate dehydrogenase (LDH) were examined and were compared between NHWs and NHBs. Other laboratory variables including Hb, serum iron, TS, TIBC, CRP, WBC, platelet count, serum albumin were also included in the analysis. Additional factors including BMI, education and cigarette smoking were also controlled for the potential confounding effects in the statistical model.

3. Statistical Analysis

a. Acute inflammation

The aim of our analyses was, first to investigate whether there is any differences in the likelihood of having elevated serum ferritin concentrations in response to acute inflammation between NHWs and NHBs. This was examined by using logistic regression models to compare the odds ratios of having elevated serum ferritin values between NHWs and NHBs controlling for the potential confounding effects.

The second goal of our analyses was to investigate whether the magnitude of having elevated serum ferritin concentrations in response to acute inflammation was also different between NHWs and NHBs. We examined this by using logistic regression analysis to compare the odds ratios per unit increment in several inflammation markers including CRP, WBC, serum albumin, lymphocyte count, monocyte count and platelet count adjusting for the potential confounders.

Logistic regression models were carried out with adjustment of several potential confounders including age, PIR, ethnicity, education, BMI, cigarette smoking, status of cancer/congestive heart failure/heart attack/diabetes mellitus/hypertension/stroke, and cardiovascular disease risk factors such as triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, and waist circumference because of their known relationship to CRP (206-209). Variables including LDL cholesterol and waist circumference were excluded from logistic regression analyses because of their unacceptable percent of missing value (LDL cholesterol: 59.65%, waist circumference: 15.54%). Multicollinearity among independents for each logistic model was tested by checking the variance inflation factor (VIF).

In our analysis, SUDAAN's t-test was used to compare the means of all continuous variables between NHWs and NHBs. SUDAAN's Chi-square test was used to compare the frequencies of all categorical variables between these two groups. Logistic regression analysis was applied for our likelihood and magnitude tests. The statistical significance level was set at a $p < 0.05$ for all analyses. Unless otherwise mentioned, sampling weights were applied to all analyses to account for the complex design effect and nonresponse.

b. Hepatitis C

Logistic regression models were applied to compare the odds ratio of having increased serum ferritin concentration ($>300\mu\text{g/L}$) between NHW and NHB HCV-infected male subjects (likelihood test). Logistic regression was also used to estimate the odds ratios of having elevated serum ferritin concentration per unit increment in several serological markers including AST, ALT, ALP, LDH and serum total bilirubin between NHWs and NHBs with adjustment of age, ethnicity, PIR, BMI, CRP and alcohol consumption (magnitude test). GGT was excluded from the analysis because of high percent of missing value (35.6%).

Descriptive statistics were computed for all variables, including means, standard error of the mean (SEM) for continuous data and frequencies for categorical variables. Logistic regression analysis was applied for our likelihood and magnitude tests. The statistical significance level was set at a $p < 0.05$ for logistic regression analysis.

RESULTS

I. Primary Analysis

Descriptive statistics

Serum ferritin (SF) difference

Crude differences in mean serum ferritin concentration were evident in all age groups and were greater in NHBs than NHWs at each age (**Fig. 1**). Overall, the mean SF concentration of NHBs was significantly higher than that of NHWs (SF difference=37.06 μ g/L, $p<0.0001$). Compared to NHBs, NHWs had significantly higher percent of subjects with normal (25-300 μ g/L) SF concentration (84.49% vs. 78.95%, $p=0.0005$) but lower percent of having elevated (>300 μ g/L) SF concentration (12.72% vs. 19.64%, $p<0.0001$) (**Table 3**).

Subject characteristics

The final data set included 3,554 males aged 20-65 years. Among them, there were 1,938 NHW males and 1,616 NHB males. The average age of NHWs was significantly older compared to NHBs. NHBs had a significantly lower PIR with greater percent of people having low income but lower percent of people having high income compared to NHWs. A significantly greater percent of NHWs remained married or living as married whereas a significantly higher percent of NHBs were unmarried. Overall, NHWs achieved a significantly higher level of education compared to NHBs. The prevalence of chronic diseases was similar between the two groups, as was the presence of arthritis, cancer (various types), congestive heart failure, diabetes mellitus, heart attack, emphysema and stroke. However, the percent of NHBs who had anemia, acute inflammation, hepatitis C and hypertension was significantly higher than NHWs. There

was no statistically significant difference between NHWs and NHBs for BMI, physical activity, and alcohol consumption. However, the number of NHWs who had moderate level of exercise was significantly higher compared to NHBs. In addition, there was a greater percent of NHBs who were current smoker, but the percent of former smoker was significantly higher among NHWs (**Table 1**).

Nutrient intakes and serum status

The data indicated that overall, the energy and nutrient intakes were higher among NHWs than NHBs, with the exceptions of protein and vitamin C. Intakes of carbohydrate, fiber, iron, zinc, copper, calcium and phosphorus were significantly higher in NHWs. Compared to NHWs, a significant higher percent of NHBs only had iron intake <10mg/d, and fewer of them had iron intakes \geq 10mg/d. The percent calorie from carbohydrate was significantly higher in NHWs whereas the percent calorie from protein was significantly lower in them. Among the serum nutrient indices, NHW subjects had a statistically higher serum retinol, serum vitamin C, serum lycopene, and serum selenium concentration, while NHB subjects had statistically higher values for serum total protein, serum β -cryptoxanthin, serum lutein/zeaxanthin, serum phosphorus and blood lead (**Table 2**).

Biochemical markers

For the hematological markers, NHWs were found to have significantly higher values for serum iron, Hb, HCT, MCV, MCH, MCHC, TIBC and TS compared to NHBs. RDW levels were significantly higher among NHBs. Compared to NHWs, there was a significantly higher percentage of NHB subjects who were anemic (Hb<13g/dL), while the percent of NHB with normal Hb value was significantly lower.

Several infection markers including CRP, lymphocyte count, plasma fibrinogen, were found significantly higher among NHBs than NHWs. The values for WBC and serum albumin were significantly lower in NHBs compared to NHWs. Among the serological markers, NHWs had statistically lower AST, ALP, GGT and LDH concentrations, while NHBs had statistically lower values for serum total bilirubin (**Table 3**).

Inferential statistics

Because there is no significant difference in the results obtained from regression models using serum ferritin concentrations as the dependent variable and regression models with log-transformed serum ferritin concentrations as the dependent variable, results of the regression analysis using serum ferritin concentration as the dependent variable were used as the final results for interpretation purposes. **Table 4** showed the results for different sub-regression models. For each sub-model, the NHB ethnicity was consistently found to be associated with higher SF concentrations compared to the NHW ethnicity after controlling for other variables. Different sub-regression models resulted in a SF difference between NHWs and NHBs ranging from 24.66 μ g/L to 47.6 μ g/L.

For the first sub-regression model containing nutrient and alcohol intakes, percent calorie intake from carbohydrate and fat as well as calcium intake were found to be inversely associated with SF concentration. For the sub-model containing serum nutrient indices, serum concentrations of total protein, retinol, α -carotene, β -cryptoxanthin, lycopene, and phosphorus were significantly associated with SF. In the sub-model for hematological markers, MCV, TIBC and log-transformed protoporphyrin were significantly associated with SF concentration after controlling for other iron status indicators. CRP was the only infection marker found to be positively associated with SF

concentration in the sub-model of infection markers. After adjustment for hepatitis C status, GGT was the only serological marker found to be significantly associated with SF concentration. Compared to the subjects who were widowed, divorced, separated or never married, being married or living as married was positively associated with SF concentration. Having an education level higher than high school was associated with a lower SF concentration compared to having an education level lower than high school.

In the two final regression models, ethnicity was no longer found to be significant after controlling for the factors within each model. The first final model running with all the significant variables obtained from each sub-regression model showed that being married, serum total protein, serum retinol, MCV and GGT were positively associated with SF concentration while %kcal from carbohydrate, calcium intake, serum α -carotene, and TIBC were inversely associated with SF concentration. After further controlling for age, BMI categories, cigarette smoking and physical activity in the second final regression model, the above-mentioned association remained significant except for being married and serum retinol, but the additional significant effect of age, %kcal from fat and BMI on SF concentration was found. Results of these were shown in **Table 5**.

Proof test

Since the results of regression analyses only identified the significant variables associated with the serum ferritin concentration, additional analysis was needed to further investigate how these variables may explain the difference in SF concentrations between NHBs and NHWs. Therefore, a proof test was carried out by using a revised regression model built on the second final regression model to further prove the contribution of serum total protein, TIBC and GGT to the observed SF difference. The model was run

after excluding the subjects with abnormal values of serum total protein, TIBC and GGT. The normal ranges for these measurements were defined as: serum total protein: 6-8.5g/dL (32); TIBC: 240-450 μ g/dL (33); GGT: <50U/L (34).

When such subjects were included in the analyses (final regression model 1 and 2), the final regression models resulted in a reduction of 25.61 μ g/L and 25.73 μ g/L from initial SF difference (37.06 μ g/L) between NHWs and NHBs, respectively. Elimination of the subjects with abnormal values of serum total protein, TIBC and GGT from the analysis resulted in a remarkable reduction of 33.11 μ g/L from initial SF difference, and the SF difference between NHWs and NHBs dropped to 3.95 μ g/L ($p=0.5886$) (**Table 6**). In the revised regression model, BMI, %kcal from carbohydrate and fat, calcium intake, and MCV were no longer found to be significantly contributing variables, however, age, serum total protein, serum α -carotene, TIBC and GGT continued to have a significant association with SF concentration (**Table 5**).

II. Supplemental Analysis

a. Acute inflammation

Descriptive statistics

The proportion of subjects who had elevated serum ferritin concentrations (>300 μ g/L) in regard to acute inflammation stratified by age and ethnicity were shown in **Fig. 2**. NHB generally showed a higher proportion of individuals with elevated serum ferritin concentrations with regard to acute inflammation than their white counterparts for each age group. The greatest ethnic differences in the percent of individuals with elevated SF concentrations were seen at age of 40 to 49 years.

The comparisons of subject characteristics between NHB and NHW were shown in **Table 7**. Among individuals with presence of acute inflammation, the percent NHBs who had elevation in SF concentrations was significantly higher than that of NHWs (35.23% vs. 21.78%, $p=0.0080$). Generally, NHBs were younger, poorer, less likely to be former smoker but more likely to smoke currently compared to NHWs. NHWs had a significantly higher percent of individuals who had higher than high school education compared to NHBs. NHBs had a significantly higher percent of individuals who had low income but a significantly lower percent of individuals with high income compared to NHWs.

Overall, there was no significant difference in disease prevalence including cancer, congestive heart failure, diabetes mellitus, heart attack, hypertension and stroke between these two groups. Values of CRP, serum albumin, platelet count, plasma fibrinogen and lymphocyte were comparable between NHWs and NHBs. However, NHWs were found to have significantly higher values of WBC and mononuclear count compared to NHBs. Compared to NHWs, NHBs had significant lower values of Hb ($p=0.0006$), but their serum ferritin concentrations were significantly higher ($p=0.0052$). Significant differences in HDL and LDL cholesterol were also found between these two groups ($p=0.0054$ and 0.0139 , respectively).

Likelihood Test

As shown in **Table 8**, NHB males with presence of acute inflammation were 2.153 times more likely (95% CI, 1.234 to 3.754) than their white counterparts to have elevations in serum ferritin ($>300\mu\text{g/L}$) after adjustment for age, ethnicity, PIR, education, BMI, smoking status, and disease status. When the relationship was further

adjusted for cardiovascular disease risks including triglycerides, total cholesterol, HDL cholesterol, systolic and diastolic blood pressure, NHBs were associated with a 2.239-fold greater risk (95% CI, 1.333 to 3.760) of having elevated serum ferritin in regard to acute inflammation than NHWs.

Magnitude Test

The odds ratios of having elevated serum ferritin concentrations associated with each increment in selected inflammation markers were calculated for NHBs with NHW as the control group (**Table 9**). Every increment of WBC, serum albumin, lymphocyte count and platelet count was associated with significantly higher odds ratios of having elevated SF concentrations among NHBs than NHWs in both reduced and full logistic models. Without further adjustment of cardiovascular disease risks, per unit change in CRP was not associated with significantly higher risk of having elevations in SF among NHBs than NHWs. When the relationship was further controlled for cardiovascular disease risks in the full logistic regression model, every unit change in CRP was associated with significantly higher odds ratios of having elevation in SF among NHBs than NHWs. However, no significant differences in the odds ratios of having elevated SF concentrations with unit change in monocyte count were found between NHBs and NHWs.

b. Hepatitis C

Descriptive statistics

Subject characteristics of HCV (hepatitis C virus) infected male participants aged ≥ 20 years old were shown in **Table 10**. Compared to NHWs, NHBs were older, poorer, leaner, with less percent of individuals who achieved higher than high school education,

and consumed more alcohol. Prevalence of congestive heart failure and hypertension was higher among NHBs, whereas prevalence of heart attack and stroke was higher among NHWs. Compared to NHWs, NHBs had a higher prevalence of having elevation in serum ferritin concentration (39.91% vs. 20.90%). The percent of HCV infected individuals who had abnormal liver enzyme values (AST or ALT >40U/L) was also higher among NHBs than NHWs (59.82% vs. 35.67%). Compared to their white counterparts, hemoglobin values were found lower among NHBs, however their SF concentrations are higher. Overall, NHBs had higher values of liver enzymes including AST, ALT, ALP, GGT, LDH and serum total bilirubin compared to NHWs.

Hepatitis C genotype distribution

Distribution of hepatitis C genotypes between NHB and NHW HCV infected males was shown in **Fig. 3**. The number of subjects carrying each HCV genotype was shown in **Table 11**. Genotype 1 was the most common genotype in both NHWs and NHBs (60.15% and 57.58%, respectively). The second most predominant genotype among NHWs and NHBs were genotype 4 (18.87%) and genotype 2 (30.60%), respectively. HCV genotype 3 was the least frequent genotype found in both NHWs and NHBs (1.18% and 3.65%, respectively).

Inferential statistics

The results of likelihood test didn't find significant differences in the odds ratios of having elevated serum ferritin concentrations between NHBs and NHWs in regard to HCV infection (**Table 12**). Unit change in selected serological markers including AST, ALT, LDH and serum total bilirubin were not associated with significantly different odds ratios of having elevation in SF between NHB and NHW HCV infected subjects. (**Table**

13). Without further adjustment for cardiovascular disease risks, per unit change in ALP was not found to be associated with significantly higher risk of having elevations in SF among NHBs than NHWs. When the relationship was further controlled for cardiovascular disease risks in the full logistic regression model, every unit change in ALP was associated with significantly higher odds ratios of having elevation in SF among NHBs than NHWs.

DISCUSSION

I. Primary Analysis

Although the higher serum ferritin concentrations have been continuously reported among blacks despite their lower hemoglobin values compared to whites, the etiology of this noted SF difference remains unknown. This study therefore served as the first attempt to explore this long-observed black-white SF difference. As a direct support to one of the overarching goals of Healthy People 2010 to eliminate health disparities among populations, our results sought to answer the question of why there is a difference in iron stores between blacks and whites, and what are the factors contributing to this difference that underlie the “iron hypothesis” (213, 214). In accordance with the results from other studies (6-9), our data indicated a continuing, significantly higher serum ferritin concentration among NHB males aged 20-65 years compared to their NHW counterparts. Overall, NHB subjects were shown to have a significantly higher SF concentration than NHW subjects. Controlling for one category of factors at a time by using different sub-regression models resulted in a SF difference ranging from 24.66 to 47.6 μ g/L between these two groups. However, after adjustment of all the significant variables obtained from each sub-regression model, the two final regression models resulted in a significant reduction in SF difference (25.61 μ g/L & 25.73 μ g/L, respectively), therefore suggesting that the putative mechanism that may underlie the marked black-white SF difference is complex.

The current results indicated that iron nutrition did not account for the SF difference between NHBs and NHWs. Instead, variables including %kcal from carbohydrate, %kcal from fat and calcium intake were found to be significantly associated with SF

concentration in the final regression models. The inverse relationship between calcium intake and ferritin concentration may be related to the fact that simultaneous ingestion of calcium inhibits iron absorption by competing for the same portion of a common absorptive pathway (186-188), and thus decreasing the amount of absorbed iron to be incorporated into the apoferritin. The observed inverse relationship between percent energy from macronutrients and SF may reflect the impact of the overall nutrition and diet on ferritin metabolism, however, more specific investigations are needed to further elucidate the mechanism underlying.

The significant associations observed between serum total protein, serum α -carotene and SF suggest a relationship between health status and SF concentration. As a two-faced protein, ferritin also serves as an acute phase reactant which concentrations in the plasma can be increased in both acute and chronic inflammation, and the elevated SF is more likely a reflection of disease activity other than iron stores. Since serum total protein is a commonly tested measurement used to evaluate overall health status, liver and kidney function as well as nutritional status, our current finding could be the reflection of the strong interaction between overall health status and ferritin metabolism. The significant inverse association observed between serum α -carotene and SF may be linked to the role of α -carotene played in the antioxidant defense system against oxidative stress induced by inflammatory stimuli (215). However, future studies are needed to clarify the biological mechanisms.

In our study, the association between iron status and ferritin metabolism was indicated by the significant associations between MCV, TIBC and serum ferritin. The effect of TIBC on SF in particular, suggested the strong relationship between body iron status and

ferritin homeostasis. TIBC measures the amount of added iron that can be specifically bound by plasma transferrin. Greater than normal level of TIBC is usually symbolic of iron deficiency, and reduced level is associated with acute inflammation, chronic infections, renal disease and malignancy (39). Therefore, the inverse relationship between TIBC and ferritin may reflect the alterations of ferritin metabolism in iron deficiency and diseases.

The observed significant association between GGT and SF may reflect the crucial linkage between hepatic well-being and ferritin economy. GGT is an enzyme which is found in hepatocytes and biliary epithelial cells. Its serum measurement provides a very sensitive indicator of the presence or absence of hepatobiliary disease, and is often used as an indicator for a variety of chronic diseases and alcoholism (212). The strong relationship observed between GGT and SF highlights the critical role the liver plays in the ferritin homeostasis. In addition, age was found positively associated with SF concentrations, and it is likely due to the higher probability of having chronic inflammatory diseases associated with older age. The current results also indicated that marital status and BMI could influence the SF concentration, however, the effect is more likely due to the nutritional and health status influenced by socioeconomic factors.

An essential part of our analysis was to uncover some of the factors that may account for the long-observed SF difference between NHWs and NHBs. This was achieved by carrying out a revised regression model (proof test) based on the hypothesis that further adjustment of the selected contributors would result in similar SF concentrations between NHWs and NHBs, or further reduce the difference in SF concentration. Serum total protein, TIBC and GGT were chosen as the contributors to be further controlled in the

proof test, given their roles of reflecting the strong associations between overall health and nutrition, iron status, hepatic well-being and serum ferritin. As expected, removal of the subjects with abnormal values of these indicators resulted in comparable SF concentrations between NHBs and NHWs, and the SF difference was no longer significant (SF difference=3.95 μ g/L, p=0.5586). Therefore, the results suggest that the differences in overall nutrition and health, iron status and hepatic well-being may be responsible for the observed difference in SF between NHBs and NHWs.

Serving as the major synthetic site of ferritin protein, liver plays a pivotal role in ferritin homeostasis. Hepatic ferritin synthesis is strongly influenced by pathophysiological conditions including inflammation and malignancy, and the inflammatory cytokines such as interleukin 1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF α) can alter the hepatic ferritin gene transcription (217). Hepatic inflammation or injury is usually accompanied by elevations in serum ferritin, and is thought to be a result of an up-regulated ferritin production as an acute phase reactant in response to inflammatory stimuli (90). Our data indicated that the overall liver function was better among NHWs compared to NHBs since several serological markers including AST, ALP, GGT and LDH were found significantly higher among NHBs. Plus the fact that hepatitis C was more prevalent among the current NHB study population and the previous findings of the higher likelihood of carrying genetic variants encoding inflammatory cytokines including IL-1, IL-6 and TNF α among blacks (25-27), the observed higher ferritin concentration in blacks may be a result of the higher frequencies of liver disease and the more aggressive cytokine-induced ferritin synthesis responsive to inflammation. But more studies are required to further assess the association.

Although the current results didn't find a significant contribution of infection to the SF difference, the role of inflammation in the etiology of black-white SF difference merits further investigation. Note that several infection markers were found significantly different between NHWs and NHBs in our analysis, thus suggesting higher frequencies of infection among blacks than whites. Interestingly, several characteristics corresponding to the definition of the anemia of chronic disease (ACD) were also found in our current NHB subjects, thereby suggesting the possible contribution of ACD to the observed SF difference. ACD is generally a normochromic or slightly microcytic anemia resulting from chronic infection, chronic immune activation, or malignancy, and is clinically characterized by reduced serum iron concentrations, TS and TIBC, with normal or elevated SF concentrations (218). ACD is distinguishable from iron deficiency anemia (IDA) because SF is reduced whereas TIBC should be high in genuine IDA. In our study, NHBs were found to have lower Hb, serum iron, MCV, TS and TIBC but higher SF concentrations compared to NHW subjects, thus suggesting a higher frequency of this condition among blacks. Unfortunately, neither bone marrow examination nor transferrin receptor, which can provide more definitive diagnosis of ACD was available in NHANES, therefore, we couldn't quantify the contribution of ACD to the observed SF difference. Moreover, our efforts of further assessing the existence and contribution of chronic inflammation to the observed SF difference between NHBs and NHWs were hampered by the limited availability of infection markers. In contrast to CRP, which is the short-term indicator of infection, erythrocyte sedimentation rate (ESR), fibrinogen and α_1 -acid glycoprotein (α_1 -AGP) are thought to best reflect the resolution, or the long-term status of the inflammation (219). However, none of them were included in our study

due to either unavailability in NHANES III (ESR & α_1 -AGP) or high percent of missing data (plasma fibrinogen). Hepcidin, a recently discovered hepatic antimicrobial protein (220-222), provide new hope to the research of ACD, however has not yet become a routinely performed test. Given the interaction of hepcidin with the iron exporter ferroportin, and its role in the regulation of iron transport and recycling, future investigation using hepcidin may shed light on the role of ACD in the etiology of the black-white SF difference in the future.

In addition to the limited availability of biomarkers, other limitations of our study should also be acknowledged. First, owing to the cross-sectional design, causal inferences of the identified factors and SF concentration cannot be drawn. Secondly, due to the potential confounding effects associated with female subjects, only male subjects were included in our study, therefore, the results cannot be inferred to the female population. Thirdly, because the data for cytokine measurements were not available in NHANES III, we were unable to determine the role of cytokine-induced ferritin synthesis in the black-white SF difference. Furthermore, because genetic information was not available in NHANES III, our current study cannot rule out the possibility that genetic polymorphism may play a role in the etiology of SF difference. Though hypothesis generating in nature, future studies should address these questions.

In summary, this study highlighted the necessity and importance of future studies that can bring more insights to this long-observed black-white serum ferritin differences. Our results suggest that the noted black-white SF difference is a result of factors including overall nutrition and health, iron status and hepatic well-being. Higher SF, low Hb and reduced TIBC level observed in blacks are consistent with the definition of ACD. More

specific investigations using cytokines, hepcidin or other infection markers are needed to further determine the role of ACD in the etiology of the SF difference between blacks and whites.

II. Supplemental Analysis

a. Acute inflammation

In the present study, we observed a significantly higher percent of NHBs with present acute inflammation had elevated SF concentrations than their NHW counterparts. And the percent of NHBs who had raised SF was consistently higher than that of NHWs at each age group. Compared to NHWs, NHBs were more likely to have elevated SF concentrations in regard to acute inflammation after adjustment for potential confounders. Therefore, the results raised a possibility that there might be different modes of responsive ferritin synthesis during inflammatory process between blacks and whites.

Cytokines directly regulate ferritin secretion both transcriptionally and posttranscriptionally. A number of cytokines including interleukin 1 alpha (IL-1 α), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF α) have been shown to up-regulate ferritin production (223-226). Proinflammatory cytokines TNF α and IL-1 α transcriptionally induce the H chain of ferritin. As a result, the selective induction of ferritin H mRNA will lead to the accumulation of a population of H-rich ferritin protein and substantially increase the content of ferritin (223-225). In primary cultured human hepatocytes, IL-1 α and IL-6 can induce a transient secretion of ferritin at 24 hours followed by a decline to baseline (226). Results from these studies suggest another possibility that the differential patterns of raised ferritin observed between blacks and

whites could be a result of different cytokine-induced ferritin synthesis during inflammation. However, prospective studies are required to test the hypothesis.

In our study, NHBs demonstrated a greater capability and propensity to have increased store iron than NHWs in regard to acute inflammation. The observed disparate pattern of ferritin increase may be a result of the different cytokine genotype distribution between blacks and whites. Several studies have shown that significantly different allelic and genotypic cytokine distribution exists between blacks and whites (25-28). Most studies reported that blacks have a greater predisposition to inflammation because of the more up-regulated inflammation cytokine genotypes found in them, IL-1 α , IL-6 and TNF α in particular. Therefore, the higher risks of having raised SF observed among inflammation-infected blacks may be the functional consequences of their predisposition to the higher level of proinflammatory cytokines which are known to stimulate ferritin secretion.

The current results also showed that every increment of selected inflammation markers was associated with higher risks of having elevated SF in NHBs than in NHWs. The stronger inflammation-ferritin relationship observed in blacks raise the possibility that the presence of amplified increase in stored iron may predispose blacks to worse clinical outcomes and poorer treatment response. Since ferritin plays a prominent role in the cytokine response and inflammatory processes, the more aggressive iron accumulation pattern reflected by raised serum ferritin in inflammation may reflect the greater intensity of inflammation and the stronger host immunoresponsiveness underlie a number of physiological and pathological processes. But further investigation is needed to confirm the association.

To date, little is known about the underlying mechanisms of the disease disparities highlighted by the strikingly higher morbidity and mortality rates among blacks. Compared with whites, blacks have approximately 1.5 times higher death rates from all causes, worse survival after myocardial infarction (10), a 2-fold-higher incidence of and morbidity from stroke (11,12), and significantly higher morbidity and mortality rates from diabetes (14), AIDS (15), and cancer (16). Given the fact that ferritin plays a pivotal role in the cellular inflammatory response and pathogenesis of disease, the question of whether there is a difference in the responsive ferritin synthesis during inflammation between blacks and whites, and whether the differences in stored iron play a role in disease disparities merits further research.

Observations over the last few decades have also identified ethnic differences in response to therapies in many diseases, including hypertension (227-229), diabetes (230), renal transplantation (231-232), heart failure (233), and depression (2342). In addition, there are striking ethnic differences in response to interferon therapy for hepatitis C (HCV). Long-term sustained responses to alpha interferon are substantially lower among black patients than white patients with hepatitis C (24,235-238). Interestingly, the fact that iron depletion by phlebotomy in patients with HCV reduces serum aminotransferase levels (239-244), and in combination with interferon, may have improved antiviral efficacy compared with interferon alone (240,242,244) suggests that increased store iron may be the cause of the reduced response to interferon. Although the presence of increased store iron is a recognized predictor of poor response to interferon monotherapy (245-252), the association between increased store iron and treatment response for other diseases has not yet been investigated adequately. Therefore, larger, prospective studies

are required to evaluate whether the different patterns of increase in stored iron are associated with different therapy response among various ethnicities.

To our knowledge, this is the first study to assess the association between acute inflammation and iron stores between blacks and whites. Despite the fact that ethnic differences in disease burden, progressions, complications, outcomes and treatment response have been observed between blacks and whites, the explanation for this remains unclear. Of great interest is to find out whether there is ethnic difference in the inflammatory process and host immuno-responsiveness that underlie a variety of clinical pathophysiological conditions. Given the pivotal role ferritin plays in the cellular inflammatory response, our study was conducted to test the hypothesis that there is ethnic difference in the relationship between acute inflammation and iron stores reflected by serum ferritin concentration between blacks and whites. Within this work, we presented evidence that blacks had a more aggressive rise in serum ferritin with regard to acute inflammation compared to whites. And the stronger inflammation-ferritin relationship observed in blacks may reflect their more robust immune response and therefore may contribute to the poorer prognosis. But more prospective data are required to further investigate the hypothesis.

Despite the significant findings of our study, our analysis is not powered to address the underlying mechanisms of the observed ethnic differences in iron stores during acute inflammation. Therefore, more specific investigation is needed to further explore the responsible biological rationale. However, our study does raise the possibility that the different patterns of iron accumulation may play a role in disease processes and result in different clinical outcomes and drug response. Future studies will address this topic.

In conclusion, the current results indicated that different patterns of iron accumulation in regard to acute inflammation exist between NHWs and NHBs. Further elucidation is needed about how ferritin regulation is perturbed in diseases, and whether increased stored iron plays a role in different clinical outcomes and drug response. To which extent that different stored iron contribute to the ethnic disparate response to disease and treatments requires further investigation. A more complete knowledge on the etiology of the differences in host inflammatory response could provide clinicians with the ability to optimize the therapeutic regimens tailored to the individual.

b. Hepatitis C

Our current data showed that hepatitis C is approximately twofold more prevalent among NHBs (6.02%) than among NHWs (2.94%). The results of HCV genotype distribution indicated that genotype 1 was the most common HCV genotype among both groups. This result is in accordance with the previous report based on NHANES III data, in which it also documented a higher prevalence of genotype 1 among NHBs with predominance of genotype 1b (253).

Previous studies have shown that raised serum ferritin was an independent predictor of non-response to antiviral treatment in patients with chronic hepatitis C infection, and HCV-infected blacks are less likely to respond to anti-HCV therapy (234-238). Of great interest is to assess whether the differences in the stored iron is responsible for the differences in antiviral treatment response among various ethnicities, especially between blacks and whites. Therefore, our analysis was carried out to investigate the relationship between HCV infection and patterns of raised serum ferritin between NHWs and NHBs.

In general, the results of our analysis showed no difference in the likelihood and magnitude of having elevated SF concentrations in regard to HCV infection between NHW and NHB males. By contrast, a recent study also using data from NHANES III suggested that NHB individuals may respond to HCV infection with an increase in iron stores reflected by both elevated serum ferritin and transferrin saturation, which may be partly responsible for the reduced efficacy of antiviral therapy (23). In this study, Ioannou et al. found that HCV-infected blacks were 5.4 times (95% CI, 1.2 to 24) more likely to have increased iron stores than nonblacks. The increased risk of elevated iron stores among HCV-positive black persons persisted despite adjusting for age, alcohol intake, gender, socioeconomic status, and BMI (odds ratio 17.8; 95% confidence interval, 5.1 to 63).

The conflicting results may be due, in part, to two reasons. First, our efforts to assess the association between hepatitis C and increased iron stores between NHWs and NHBs may have been hampered by the paucity of HCV infected subjects included in our analysis. Despite the significant findings that Ioannou et al. found from their study, the extremely high upper CI values are suggestive of inadequate statistical power and small sample size. Therefore, the stability and reliability of their statistical analyses are questionable. Secondly, both studies were limited by the fact that hepatic iron index, which is a better indicator of body iron stores and predictor of IFN response, was not available in NHANES III. Therefore, future study by using hepatic iron concentration is required to further determine the relationship between iron stores and the course of HCV, as well as the response to therapy.

Although the HCV genotype information was available in NHANES III, the data was not sufficient enough for us to further investigate the relationship between the ethnic differences in genotype predominance and the increase in iron stores. However, emerging data have shown that genotype is the most critical predictor of anti-HCV treatment response. Studies have clearly indicated that patients with genotypes 1a or 1b have poorer responses to treatment with either IFN monotherapy, or standard or pegylated IFN plus ribavirin combination therapy than patients with genotypes 2 or 3 (254-256). Therefore, the linkage between HCV genotypes and the responsive change in iron stores among different ethnicities merits further investigation.

Despite the fact that we didn't find significant results in our current analysis, the role of iron accumulation in hepatitis C is worthy of further investigation. Adequately powered and sized prospective trials are needed to further investigate the hypothesis that differences in the stored iron may be responsible for the differences in anti-HCV therapy response among various ethnicities.

APPENDICIES

TABLE 1
Subject characteristics in NHANES III non-Hispanic white and non-Hispanic black males, aged 20-65 yrs.¹

Characteristics	non-Hispanic white (n=1938)			non-Hispanic black (n=1616)			p
	n	mean	SEM	n	mean	SEM	
Age (yr)	1938	39.73	0.38	1616	37.49	0.37	<0.0001*
Education (yr)	1938	13.32	0.10	1616	12.61	0.26	0.0062*
≤12 yr (%)	387	16.87	1.22	538	29.30	1.64	<0.0001*
>12 yr (%)	1551	83.13	1.22	1078	70.70	1.64	<0.0001*
Poverty income ratio (PIR)	1852	3.55	0.09	1486	2.29	0.07	<0.0001*
<1.3 (%)	296	14.18	1.08	647	37.47	1.95	<0.0001*
1.3-3.5 (%)	846	41.67	1.54	696	43.01	1.71	0.5491
≥3.5 (%)	796	44.15	1.80	273	19.42	1.36	<0.0001*
Marital status							
married (%)	1460	73.21	1.51	863	51.59	1.46	<0.0001*
unmarried (%)	477	26.79	1.51	749	48.41	1.51	<0.0001*
Prevalence (%)							
anemia	71	3.04	0.79	154	8.53	0.82	<0.0001*
elevated SF concentration	274	12.72	0.87	324	19.64	1.25	<0.0001*
acute inflammation	82	3.50	0.51	117	6.37	0.60	0.0007*
congestive heart failure	40	1.23	0.22	36	1.94	0.36	0.1026
diabetes mellitus	125	5.12	0.56	125	6.82	0.60	0.0343
heart attack	86	2.90	0.40	41	2.31	0.40	0.3289
hepatitis C	46	2.94	0.55	86	6.02	0.93	0.0041*
hypertension	358	15.16	1.16	375	20.69	1.03	0.0016*
emphysema	0	0		0	0		
stroke	22	0.96	0.23	22	0.98	0.25	0.9573
BMI (kg/m ²)	1938	26.71	0.15	1613	26.62	0.14	0.6814
<18.5 (%)	21	0.99	0.24	25	1.42	0.29	0.2665
18.5-25 (%)	738	39.09	1.33	662	41.14	1.32	0.2811
25-30 (%)	776	40.11	1.18	575	36.45	1.08	0.0535
≥30 (%)	403	19.80	0.86	351	20.98	1.09	0.4362
Physical activity	1938	231.89	54.61	1616	253.73	49.50	0.7639
sedentary (%)	357	16.67	1.12	345	19.88	1.42	0.0278
moderate (%)	830	41.88	1.32	579	35.65	1.13	0.0006*
active (%)	745	41.45	1.56	688	44.47	1.50	0.1514
Alcohol (drinks/mo)	1938	18.40	3.14	1616	18.42	2.00	0.9954
non-drinker (%)	666	30.80	1.97	563	33.19	1.33	0.3636
light drinker (%)	970	53.44	1.61	773	48.98	1.67	0.0495
moderate drinker (%)	222	11.59	1.14	187	12.30	1.15	0.6801
heavy drinker (%)	80	4.17	0.52	93	5.53	0.54	0.0845
Smoking status							
non smoker (%)	640	35.79	1.23	623	40.26	1.67	0.0465
former smoker (%)	650	29.77	1.29	302	18.12	1.21	<0.0001*
current smoker (%)	648	34.44	1.43	690	41.62	1.28	0.0007*

¹ The means of all continuous variables were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's t-test. The frequencies of all categorical variables were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's Chi-square test.

* Statistical significance is set at a p<0.01.

None of the subjects had arthritis or cancer, therefore data were not showed in the table.

TABLE 2

Dietary intake and serum nutrient status among NHANES III non-Hispanic white and non-Hispanic black males, aged 20-65 yrs.¹

	non-Hispanic white (n=1938)			non-Hispanic black (n=1616)			p
	n	mean	SEM	n	mean	SEM	
Dietary intakes							
total energy intake (kcal)	1873	2808.51	31.72	1557	2702.14	44.44	0.0127
carbohydrate (g)	1873	331.14	3.96	1557	305.90	5.18	<0.0001*
protein (g)	1873	103.53	1.62	1557	104.15	1.89	0.7722
total fat (g)	1873	110.02	2.14	1557	106.67	2.26	0.1741
%kcal from carbohydrate (%)	1873	47.89	0.52	1557	46.16	0.33	0.0042*
%kcal from protein (%)	1873	15.07	0.12	1557	15.69	0.16	0.0047*
%kcal from fat (%)	1873	34.70	0.36	1557	34.37	0.22	0.3331
fiber (g)	1873	19.79	0.25	1557	16.75	0.32	<0.0001*
dietary iron (mg)	1873	19.17	0.29	1557	17.11	0.40	<0.0001*
<10 (%)	373	18.43	1.16	474	27.42	1.10	<0.0001*
≥10 (%)	1565	81.57	1.16	1142	72.76	1.10	<0.0001*
vitamin A (RE)	1873	1154.94	40.18	1557	1067.51	72.69	0.3298
carotenes (RE)	1873	531.42	27.50	1557	447.64	23.70	0.0288
vitamin C (mg)	1873	113.69	3.77	1557	123.66	4.61	0.1116
zinc (mg)	1873	15.14	0.18	1557	14.05	0.33	0.0034*
copper (mg)	1873	1.70	0.02	1557	1.48	0.44	<0.0001*
calcium (mg)	1873	1056.62	20.83	1557	779.14	17.27	<0.0001*
phosphorus (mg)	1873	1650.98	20.76	1557	1435.74	26.73	<0.0001*
Serum nutrient status							
total protein (g/dL)	1907	7.32	0.02	1575	7.59	0.02	<0.0001*
serum retinol (µg/dL)	1919	63.06	0.51	1599	58.65	0.45	<0.0001*
vitamin C (mg/dL)	1875	0.67	0.02	1542	0.55	0.01	<0.0001*
α-carotene (µg/dL)	1919	3.99	0.01	1599	3.47	0.21	0.0222
β-carotene (µg/dL)	1919	16.25	0.48	1599	15.79	0.56	0.5345
β-cryptoxanthin (µg/dL)	1919	8.10	0.14	1599	8.93	0.23	0.0022*
lutein/zeaxanthin (µg/dL)	1919	20.18	0.34	1599	24.11	0.57	<0.0001*
lycopene (µg/dL)	1919	26.69	0.46	1599	24.84	0.38	0.0016*
total calcium (mg/dL)	1907	9.34	0.02	1575	9.37	0.02	0.2131
phosphorus (mg/dL)	1907	3.36	0.02	1575	3.46	0.17	<0.0001*
selenium (ng/mL)	1898	128.15	1.25	1570	121.11	0.79	<0.0001*
blood lead (µg/dL)	1937	4.11	0.18	1616	5.13	0.18	<0.0001*

¹ The means of all continuous variables were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's t-test. The frequencies were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's Chi-square test.

* Statistical significance is set at a p<0.01.

TABLE 3Biochemical markers among NHANES III non-Hispanic white and non-Hispanic black males, aged 20-65 yrs.¹

Characteristics	non-Hispanic white (n=1938)			non-Hispanic black (n=1616)			p
	n	mean	SEM	n	mean	SEM	
Hematological markers							
serum iron (µg/dL)	1936	100.40	0.93	1614	93.08	1.00	<0.0001*
serum ferritin (µg/L) ²	1938	173.30	3.90	1616	210.36	6.28	<0.0001*
<25 (%)	57	2.80	0.39	27	1.41	0.34	0.0157
25-300 (%)	1607	84.49	0.95	1265	78.95	1.23	0.0005*
>300 (%)	274	12.72	0.87	324	19.64	1.25	<0.0001*
hemoglobin (g/dL)	1905	15.26	0.04	1600	14.56	0.04	<0.0001*
<13 (%)	71	3.04	0.79	154	8.53	0.82	<0.0001*
13-18 (%)	1859	96.60	0.82	1459	91.31	0.85	<0.0001*
hematocrit (%)	1905	44.82	0.11	1600	43.63	0.10	<0.0001*
RBC (10 ⁶ cells/mm ³)	1905	4.98	0.02	1600	4.97	0.02	0.7827
RDW (%)	1905	12.75	0.02	1600	13.34	0.04	<0.0001*
MCV (SI, fL)	1905	90.21	0.18	1600	88.12	0.21	<0.0001*
MCH (SI, pg)	1905	30.72	0.06	1600	29.43	0.08	<0.0001*
MCHC (SI)	1905	34.05	0.05	1600	33.38	0.05	<0.0001*
TIBC (µg/dL)	1935	349.21	2.48	1612	338.58	2.32	0.0004*
TS (%)	1933	29.25	0.32	1611	27.90	0.31	0.0069*
protoporphyrin (µg/dL)	1928	41.78	0.40	1606	41.65	0.47	0.8007
Infection markers							
CRP (mg/dL)	1916	0.33	0.01	1592	0.40	0.02	0.0011*
WBC (10 ³ cells/mm ³)	1905	7.29	0.06	1600	6.48	0.06	<0.0001*
lymphocyte (10 ³ cells/mm ³)	1904	2.21	0.02	1600	2.35	0.02	<0.0001*
mononuclear (10 ³ cells/mm ³)	1885	0.44	0.01	1562	0.42	0.01	0.0233
platelet count (10 ³ cells/mm ³)	1904	258.23	2.76	1600	263.31	2.43	0.0428
plasma fibrinogen (mg/dL)	1091	286.41	3.72	686	302.10	4.19	0.0040*
serum albumin (g/dL)	1907	4.35	0.02	1575	4.19	0.02	<0.0001*
Serological markers							
AST SI(U/L)	1907	22.89	0.32	1575	26.96	0.54	<0.0001*
ALT SI (U/L)	1907	21.49	0.73	1575	21.80	0.55	0.7051
ALP SI(U/L)	1907	82.15	0.88	1575	87.27	1.33	<0.0037*
GGT SI(U/L)	1513	33.37	1.29	1294	53.94	3.50	0.0001*
serum total bilirubin (mg/dL)	1907	0.74	0.01	1575	0.65	0.01	<0.0001*
LDH SI(U/L)	1907	155.20	2.38	1574	166.47	1.87	0.0001*

¹ The means of all continuous variables were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's t-test. The frequencies were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's Chi-square test.

² Arithmetic means of serum ferritin were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's t-test.

* Statistical significance is set at a p<0.01.

TABLE 4

Multiple regression sub-models with serum ferritin concentration as dependent variable.

# model IVs [‡]	R ² (n)	NHW LS means of SF (µg/L) ¹	NHB LS means of SF (µg/L) ²	Difference in SF (µg/L)	Significant IVs [‡]	β	P ⁷
Model #1							
ethnicity, %kcal from protein, %kcal from CHO, %kcal from fat, fiber, vitamin A, vitamin C, carotene, calcium, iron, zinc, copper, alcohol	0.03028 (n=3430)	175.53	200.86	25.33	ethnicity	25.3268 ³	0.0019
					%kcal from CHO	-1.4122	0.0034
					%kcal from fat	-1.2456	0.0163
					calcium intake	-0.0156	<0.0001
Model #2							
ethnicity, serum total protein, serum retinol, serum vitamin C, α- carotene, β-carotene, β- cryptoxanthin, lutein/zeaxanthin, lycopene, serum calcium, selenium, lead	0.0555 (n=3359)	174.31	201.09	26.78	ethnicity	26.7809 ³	0.0038
					serum total protein	27.4232	0.0316
					serum retinol	1.3795	<0.0001
					serum α-carotene	-3.2826	0.0037
					serum β-cryptoxanthin	-1.6389	0.0139
					serum lycopene	-0.7363	0.0186
					serum phosphorus	-15.6600	0.0019
Model #3							
ethnicity, serum iron, hemoglobin, RBC, RDW, MCV, TIBC, TS, log-protoporphyrin	0.0614 (n=3475)	172.29	219.89	47.6	ethnicity	47.6217 ³	<0.0001
					MCV	5.2860	<0.0001
					TIBC	-0.4826	<0.0001
					log-protoporphyrin ⁴	35.1389	0.0379
Model #4							
ethnicity, CRP, WBC, lymphocyte count, mononuclear count, platelet count, serum albumin	0.0143 (n=3373)	173.69	206.01	32.32	ethnicity	32.3209 ³	0.0001
					C-reactive protein	13.8557	0.034
Model #5							
ethnicity, AST, ALT, ALP, GGT, LDH, hepatitis C status	0.1155 (n=2786)	175.23	199.89	24.66	ethnicity	24.6633 ³	0.0018
					γ glutamyl transferase	0.6800	<0.0001
Model #6							
ethnicity, education, marital status, poverty income ratio	0.0191 (n=3549)	172.56	216.02	43.46	ethnicity	43.7508 ³	<0.0001
					marital status	25.1305 ⁵	<0.0001
					education	-22.8386 ⁶	0.0084

1. Least square means of serum ferritin (SF) were calculated for non-Hispanic white (NHW) subjects by using SUDAAN.

2. Least square means of serum ferritin (SF) were calculated for non-Hispanic black (NHB) subjects by using SUDAAN.

3. β coefficient corresponding to the change in serum ferritin concentrations (µg/L) between non-Hispanic black and non-Hispanic white (reference group).

4. Log transformation was used to correct highly right-skewed data distribution of serum protoporphyrin.

5. β coefficient corresponding to the change in serum ferritin concentrations (µg/L) between married and non-married participants (reference group).

6. β coefficient corresponding to the change in serum ferritin concentrations (µg/L) between participants with education level >12yr and ≤12 yr (reference group).

7. Statistical significance is set at a p<0.05. Results were obtained by using SUDAAN regression analysis.

‡ IV: independent variable

TABLE 5Final regression models and revised model with serum ferritin concentration as dependent variable¹.

Independent variables (IV)	Model #1 (n=2648)		Model #2 (n=2639)		Revised Model ² (n=2090)	
	β	p	β	p	β	p
Age	---	---	1.3882	<0.0001*	1.4823	<0.0001*
Ethnicity						
non-Hispanic white†	---	---	---	---	---	---
non-Hispanic black	11.4513	0.2131	11.3958	0.1893	4.1362	0.5719
Marital status						
married	21.6943	0.0021*	7.9840	0.2484	0.5578	0.9372
unmarried†	---	---	---	---	---	---
Education						
>12 yr	-2.6134	0.7896	-2.0193	0.8586	-13.4661	0.3044
≤12 yr	---	---	---	---	---	---
BMI (kg/m ²)				0.0012*		0.0239
<18.5†	---	---	---	---	---	---
18.5-25	---	---	-12.3747	0.6697	-0.4125	0.9791
25-30	---	---	8.7398	0.7632	11.6808	0.4770
≥30	---	---	38.3414	0.2054	36.0558	0.0431
Cigarette smoking						
non-smoker	---	---	16.7856	0.0708	14.8545	0.1093
former smoker	---	---	8.0475	0.2743	7.2396	0.3380
current smoker†	---	---	---	---	---	---
Physical activity				0.2045		0.2858
sedentary†	---	---	---	---	---	---
moderate	---	---	-8.1183	0.4358	-7.5184	0.4591
active	---	---	-15.5823	0.0923	-13.4781	0.1229
%kcal from carbohydrate	-1.1833	0.0039*	-1.3162	0.0013*	-0.7365	0.0391
%kcal from fat	-0.9011	0.0809	-1.2506	0.0091*	-0.6607	0.1515
calcium intake	-0.0166	<0.0001*	-0.0133	0.0013*	-0.0114	0.0153
serum total protein	45.5401	<0.0001*	50.3147	<0.0001*	36.6326	0.0007*
serum retinol	1.3217	0.0005*	1.0112	0.0141	1.1708	0.0121
serum α -carotene	-2.0959	0.0017*	-2.5840	0.0013*	-2.4820	0.0013*
serum β -cryptoxanthin	-1.1107	0.1333	-0.9883	0.2099	-0.5355	0.4740
serum lycopene	-0.3281	0.3932	-0.1369	0.7221	0.2394	0.5702
serum phosphorus	-8.4969	0.1629	-2.1725	0.7177	2.5227	0.7016
MCV	3.8530	0.0003*	4.2271	0.0001*	2.2118	0.0143
TIBC	-0.6895	<0.0001*	-0.6765	<0.0001*	-0.6047	<0.0001*
log-protoporphyrin	11.3513	0.4463	-14.3476	0.3964	-14.3616	0.4321
GGT	0.6621	0.0004*	0.6014	0.0007*	1.7255	<0.0001*
CRP	6.3389	0.2953	1.6109	0.8309	1.5287	0.8417

¹ Results of final regression models obtained using SUDAAN regression analysis. Final model #1 contained all significant variables from each sub-regression model. Final model #2 contained all variables in final model #1 with further adjustment for age, BMI, cigarette smoking and physical activity.

² Revised model was run based on final regression model #2 after excluding subjects with abnormal values of TIBC, serum total protein and GGT (normal TIBC: 240-450 μ g/dL, normal serum total protein: 6-8.5g/dL, normal GGT: <50 (U/L).

† Reference group.

* Statistical significance is set at a p<0.01.

TABLE 6

Comparisons of least square means (LS means) of serum ferritin concentration among final regression and revised models.

# Model	n	R ²	NHW LS means of SF (µg/L) ¹	NHB LS means of SF (µg/L) ²	Difference in SF (µg/L)	p
Model 1	2648	0.1954	178.42	189.87	11.45	0.2131
Model 2	2639	0.2271	178.77	190.10	11.33	0.1888
Revised Model³	2090	0.1751	164.14	168.09	3.95	0.5886

^{1.} Least square means of serum ferritin (SF) were calculated for non-Hispanic white (NHW) subjects by using SUDAAN.

^{2.} Least square means of serum ferritin (SF) were calculated for non-Hispanic black (NHB) subjects by using SUDAAN.

^{3.} Revised model was run based on final regression model #2 after excluding subjects with abnormal values of TIBC, serum total protein and GGT (normal TIBC: 240-450µg/dL, normal serum total protein: 6-8.5g/dL, normal GGT: <50 (U/L).

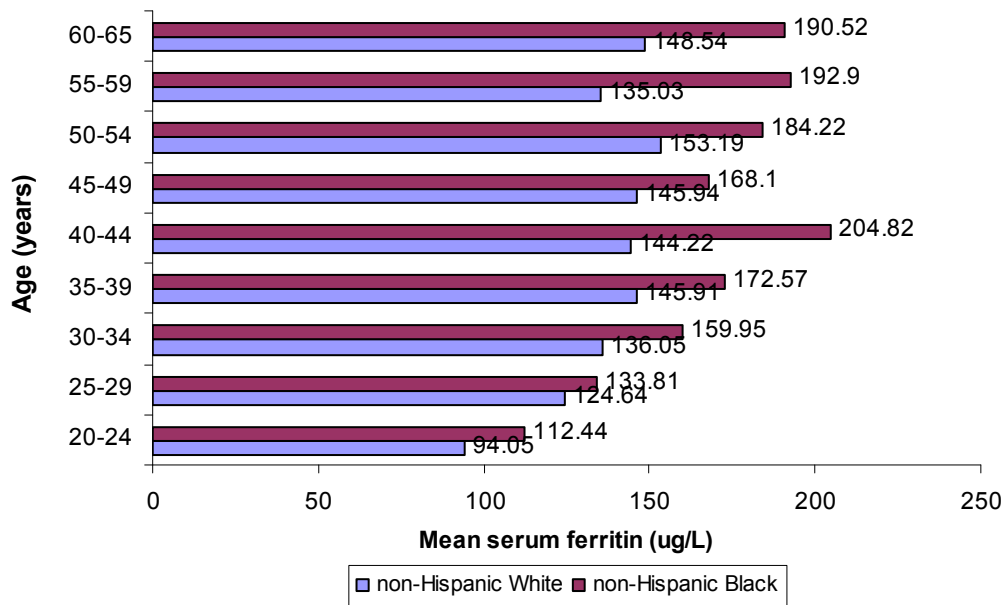


Fig. 1 Geometric means of serum ferritin (µg/L) among NHANES III non-Hispanic white and non-Hispanic black male subjects stratified by age.

TABLE 7Subject characteristics of NHANES III acute inflammation present non-Hispanic black and non-Hispanic white males aged ≥ 20 yrs.¹

Characteristics	non-Hispanic White (n=235)			non-Hispanic Black (n=164)			p
	n	mean	SEM	n	mean	SEM	
age (y)	235	55.9621	2.0883	164	50.7002	1.2359	0.0534
Education (yr)	235	12.0015	0.2873	164	12.1766	1.1897	0.8920
≤ 12 yr (%)	114	33.7586	4.2168	99	50.5377	3.5186	0.0120*
> 12 yr (%)	121	66.2414	4.2168	65	49.4623	3.5186	0.0120*
Poverty income ratio (PIR)	217	2.9436	0.1418	149	1.9458	0.1479	0.0004*
< 1.3 (%)	69	26.9145	3.9589	85	45.7873	5.5094	0.0152*
1.3-3.5 (%)	106	46.0655	5.1400	61	41.2287	5.2099	0.5076
≥ 3.5 (%)	60	27.0200	3.5356	18	12.9840	3.3683	0.0187*
Prevalence (%)							
congestive heart failure	22	5.6943	2.1255	14	9.6592	3.3689	0.3555
diabetes mellitus	4	1.2063	0.6389	8	4.8673	1.9725	0.0606
heart attack	42	12.7124	2.8343	14	8.9879	2.7328	0.4045
hypertension	82	28.3035	4.5524	65	33.9806	3.3924	0.3595
stroke	26	6.3637	0.9380	14	6.1220	1.5049	0.9002
BMI (kg/m ²)	235	27.4994	0.5037	164	28.4522	0.6232	0.2855
< 18.5 (%)	8	2.0194	0.8163	6	3.3395	1.4250	0.4426
18.5-25 (%)	82	33.0797	5.1274	53	28.0761	3.6765	0.4904
25-30 (%)	96	40.7493	4.7207	54	37.1665	5.3010	0.6433
≥ 30 (%)	49	24.1516	3.1342	51	31.4179	3.8376	0.2128
waist circumference (cm)	190	100.7669	0.9919	147	99.3081	1.5467	0.4641
Smoking status (%)							
non smoker	57	20.6656	2.8065	43	25.9921	2.5299	0.3299
former smoker	112	37.2360	3.9601	37	17.8888	2.7336	0.0007*
current smoker	66	42.0984	4.5366	84	56.1191	3.7509	0.0452*
c-reactive protein (mg/dL)	235	2.2686	0.1462	164	2.1940	0.1243	0.7107
serum albumin (g/dL)	232	3.9732	0.0490	163	3.8536	0.0317	0.0548
WBC (10^3 cells/mm ³)	235	9.1721	0.1805	163	7.4799	0.1770	< 0.0001 *
platelet count (10^3 cells/mm ³)	235	281.6856	4.9016	163	282.0639	8.4728	0.9693
plasma fibrinogen (mg/dL)	216	668.0087	11.4567	131	614.7575	11.2375	0.7441
lymphocyte (10^3 cells/mm ³)	235	2.1838	0.0685	163	2.3668	0.0863	0.0954
mononuclear (10^3 cells/mm ³)	233	0.5584	0.0129	160	0.4692	0.0156	0.0007*
hemoglobin (g/dL)	235	14.6319	0.1351	163	13.8277	0.1171	0.0006*
serum iron (μ g/L)	234	62.0671	3.0403	164	61.1701	2.7020	0.8309
serum ferritin (μ g/L)	235	212.1929	11.2600	164	274.6356	14.9006	0.0052*
25-300 (%)	172	75.4021	2.9997	105	63.1964	3.6817	0.0214*
> 300 (%)	54	21.7790	2.6778	56	35.2257	3.6025	0.0080*
transferrin saturation (%)	234	19.6342	0.9648	164	19.8068	0.8975	0.8972
TIBC	234	329.3691	4.5618	164	315.1581	5.0842	0.0625
triglycerides (mg/dL)	233	162.3160	9.8269	164	141.8698	6.0820	0.1581
total cholesterol (mg/dL)	235	198.5622	3.6845	164	206.5248	5.1076	0.2670
HDL cholesterol (mg/dL)	232	41.9932	0.9229	164	47.6259	1.5877	0.0054*
LDL cholesterol (mg/dL)	89	122.6330	3.4264	72	144.1864	7.0443	0.0139*
Plasma glucose (mg/dL)	207	104.5986	2.3103	159	115.7463	5.4416	0.0770
systolic blood pressure (mmHg)	206	130.2366	1.7616	158	133.0782	1.1767	0.1989
diastolic blood pressure (mmHg)	206	76.6417	0.7133	158	78.3303	1.0381	0.1675

¹ The means of all continuous variables were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's t-test. The frequencies of all categorical variables were compared between non-Hispanic white and non-Hispanic black male subjects using SUDAAN's Chi-square test.

* Statistical significance was set at a $p < 0.05$

None of the subjects had cancer, therefore, data were not showed in table.

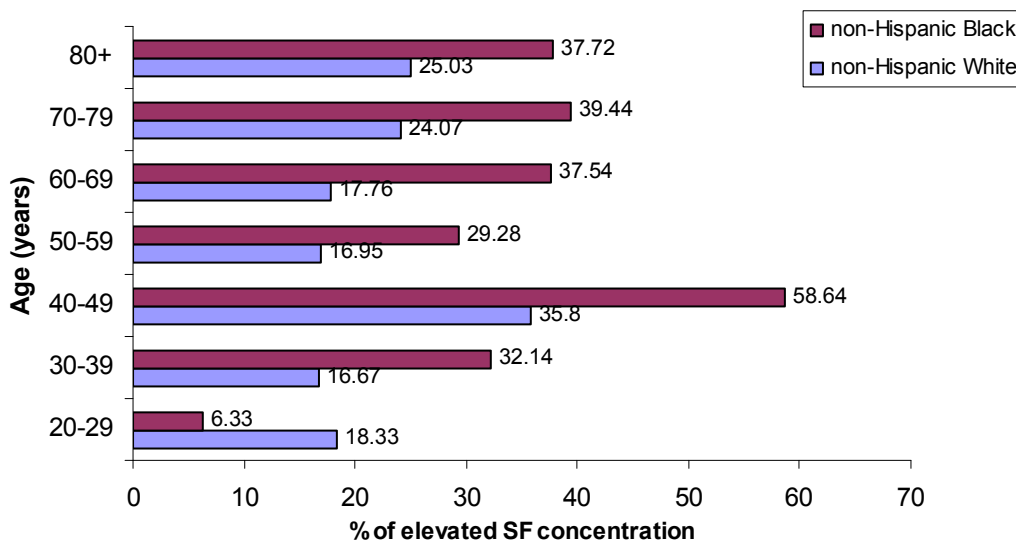


Fig. 2 The proportion of acute inflammation present subjects who had elevated serum ferritin concentration (>300µg/L), stratified by age and ethnicity.

TABLE 8

Risks of having elevated SF concentrations between NHW and NHB acute inflammation present NHANES III male subjects aged ≥20yrs.¹

# Model	n	non-Hispanic Black (NHB) Odds (95% CI)
Model 1 ²	366	2.153 (1.234 3.754)
Model 2 ³	333	2.239 (1.333 3.760)

¹. Comparisons of adjusted odds ratios of having elevated serum ferritin (SF) concentrations in response to acute inflammation between non-Hispanic white (reference group) and non-Hispanic black male subjects. Elevated serum ferritin concentration defined as >300µg/L for male.

². Model 1 adjusted for age, ethnicity, PIR, education, BMI, smoking status, chronic diseases including cancer, congestive heart failure, heart attack, hypertension, diabetes and stroke.

³. Model 2 further adjusted for cardiovascular risk factors including triglycerides, total cholesterol, HDL cholesterol, systolic and diastolic blood pressure in addition to the effects mentioned in footnote 2.

TABLE 9

Risk of having elevated SF concentrations per unit change in inflammation markers between NHW and NHB acute inflammation present NHANES III male subjects aged ≥20yrs.¹

Inflammation Marker	Model 1 ²		Model 2 ³	
	n	$\hat{\beta}$ (p) [†]	n	$\hat{\beta}$ (p) [†]
c-reactive protein	366	0.0991(0.3556)	333	0.2070(0.0005)*
WBC	365	0.0558(<0.0001)*	332	0.0536(0.0001)*
serum albumin	364	0.0831(0.0201)*	331	0.0894(0.0031)*
lymphocyte count	365	0.1448(0.0040)*	332	0.1398(0.0038)*
monocyte count	361	0.3823(0.0987)	328	0.3183(0.1264)
platelet count	365	0.0015(0.0002)*	332	0.0016(0.0003)*

¹. Logistic regression models were used to estimate the magnitude of serum ferritin (SF) elevation associated with one unit change in inflammation markers.

². Model 1 adjusted for age, ethnicity, PIR, education, BMI, smoking status, chronic diseases including cancer, congestive heart failure, heart attack, hypertension, diabetes and stroke.

³. Model 2 further adjusted for cardiovascular risk factors including triglycerides, total cholesterol, HDL cholesterol, systolic and diastolic blood pressure in addition to the effects mentioned in footnote 2.

[†] P value corresponding to the comparisons between the β of non-Hispanic black and non-Hispanic white (reference group), respectively.

* Statistical significance set as p<0.05.

TABLE 10Subject characteristics of NHANES III hepatitis C-infected non-Hispanic black and non-Hispanic white males aged ≥ 20 yrs.¹

Characteristics	non-Hispanic White (n=62)			non-Hispanic Black (n=92)		
	n	mean	SEM	n	mean	SEM
age (y)	62	38.8805	1.4053	92	40.4501	1.1217
Education (yr)	62	11.3624	0.3170	92	11.7966	0.5585
≤ 12 yr (%)	25	41.7095	6.6099	39	43.0889	5.0522
> 12 yr (%)	37	58.2905	6.6099	53	56.9111	5.0522
Poverty income ratio (PIR)	59	2.1518	0.2167	89	1.6378	0.2074
< 1.3 (%)	18	29.8729	4.6256	53	55.1676	7.0804
1.3-3.5 (%)	35	54.4031	5.4696	32	35.7660	5.3583
≥ 3.5 (%)	9	15.7240	3.4509	7	9.0664	2.3836
Prevalence (%)						
cancer (various types)	0	0	0	0	0	0
congestive heart failure	2	2.0423	0.5390	2	3.5661	2.3752
diabetes mellitus	0	0	0	0	0	0
heart attack	9	6.7880	3.6203	2	2.7044	2.1657
hypertension	11	6.8267	1.6187	21	18.6453	3.7319
stroke	2	1.7920	0.1412	2	0.9648	0.7055
abnormal liver enzymes‡	21	35.6880	4.8662	50	59.8186	5.1176
BMI (kg/m ²)	62	25.2357	0.5724	92	24.2422	0.3570
< 18.5 (%)	2	1.9888	1.8954	1	0.7278	0.7287
18.5-25 (%)	31	56.7926	3.9262	59	64.4130	3.5763
25-30 (%)	20	26.6847	3.9150	20	24.0277	3.7566
≥ 30 (%)	9	14.5346	1.8486	12	10.8315	3.0202
alcohol consumption (drinks/mo)†	62	11.7553	4.2642	92	20.1051	3.6027
non-drinker (%)	34	45.8474	4.1342	23	22.5689	4.6266
light drinker (%)	25	43.6480	3.8403	55	61.0375	3.8391
moderate drinker (%)	2	2.2530	0.4247	9	8.5821	3.1488
heavy drinker (%)	1	8.2516	0.6500	5	7.8115	4.6659
c-reactive protein (mg/dL)	62	0.2542	0.0089	92	0.5426	0.1086
WBC (10 ³ cells/mm ³)	60	7.8289	0.2276	90	6.3984	0.2275
platelet count (10 ³ cells/mm ³)	60	246.6482	10.3299	90	237.3385	9.6898
serum albumin (g/dL)	62	4.2899	0.0321	90	4.0293	0.0457
hemoglobin (g/dL)	60	15.3430	0.2034	90	14.4465	0.1816
serum iron (μ g/L)	62	97.8023	5.4525	92	112.6925	5.8364
serum ferritin (μ g/L)	62	198.5646	25.3786	92	310.3259	32.9399
< 25 (%)	1	3.6891	3.6123	1	0.7926	0.0864
25-300 (%)	50	75.4120	3.6245	59	59.9013	5.3947
> 300 (%)	11	20.8990	1.6677	32	39.3061	5.4506
transferrin saturation (%)	62	27.6997	1.6530	91	32.4767	2.0632
TIBC	62	357.7358	4.7743	91	353.8499	8.1383
AST SI(U/L)	62	33.8477	2.1921	90	54.9738	5.8770
ALT SI (U/L)	62	35.9374	3.1056	90	46.3029	3.3367
ALP SI(U/L)	62	85.5920	2.7759	90	90.2533	3.2261
GGT SI(U/L)	49	69.4828	9.2045	74	127.0551	29.8752
LDH SI(U/L)	62	167.8463	5.5962	89	174.1724	3.0130
serum total bilirubin (mg/dL)	62	0.6407	0.0313	90	0.6953	0.0277

‡ Serum aspartate or alanine aminotransferase above 40U/L.

† Alcohol consumption was further divided into 4 categories: non-drinker (0 drink/mo), light drinker (1-30 drinks/mo), moderate drinker (31-60 drinks/mo), heavy drinker (> 60 drinks/mo).

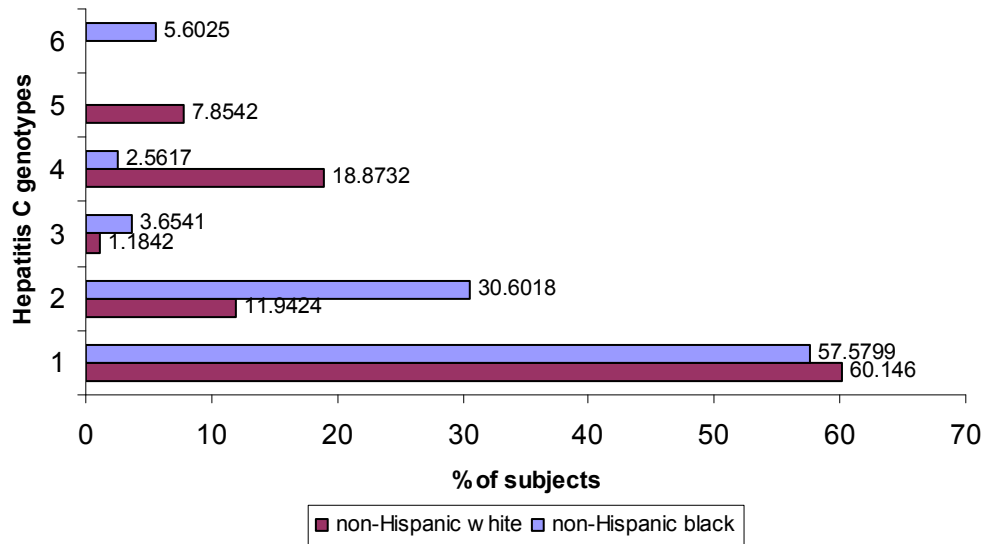


Fig. 3 Distribution of hepatitis C genotypes between non-Hispanic white and non-Hispanic black NHANES III male subjects aged ≥ 20 .

TABLE 11
Distribution of hepatitis C genotypes between non-Hispanic white and non-Hispanic black NHANES III male participants aged ≥ 20 .

hepatitis C genotypes	non-Hispanic white		non-Hispanic black	
	n	%	n	%
1	19	60.15	43	57.58
2	7	11.94	26	30.60
3	1	1.18	2	3.65
4	7	18.87	3	2.56
5	2	7.85	0	0
6	0	0	3	5.60

TABLE 12
Risk of having elevated SF concentrations between NHW and NHB hepatitis C infected NHANES III male participants aged ≥ 20 .¹

# Model	n	non-Hispanic Black (NHB) Odds (95% CI)
Model 1 ²	148	1.626 (0.682 3.877)
Model 2 ³	145	1.910 (0.765 4.768)

^{1.} Comparisons of adjusted odds ratios of having elevated serum ferritin (SF) concentrations in response to hepatitis C between non-Hispanic white (reference group) and non-Hispanic black male subjects. Elevated serum ferritin concentration defined as $>300\mu\text{g/L}$ for male.

^{2.} Model 1 adjusted for age, ethnicity, PIR, BMI, C-reactive protein and alcohol consumption.

^{3.} Model 2 further adjusted for serological markers including AST, ALT, ALP, LDH and serum total bilirubin in addition to the effects mentioned in footnote 2.

TABLE 13

Risk of having elevated SF concentrations per unit change in serological markers between NHW and NHB hepatitis C infected NHANES III male subjects aged ≥ 20 yrs.¹

Inflammation Marker	Model 1 ²		Model 2 ³	
	n	$\hat{\beta}$ (p) [†]	n	$\hat{\beta}$ (p) [†]
aspartate aminotransferase (AST)	146	0.00301(0.4012)	145	0.00519(0.3412)
alanine aminotransferase (ALT)	146	0.0013(0.7363)	145	0.00158(0.6859)
alkaline phosphatase (ALP)	146	0.00388(0.0595)	145	0.00452(0.0435)*
lactate dehydrogenase (LDH)	145	0.00201(0.0892)	145	0.00159(0.02120)
serum total bilirubin	146	0.3681(0.2276)	145	0.4032(0.2578)

^{1.} Logistic regression models were used to estimate the magnitude of serum ferritin (SF) elevation associated with one unit change in serological markers.

^{2.} Model 1 adjusted for age, ethnicity, education, PIR, BMI, C-reactive protein and alcohol consumption.

^{3.} Model 2 further adjusted for other serological markers including AST, ALT, ALP, LDH and serum total bilirubin in addition to the effects mentioned in footnote 2.

[†] *P* value corresponding to the comparisons between the β of non-Hispanic black and non-Hispanic white (reference group), respectively.

* Statistical significance set as $p < 0.05$.

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