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Comparing Serum Ferritin Values from Different Population Surveys

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This report compares serum ferritin data collected in recently conducted Health and Nutrition Examination Surveys by age, sex, and ethnic group. Serum ferritin assays and selected confounding variables are examined to explore the basis of the difference in serum ferritin values obtained from the different surveys.

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Symbols

- - -	Data not available
. . .	Category not applicable
–	Quantity zero
0.0	Quantity more than zero but less than 0.05
Z	Quantity more than zero but less than 500 where numbers are rounded to thousands
*	Figure does not meet standard of reliability or precision

Comparing Serum Ferritin Values from Different Population Surveys

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Introduction

Evaluating and monitoring the iron status of the U.S. population is an important goal of the nutrition component of the various Health and Nutrition Examination Surveys (HANES) conducted by the National Center for Health Statistics. The ability to perform this function has been enhanced with the addition of serum ferritin (SF) measurements in the three HANES surveys conducted since 1976: The second National Health and Nutrition Examination Survey (NHANES II), conducted in 1976–80; the Hispanic Health and Nutrition Examination Survey (HHANES), conducted in 1982–84; and the third National Health and Nutrition Examination Survey (NHANES III), which began in 1988 and will be completed in 1994.

Because SF is related to body iron stores in healthy individuals (1), its inclusion in HANES allows an assessment of the iron-replete segment of the population as well as the iron-deficient segment (2). Interest in the iron status of the iron-replete segment of the population has increased lately because of recent reports that the gene frequency for hemochromatosis may be higher than previously suspected (3) and that body iron stores may be related to cancer risk (4).

SF has now been measured in more than one HANES; therefore, it should be possible to compare SF data from the different surveys to assess secular trends or compare different populations.

Preliminary analyses were done to compare iron status of Hispanic persons tested in HHANES (1982–84) with that of non-Hispanic white and Hispanic persons tested in NHANES II (1976–80). The SF values were found to be higher for Hispanic persons from HHANES than for either non-Hispanic white or Hispanic persons from NHANES II, especially among males.

In the present study, the serum ferritin distributions from the two surveys were compared by age, sex, and ethnic group to determine whether the difference in SF values noted for males was present in other age-sex groups. In addition, SF data from three pilot studies conducted for NHANES III were examined. Other relevant variables related to iron status, liver function, and socioeconomic status were compared to explore the basis for the SF differences among the surveys.

An understanding of the difference between the SF distributions found in NHANES II and HHANES is important, as SF data from HANES are used to monitor iron status in the United States and to generate reference data for different population subgroups. More generally, the study illustrates factors that need to be considered in comparing SF values obtained from different surveys and suggests ways to reduce these problems in future studies.

All abbreviations used in this report are spelled out in the text when they are first mentioned. However, because there are so many abbreviations, they are also listed in the appendix.

Methods

Population samples

NHANES II was a survey of the civilian noninstitutionalized U.S. population ages 6 months–74 years (5). HHANES was a survey of civilian noninstitutionalized persons ages 6 months–74 years from three Hispanic subpopulations living in selected areas of the United States: Mexican-Americans from Arizona, California, Colorado, New Mexico, and Texas; Cubans from Dade County, Florida; and Puerto Ricans from the New York City metropolitan area (6). The samples used in the three pilot studies for NHANES III were not representative of the U.S. population or of the total metropolitan area where the study was performed. The first NHANES III pilot study was conducted in the Washington, D.C., metropolitan area in October–December 1987; a convenience sample of 492 community volunteers was examined. The second pilot study was conducted in Tampa, Florida, in February–April 1988; a probability sample of 329 individuals from selected locations in the metropolitan area was examined. The third pilot study was conducted in the Washington, D.C., area in September–November, 1988; a probability sample of 776 individuals from selected locations was examined. The first (Washington, D.C.) and second (Tampa) pilot studies were of primarily non-Hispanic persons, whereas the third pilot study (Washington, D.C.) included Hispanic persons, who were mostly from Central American countries such as El Salvador, Nicaragua, and Guatemala.

Certain exclusion criteria were used to derive the analytic samples from the three surveys used in this study. Individuals with missing values for the iron status indicators examined in this study were deleted from the analyses of that particular indicator only. Children under 5 years of age were excluded from the NHANES II and HHANES analytic samples to limit analyses to children who had provided venous blood samples in both surveys and to form age categories consistent with previous analyses of iron status indicators from both surveys (7–9). Individuals under 20 years of age or 65 years and over were excluded from the analyses of the NHANES III pilot study samples because there were too few individuals in these age ranges to permit analysis. Pregnant women were excluded from the NHANES II and HHANES analytic samples, as pregnancy affects the interpretation of iron status indicators. Pregnancy status data for the NHANES III pilot study samples were not available at the time of this study; therefore, pregnant women could not be excluded from the analytic sample for the pilot studies.

The analytic sample from NHANES II was restricted to white persons, who were in turn categorized as non-Hispanic or Hispanic, based on self-reported family ancestry or national origin. Hispanic persons were defined as those whose self-reported family ancestry or national origin was one of the following: Chicano, Mexicano, Mexican, Mexican-American, other Spanish, Central or South American, Puerto Rican, or Cuban. The remaining white persons were defined as non-Hispanic white. Of the 16,965 persons ages 5–74 years examined in NHANES II, 14,347 remained in the analytic sample after the exclusions had been made. The analytic sample consisted of 13,473 non-Hispanic white persons and 874 Hispanic persons.

Because of sampling rules that allowed all members of a family with at least one Hispanic member to be eligible, some non-Hispanic persons were included in HHANES. Therefore, it was also necessary to use self-reported family ancestry or origin to restrict the analytic sample from HHANES to Mexican-Americans, Cubans, and Puerto Ricans. There were 9,853 persons ages 5–74 years examined in HHANES, of whom 9,570 remained in the analytic sample after non-Hispanic persons and pregnant women were excluded. The analytic sample consisted of 6,087 Mexican-Americans, 2,281 Puerto Ricans, and 1,202 Cubans.

To preserve an adequate sample size, the analytic sample from the NHANES III pilot studies included persons of all races. Self-reported race and national origin were available from two of the pilot studies; the analytic samples from these studies were subdivided into non-Hispanic white and Hispanic persons. The analytic samples of adults ages 20–64 years from the NHANES III pilot studies consisted of 334 individuals examined in the 1987 Washington, D.C., pilot study; 157 individuals examined in the Tampa pilot study; and 160 individuals examined in the 1988 Washington, D.C., pilot study.

Iron status

Serum ferritin

In HHANES and the NHANES III pilot studies, serum ferritin (SF) was measured for all individuals over a

certain age (4 years and over in HHANES and 1 year and over in NHANES III), but in NHANES II, SF was measured only for a subsample of 5,157 individuals. Approximately 30 percent of the subsample was composed of the special hematologic subgroup selected because of an abnormal red blood cell (RBC) count, hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), or white blood cell count. The remainder of the SF subsample was selected randomly from the hematologically normal NHANES II sample. Although an unusual selection process was used for the SF subsample, use of the appropriate sample weights in statistical analyses produced SF data that were representative of the U.S. population at the time of NHANES II (7). Analyses done for the present study (not shown in this report) indicate that inclusion of the special hematologic subgroup in the NHANES II SF subsample did not disproportionately lower the SF values for the survey and thus could not explain the SF difference noted between NHANES II and HHANES. Of the 14,347 individuals in the analytic sample from NHANES II used in the present study, ferritin determinations were made for 3,944.

In NHANES II, SF was measured at the University of Kansas (UK) Medical Center (2,7) using a two-site immunoradiometric assay (IRMA). The Lowry technique (10) was used to standardize ferritin protein against bovine serum albumin. A single batch of recrystallized human ferritin from liver and spleen was injected into rabbits to obtain antibodies and was also used to produce standards which were diluted to 1,000 micrograms per liter (μg/L) in buffered 5-percent bovine serum albumin and stored at −20 °C. All assays were performed in triplicate at an initial dilution of 1:20. Samples with SF less than 20 μg/L or greater than 200 μg/L were reassayed at dilutions of 1:10 or 1:1,000, respectively, with diluted standards containing equivalent concentrations of normal rabbit serum.

Quality control procedures in NHANES II consisted of assays of samples from bench control pools of human blood produced at the UK Medical Center and blind quality control samples supplied by the Centers for Disease Control. In addition, the ferritin standard from the Iron Panel of the International Committee for Standardization in Hematology (ICSH) was available at the hematology laboratory at the UK Medical Center before it was released for general use, so comparisons with this standard were also made. The value for the initial bench quality control pool was observed to decrease with time, possibly because of storage at −20 °C rather than −70 °C. Thus, at the conclusion of NHANES II, SF measurements in each assay were adjusted to an overall average for a quality control pool of normal serum stored at −70 °C. The validity of applying this correction was tested by repeat analysis of samples assayed throughout the study and by examination of the data from blind quality control pools over time.

In HHANES and the NHANES III pilot studies, SF was measured at the Centers for Disease Control (11) with the "Quantimune Ferritin IRMA kit" (BioRad Laboratories, Hercules, California). The assay was a single-incubation, two-site IRMA based on assays described by Addison et al. (1) and Miles et al. (12). This kit was selected after an extensive evaluation of commercially available products. The accuracy of the kit was confirmed by using materials supplied by the hematology laboratory at the UK Medical Center, as well as the ICSH ferritin reference material supplied by the National Institute for Biological Standards and Controls, London, United Kingdom.

Quality control procedures in HHANES and the NHANES III pilot studies consisted of (a) assays of samples from bench quality control pools prepared at three levels ("normal," "low," and "high"), which were measured two–four times in each analytic run, and (b) assays of samples from blind quality control pools prepared at two levels ("low normal" and "high normal"), which were randomly incorporated with every 20 serum samples.

Comparison of serum ferritin assays

A direct comparison of the SF assays used in NHANES II and HHANES was not possible because the original two-site IRMA method used in NHANES II could not be reconstructed at the time of HHANES. The two-site IRMA used at the UK Medical Center in 1982–84 and the Quantimune kit assay were each independently compared with the two-site, enzyme-linked immunoassay (EIA) based on monoclonal antibodies used at the UK Medical Center. The assay protocol for the two-site EIA and its comparison with the two-site IRMA used at the UK Medical Center have been described (13). Both assays were used to measure SF in sera obtained from 39 normal subjects.

The Quantimune IRMA kit and the two-site EIA were compared by measuring SF on the same 42 serum samples collected in HHANES and on 50 serum samples collected in the NHANES III pilot studies. At the beginning of the SF analyses for HHANES (November 1984), 22 HHANES samples were exchanged; 20 were exchanged at the end of the SF analyses (January 1986). The 50 blood samples from the NHANES III pilot studies were exchanged in August 1988.

Other iron status indicators

Assays for HB, HCT, serum iron (SI), total iron-binding capacity (TIBC), and erythrocyte protoporphyrin (EP) were performed at the Centers for Disease Control or in the mobile examination centers used in all surveys. Details of the procedures used in NHANES II and HHANES for blood collection, specimen storage, assays for each indicator, and quality control have been published elsewhere (11,14). Values for transferrin saturation (TS) were calculated by dividing SI by TIBC, and MCV values were calculated by dividing HCT by RBC. The same assay procedures, except for SI, were used in the NHANES III pilot studies. The protocol for SI was

modified by adding thiourea to complex copper, which resulted in slightly lower SI, and hence lower TS values, for the NHANES III pilot studies than for HHANES or NHANES II. HCT, EP, and TS data were available from the NHANES III pilot studies at the time of the present study.

Impaired iron status

Analyses performed to examine the relationship between SF and other selected iron status indicators in the low range of their distributions were restricted to women ages 20–44 years because this group had a sufficiently large prevalence of low values. Hispanic and non-Hispanic white females who had either a low HB value (less than 119 grams per liter) or impaired iron status based on two models that used multiple iron status indicators were compared in terms of mean SF and percent with SF less than 12 μg/L. Both models were developed by an expert panel for use with NHANES II data (7,8). The first model, called the MCV model, is based on MCV, TS, and EP; the second model, called the ferritin model, is based on SF, TS, and EP. To be considered iron impaired by either model, an individual must have an abnormal value for at least two of the three iron status indicators included in that model. The age-specific cutoff values that indicate abnormality for each indicator have been published elsewhere (7,8).

Liver function

Three types of data related to liver function were available from at least one of the HANES: Selected serum analyte levels, self-reported liver disease, and frequency of alcohol consumption.

Serum total bilirubin and aspartate aminotransferase were measured at the Primate Research Institute of the New Mexico State University in HHANES and in the NHANES III pilot studies as part of the Centrifichem Biochemistry Profile (15). Comparable data from a representative non-Hispanic white sample were not available from NHANES II, as these serum analytes were measured only for a small subsample with elevated bile acid levels.

Self-reported liver disease was defined as positive when the respondent said that a physician had diagnosed cirrhosis of the liver, hepatitis, or jaundice.

Alcohol intake was derived from the food frequency instrument used in NHANES II and HHANES. (The 24-hour recall data base for HHANES was not available at the time of the present study.) The food frequency assessment was of the usual number of servings of beer, wine, and hard liquor consumed per time unit (for example, day, week, or month) over a 3-month period and could not be used to calculate ounces of intake.

Poverty status

Poverty status, as defined by the poverty income ratio, was used as an indicator of the socioeconomic status of the respondents in NHANES II and HHANES. The poverty income ratio is calculated by dividing the total family income by the poverty threshold for a family of that size (16). Persons whose poverty income ratio was less than 1.00 were considered "poor"; persons whose poverty income ratio was 1.00 or greater were considered "nonpoor."

Data analysis

All statistics were calculated using SAS (17). A natural logarithm transformation of SF values was used to calculate geometric means to account for the skewed distribution of this indicator. To account for the complex design of NHANES II and HHANES, sample weights were used when calculating descriptive statistics; sample weights were not available for data from the NHANES III pilot studies, as these studies did not employ representative samples. The use of sample weights provides estimates that represent the targeted U.S. subpopulation at the midpoint of the respective survey. Variances were calculated using an average design effect to modify the subgroup variances calculated under the assumption of simple random sampling. The rationale for this approach has been described elsewhere (9). The average design effects used for non-Hispanic white persons from NHANES II and for Mexican-Americans, Cubans, and Puerto Ricans from HHANES were 1.2, 1.2, 1.1, and 0.9, respectively. Geometric mean SF values were compared between population groups within an age and sex category with a *t*-test. Because multiple comparisons were made, the critical *t*-value was calculated using the Bonferroni approach (18).

Findings

Serum ferritin distributions

The SF levels for non-Hispanic white and Hispanic persons from NHANES II, HHANES, and the NHANES III pilot studies are shown in tables 1 and 2 for children and adolescents and for adults ages 20–74 years, respectively. Because of small sample sizes, data for Hispanic persons from NHANES II are limited to medians and a few selected percentiles for most age-sex categories. Data for individuals from the NHANES III pilot studies are further limited to adults only.

In all age-sex groups, the SF values for Hispanic persons from HHANES were higher than those for non-Hispanic white persons from NHANES II. The SF values for adult Hispanic males from HHANES were about 20–50 percent higher than those for non-Hispanic white males from NHANES II at all percentiles. When averaged over the three Hispanic groups (Mexican-American, Cuban, and Puerto Rican), the geometric mean SF values for Hispanic males from HHANES were approximately 55 μg/L higher than those for non-Hispanic white males from NHANES II in the three adult age groups (20–44, 45–64, and 65–74 years). The differences in geometric means were statistically significant in all but one age-ethnic group comparison. SF values for Hispanic and non-Hispanic white adult males from the NHANES III pilot studies were similar to those for Hispanic males from HHANES; SF values for adult Hispanic males from NHANES II were comparable to those for non-Hispanic white males from NHANES II. Preliminary SF data (not shown in this report) for non-Hispanic white and Hispanic males from the first 11 locations of NHANES III were similar to those seen in the NHANES III pilot studies, which suggests that the SF levels observed in the NHANES III pilot studies were not an aberration.

SF values for adult females from NHANES II and HHANES were similar at percentiles below the median but diverged at percentiles above the median. Geometric mean values did not differ statistically between Hispanic and non-Hispanic white females from the two surveys in any age group. Geometric means from HHANES for Hispanic females under 65 years of age were 3–7 μg/L higher than those for non-Hispanic white females from NHANES II in comparable age groups; geometric means for elderly Hispanic females from HHANES were approximately 27 μg/L higher than those for elderly non-Hispanic white females from NHANES II. SF values for non-Hispanic white and Hispanic females from the NHANES III pilot studies were similar to those of Hispanic females from HHANES. Although SF values for older Hispanic females from NHANES II were similar to those of non-Hispanic white females from that survey, values for young adult Hispanic females from NHANES II were closer to those of young adult Hispanic females from HHANES.

The pattern of differences between SF data from NHANES II and HHANES among children and adolescents was similar to that described for females (table 1).

Serum ferritin assay comparison

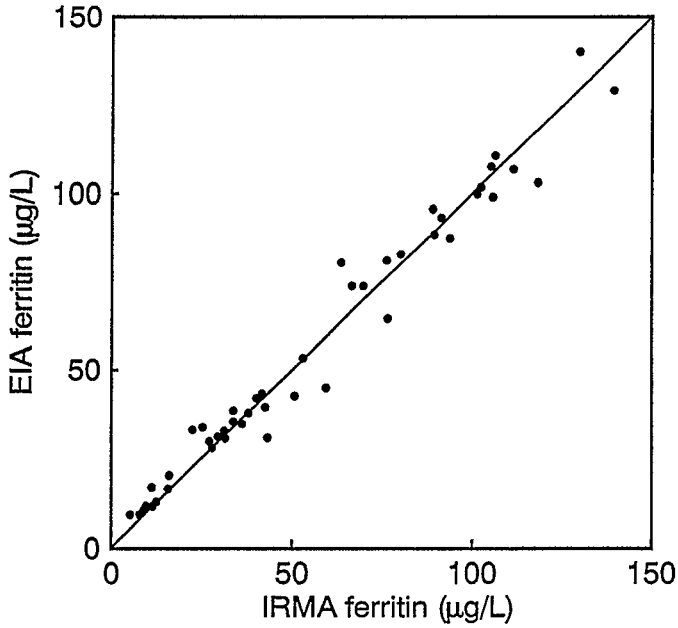
Results of the comparison of the EIA and two-site IRMA used at the UK Medical Center conducted by Flowers et al. (13) are shown in figure 1. The ferritin concentrations in the serum samples ranged from 4–150 μg/L. The correlation between values from the two assays was 0.987. Flowers et al. (13) concluded that the two methods were comparable.

Results of the comparison of SF assays used in HHANES and the NHANES III pilot studies with the EIA used at the UK Medical Center are shown in figures 2 and 3, respectively. The 42 serum samples from HHANES used in the comparison study had ferritin concentrations ranging from 1–1,440 μg/L ($\bar{X}$= 159 μg/L), and the ferritin levels in the 50 serum samples from the NHANES III pilot studies ranged from 10–689 μg/L when the two-site IRMA commercial kit assay was used. In general, values obtained with the commercial kit used in the two HANES were slightly lower than those produced by the EIA, but the difference was not statistically significant. The correlations between SF obtained with the commercial kit IRMA and the EIA were 0.987 and 0.969 in the studies using the HHANES and NHANES III pilot study serum samples, respectively. Thus, based on the 92 samples exchanged, the methods appear comparable.

Related analyses

Other iron status indicators

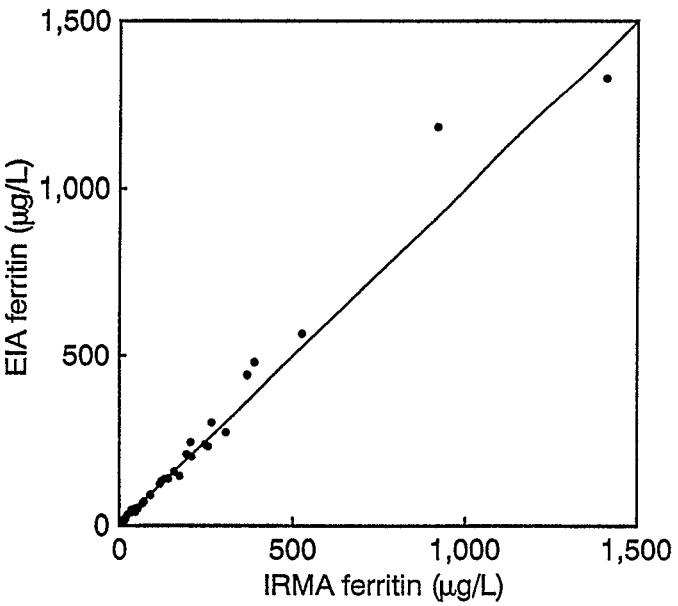
Distributions of five selected iron status indicators for young Hispanic adults from HHANES and young non-Hispanic white adults from NHANES II are shown in



NOTES: µg/L is micrograms per liter. The solid line represents the line of identity.

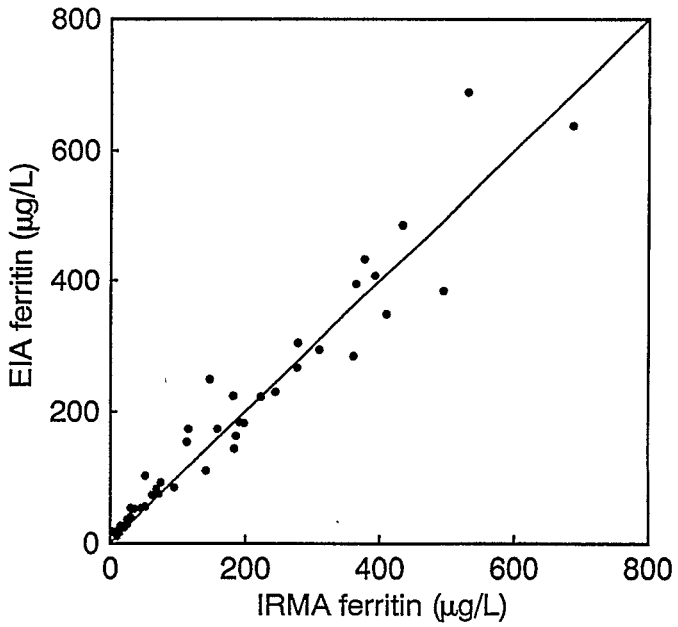
SOURCE: Flowers CA, Kuizon M, Beard JL, et al. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol 23:141–51. 1986. Copyright notice: © 1986. Wiley-Liss, a division of John Wiley and Sons, Inc. Reprinted by permission of Wiley-Liss, a division of John Wiley and Sons, Inc.

Figure 1. Comparison of serum ferritin values measured with immunoradiometric assay (IRMA) and enzyme-linked immunoassay (EIA), used at University of Kansas Medical Center



NOTES: HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84. µg/L is micrograms per liter. The solid line represents the line of identity. Regression equation: EIA ferritin = 2.9 +1.1*IRMA ferritin.

Figure 2. Comparison of serum ferritin values of HHANES sera measured with immunoradiometric assay (IRMA), used in HHANES, and enzyme-linked immunoassay (EIA), used at University of Kansas Medical Center



NOTES: The NHANES III (third National Health and Nutrition Examination Survey) pilot studies were conducted in 1987–88; HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84. µg/L is micrograms per liter. The solid line represents the line of identity. Regression equation: EIA ferritin = 11.3 + 0.98*IRMA ferritin.

Figure 3. Comparison of serum ferritin values of sera from NHANES III pilot studies measured with immunoradiometric assay (IRMA), used in HHANES, and enzyme-linked immunoassay (EIA), used at University of Kansas Medical Center

table 3. Data from the NHANES III pilot studies for EP, TS, and HCT are also shown. These indicators were compared for Hispanic and non-Hispanic white persons in all age-sex groups. Results for only one age group are presented as an example because distributions for these indicators were similar for Hispanic and non-Hispanic white persons in all age-sex groups. The age group 20–44 years was chosen because males of this age generally have a very low prevalence of iron deficiency or inflammatory disease and females have a high prevalence of iron deficiency. In general, median EP values for non-Hispanic white persons from NHANES II tended to be slightly higher than both the values for Hispanic persons from HHANES and the values for males and females ages 20–44 years from the NHANES III pilot studies. Median TS and HB values were slightly higher for Hispanic males than for non-Hispanic white males but were lower or about the same for Hispanic females compared with non-Hispanic white females. MCV and HCT values were equivalent for Hispanic and non-Hispanic white persons of both sexes. Thus, a clear difference in these other iron status indicators consistent with the SF difference between HHANES and NHANES II was not evident in this age group.

Mean SF values and prevalences with SF less than 12 µg/L for Hispanic and non-Hispanic white females 20–44 years of age with low HB or impaired iron status based on the MCV model are shown in table 4. In general, Puerto

Rican females with low HB or impaired iron status had mean SF and percent low SF values similar to those for non-Hispanic white females with these conditions. Mexican-American females had a lower mean SF value and a similar or slightly higher prevalence with low SF than non-Hispanic white females. The small number of Cuban females precludes reliable estimates.

Table 5 contains the prevalences of low HB among young adult Hispanic and non-Hispanic white females with either low SF or impaired iron status based on the ferritin model. Prevalences of low HB among females with these conditions were similar among ethnic groups.

Liver function

To assess indirectly whether abnormal liver function among Hispanic males, as reflected by serum aspartate aminotransferase (AST) and bilirubin, could explain their higher SF values, SF levels of these males were calculated before and after excluding persons with the following AST or bilirubin levels: (a) above the manufacturer's normal range for the method used in HHANES; (b) above the value of the 95th percentile for the analyte in the first National Health and Nutrition Examination Survey (NHANES I), which was conducted in 1971–75; and (c) above the value of the 75th percentile for the analyte in NHANES I, which represented an extreme definition of "high" values. Results are shown in table 6. With one exception (AST for Mexican-American males 65–74 years of age), the median SF levels for the Hispanic males were still greater than those for non-Hispanic white males in corresponding age categories. Thus, excluding persons with varying degrees of "high" serum AST or bilirubin levels did not remove the difference in SF values.

Results for the other variables related to liver function available from HHANES (that is, self-reported liver disease and alcohol consumption) did not indicate that differences in these variables could account for the SF difference. For example, the difference in SF values for Hispanic and non-Hispanic white persons remained after excluding individuals with self-reported liver disease (data not shown in this report). Problems with self-reporting of health conditions and the small number of individuals who reported these conditions may have influenced the result. Differences in the reported frequency of alcohol intake were confined to beer intake among males, and the pattern of differences between Hispanic and non-Hispanic white persons was not consistent across age categories (data not shown in this report).

Poverty status

SF levels for Hispanic and non-Hispanic white persons are shown by poverty status, age, and sex in table 7. Accounting for poverty status did not remove the difference in SF levels between Hispanic and non-Hispanic white persons. The magnitude of the differences in means and medians was similar or greater than had been observed before stratifying by poverty in a majority of the age, sex, and poverty categories.

Discussion

Interpretation of the difference in SF values between NHANES II and HHANES is complicated for several reasons:

- The ethnic composition of the populations sampled in the two surveys is different; therefore the SF difference may reflect a difference in iron stores or in confounding factors such as liver or inflammatory disease between the ethnic groups.
- The SF assays differed between surveys, although both were based on the two-site immunoradiometric assay (IRMA) developed by Miles et al. (12).
- The surveys were conducted at two time points, so secular trends in the overall U.S. population could have occurred.

Because the present study is retrospective, the potential reasons for the SF difference between NHANES II and HHANES can only indirectly be assessed. A genetic difference between ethnic groups seems unlikely given the similarities of SF values between (a) Hispanic persons and non-Hispanic white persons in NHANES II and (b) Hispanic persons from HHANES and the non-Hispanic sample from the NHANES III pilot studies. The similarity of SF values between ethnic groups sampled in the same survey also argues against population differences in confounding factors, such as inflammatory disease, liver function, or economic status, unless the prevalence of the confounding factor changed between surveys in both the Hispanic and the non-Hispanic white populations.

Differences in selected confounding factors between populations were not evident in the other limited types of assessments that were possible with the available data. For example, values for other iron status indicators that are affected by inflammation and infection, such as TS and HB, were generally similar in comparable age-sex groups in both surveys. This similarity must be interpreted with some caution, as these indicators may be less affected by subclinical chronic infections or inflammatory states than SF is. After the exclusion of those Hispanics in HHANES with "high" levels of serum AST or bilirubin, as defined by various cutoff values, SF values of Hispanic persons were still higher than those of non-Hispanic white persons from NHANES II, which suggests that differences in liver function did not account for the higher SF values observed in HHANES. Finally, the higher SF values in HHANES still remained after stratifying by poverty status, a finding that does not support a socioeconomic explanation for the SF difference.

The lack of a direct comparison study between the SF assays used in NHANES II and in HHANES makes it impossible to completely rule out a methodologic basis for the SF difference. Considerable variation in serum ferritin measurements by different assays has been reported (19,20). An indirect comparison of the SF assays used in the two HANES could be construed from the correlation of values produced by the monoclonal EIA method used at the UK Medical Center with values from the two-site IRMA method used in this laboratory in the mid-1980's, because values obtained with the HHANES assay also correlated well with the monoclonal EIA.

The comparison study between the EIA and the two-site IRMA used at the UK Medical Center did not include SF values greater than 150 μg/L, which correspond to the upper half of the SF distribution for Hispanic males. Thus, the possibility that the assays diverged in the higher portion of the SF distribution cannot be assessed. This type of divergence might explain the inconsistency of the SF difference between NHANES II and HHANES for males and females. Divergence of SF in the higher portion of the distribution has been observed for other ferritin assays (21).

Data from the two surveys that would confirm that the SF difference reflects a true change in iron stores are essentially lacking; direct methods of measuring body iron stores, such as liver biopsy or bone marrow aspirate, are not feasible in population surveys. Differences in other iron status indicators consistent with increased body iron stores were not observed between non-Hispanic white persons from NHANES II and Hispanic persons from HHANES, but these indicators are only indirectly related to body iron stores. SF is the only indicator related to body iron stores, at least in healthy individuals, that is applicable in a population survey, and some aspects of its relationship to these stores remain unresolved. For example, it is not known whether SF values are affected by the distribution of iron stores between parenchymal cells or the monocyte/macrophage (reticuloendothelial) system. SF may also vary depending on the type of iron storage molecule, for example, ferritin or hemosiderin. Finally, SF values may reflect the concentration of iron stores rather

than the absolute amount, in which case it may be more valid to express SF as a ratio of the peripheral blood value to total body size or lean body mass.

Data from other studies to support a true change in iron stores in the population are both limited and inconclusive. SF data from other studies of large groups of healthy males in the United States have not been published. The recent decline in the prevalence of anemia noted among infants has been linked with improved infant feeding practices (22–28) and likely cannot be generalized beyond this age group. The increase in per capita iron content of the U.S. food supply that has occurred in recent years has not been reflected in mean dietary iron intakes from food reported in national surveys (29). The inconsistency between trends in per capita iron availability and dietary iron intake of individuals has been attributed to the difficulty in assessing intake of individuals based on surveys that use different methods over time (29). In addition, data on quantitative nutrient intake from supplements have not been collected in these dietary surveys to date, so total iron intake (from diet and supplements) cannot be assessed. More importantly, estimates of the total amount of bioavailable iron consumed are lacking. Limited trend data suggest use of more bioavailable iron compounds in food fortification (30,31). Whether increased use of bioavailable iron in fortification and enrichment could account for the systematic shift in SF values observed for males from HHANES and the NHANES III pilot studies is unclear. For example, SF levels of Swedish males, collected after 10 years of iron fortification of flour at a level two times higher than in the United States, were similar to those observed for non-Hispanic white males ages 20–44 years in NHANES II (32).

In summary, an explanation of the SF difference between NHANES II and HHANES cannot be conclusively identified because of the retrospective nature of the present study. The lack of evidence for differences in confounding factors or assays between NHANES II and HHANES suggests that iron stores, as reflected by SF, may have improved in the iron-replete segment of the population between surveys. A firm conclusion must await confirmation from other studies. Until further confirmation is available, the validity of comparing SF data from NHANES II with data from HHANES is uncertain. Comparing SF data between subgroups within any one survey are valid, however. In addition, the similarity of the relationships between low HB, impaired iron status (defined by the MCV and ferritin models), and SF between Hispanic and non-Hispanic white females suggests that cutoff values used for NHANES II to define the low SF range are appropriate for HHANES as well. NHANES III plans include an analysis of SF levels of patients with clinically diagnosed iron deficiency anemia to resolve the issue of appropriate cutoff values.

The difficulty in identifying the reason for the higher SF values in HHANES and the NHANES III pilot studies underscores the need to calibrate the ferritin assays performed in different studies across a wide range of SF values. Part of this calibration should involve use of the ICSH reference standard, which is being continued in NHANES III. Additionally, comparison with the monoclonal EIA used at the UK Medical Center (under consideration as an ICSH reference assay) will be continued in NHANES III. Methods for storing standards, assay reagents, and serum samples from study subjects in a stable manner over long time periods need to be developed so that future assay comparisons can be performed if necessary.

In addition, information on factors that can affect the interpretation of SF needs to be collected. For example, in NHANES III, serum AST and bilirubin are being measured on the entire sample ages 12 years and over, which will provide information on liver function for the total sample rather than on a special subsample, as in NHANES II. Measurement of C-reactive protein has also been added to NHANES III to help identify individuals with inflammation. Data on these types of confounding factors will improve interpretation of the SF data by more clearly defining the study sample.

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List of detailed tables

Table 1. Serum ferritin levels of persons 5–19 years of age, by sex, age, and national origin: NHANES II and HHANES

Characteristic	Number of examined persons	Mean	Geometric mean	Percentile				
				5th	25th	50th	75th	95th
Both sexes		Micrograms per liter						
5–10 years:								
NHANES II:								
Non-Hispanic white	365	21.0	[a,b,c]17.3	6	12	19	26	43
Hispanic	50	24.1	20.4	*	12	17	32	*
HHANES:								
Mexican-American	901	30.4	[a]25.1	8	17	26	39	66
Puerto Rican	284	35.4	[b]30.5	12	22	31	42	79
Cuban	79	43.3	[c]38.7	*	30	41	57	*
Male								
11–14 years:								
NHANES II:								
Non-Hispanic white	111	24.0	[a,b,c]17.0	4	10	18	27	59
Hispanic	16	*	*	*	*	17	*	*
HHANES:								
Mexican-American	356	31.0	[a]24.2	6	17	26	37	67
Puerto Rican	126	33.3	[b]28.0	10	18	28	44	73
Cuban	47	46.5	[c]39.6	*	26	37	50	*
15–19 years:								
NHANES II:								
Non-Hispanic white	176	47.3	[a,b,c]34.6	11	21	33	54	95
Hispanic	7	*	*	*	*	*	*	*
HHANES:								
Mexican-American	277	68.8	[a]50.6	13	30	53	85	177
Puerto Rican	165	66.2	[b]53.7	19	34	55	94	143
Cuban	54	85.1	[c]64.1	*	44	67	116	*
Female								
11–14 years:								
NHANES II:								
Non-Hispanic white	111	21.5	18.2	6	14	18	28	47
Hispanic	14	*	*	*	*	23	*	*
HHANES:								
Mexican-American	320	26.2	19.6	5	13	22	34	67
Puerto Rican	128	28.9	21.5	6	14	22	35	75
Cuban	40	33.1	24.6	*	11	31	45	*
15–19 years:								
NHANES II:								
Non-Hispanic white	197	24.2	[a]16.3	3	8	19	33	61
Hispanic	15	*	*	*	*	22	*	*
HHANES:								
Mexican-American	313	27.7	17.6	2	10	21	36	74
Puerto Rican	160	33.4	[a]23.3	3	14	28	45	72
Cuban	40	37.1	24.4	*	14	25	55	*

[a,b,c]Geometric means bearing common superscripts within an age-sex category differ statistically ($p < 0.05$); geometric means bearing different superscripts or lacking a superscript do not differ significantly. Comparisons were not made with serum ferritin values for Hispanic persons from NHANES II.

NOTES: NHANES II is the second National Health and Nutrition Examination Survey, conducted in 1976–80; HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84. Data are weighted.

Table 2. Serum ferritin levels of persons 20–74 years of age, by sex, age, and national origin: NHANES II, HHANES, and NHANES III pilot studies

Characteristic	Number of examined persons	Mean	Geometric mean	Percentile				
				5th	25th	50th	75th	95th
		Micrograms per liter						
Male								
20–44 years:								
NHANES II:								
Non-Hispanic white	555	101.3	[a,b,c]80.5	25	53	88	127	228
Hispanic	41	84.0	70.4	*	44	76	99	*
HHANES:								
Mexican-American	771	148.5	[a]119.7	43	82	126	190	340
Puerto Rican	210	159.6	[b]131.8	40	83	147	213	355
Cuban	132	173.0	[c]148.8	60	99	153	212	367
NHANES III pilot studies:								
All persons	159	160.6	117.8	19	77	137	199	408
Non-Hispanic white	37	181.7	140.0	*	84	152	228	*
Hispanic	27	172.9	109.1	*	64	107	201	*
45–64 years:								
NHANES II:								
Non-Hispanic white	441	126.2	[a,b,c]93.0	19	60	97	161	332
Hispanic	18	*	*	*	*	135	*	*
HHANES:								
Mexican-American	388	201.8	[a]144.7	35	93	159	264	467
Puerto Rican	166	203.9	[b]166.1	48	126	196	249	410
Cuban	180	203.5	[c]153.9	35	104	158	256	412
NHANES III pilot studies:								
All persons	126	215.2	148.7	28	90	176	312	509
Non-Hispanic white	20	*	*	*	103	181	375	*
Hispanic	7	*	*	*	*	*	*	*
65–74 years:								
NHANES II:								
Non-Hispanic white	325	133.3	[a]91.0	19	55	96	166	367
Hispanic	4	*	*	*	*	*	*	*
HHANES:								
Mexican-American	70	192.5	123.3	*	62	117	265	*
Puerto Rican	22	*	*	*	105	188	226	*
Cuban	38	214.7	[a]168.4	*	92	193	308	*
NHANES III pilot studies:								
All persons	39	178.2	114.5	*	74	142	258	*
Non-Hispanic white	23	*	*	*	55	148	232	*
Hispanic	2	*	*	*	*	*	*	*
Female								
20–44 years:								
NHANES II:								
Non-Hispanic white	627	38.5	24.8	4	13	27	47	110
Hispanic	59	44.1	24.0	*	18	30	58	*
HHANES:								
Mexican-American	914	41.4	22.7	2	11	26	56	127
Puerto Rican	378	50.6	31.9	4	18	34	68	147
Cuban	177	45.2	29.0	3	16	37	62	122
NHANES III pilot studies:								
All persons	206	52.5	31.6	3	18	36	74	140
Non-Hispanic white	28	40.1	25.7	*	14	36	55	*
Hispanic	28	51.4	32.4	*	21	35	67	*
45–64 years:								
NHANES II:								
Non-Hispanic white	464	75.2	53.8	11	32	62	102	175
Hispanic	22	*	*	*	27	42	60	*
HHANES:								
Mexican-American	495	105.2	64.4	6	38	82	140	285
Puerto Rican	252	107.1	62.2	6	32	73	141	286
Cuban	197	85.0	55.8	6	39	72	111	229
NHANES III pilot studies:								
All persons	145	101.5	60.6	6	32	70	135	330
Non-Hispanic white	27	101.4	69.1	*	36	84	147	*
Hispanic	9	*	*	*	*	*	*	*
65–74 years:								
NHANES II:								
Non-Hispanic white	344	97.5	69.3	17	44	73	122	311
Hispanic	8	*	*	*	*	*	*	*
HHANES:								
Mexican-American	98	137.4	85.0	*	50	94	156	*
Puerto Rican	49	139.7	101.4	*	60	109	241	*
Cuban	57	122.7	89.0	*	59	104	160	*
NHANES III pilot studies:								
All persons	43	137.6	102.0	*	53	101	191	*
Non-Hispanic white	20	*	*	*	49	110	210	*
Hispanic	3	*	*	*	*	*	*	*

[a,b,c]Geometric means bearing common superscripts within an age-sex category differ statistically ($p < 0.05$); geometric means bearing different superscripts or lacking a superscript do not differ significantly. Comparisons were not made with serum ferritin values for Hispanic persons from NHANES II or for persons from the NHANES III pilot studies.

NOTES: NHANES II is the second National Health and Nutrition Examination Survey, conducted in 1976–80; HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84; the NHANES III (third National Health and Nutrition Examination Survey) pilot studies were conducted in 1987–88. Data from NHANES II and HHANES are weighted; data from the NHANES III pilot studies are unweighted.

Table 3. Levels of selected iron status indicators of persons 20–44 years of age, by sex and national origin: NHANES II, HHANES, and NHANES III pilot studies

Characteristic	Mean	Percentile				
		5th	25th	50th	75th	95th
Male						
Erythrocyte protoporphyrin:			Micromoles per liter red blood cells			
NHANES II:						
Non-Hispanic white	0.84	0.59	0.71	0.80	0.93	1.14
HHANES:						
Mexican-American	0.77	0.50	0.61	0.71	0.84	1.19
Puerto Rican	0.75	0.48	0 61	0.69	0.87	1.16
Cuban	0.78	0.50	0 61	0.66	0.78	0.98
NHANES III pilot studies:						
All persons	0.75	*	0.62	0.73	0.87	*
White	0.73	*	0.62	0.75	0.85	*
Transferrin saturation:			Percent			
NHANES II:						
Non-Hispanic white	30	16	23	29	36	48
HHANES:						
Mexican-American	32	16	23	30	38	54
Puerto Rican	31	14	22	29	38	51
Cuban	33	19	26	32	39	50
NHANES III pilot studies:						
All persons	25	*	19	22	28	*
White	*	*	*	*	*	*
Mean corpuscular volume:			Femtoliters			
NHANES II:						
Non-Hispanic white	89	82	86	89	92	97
HHANES:						
Mexican-American	87	80	84	87	90	94
Puerto Rican	88	81	85	88	90	96
Cuban	87	79	84	87	89	90
Hemoglobin:			Grams per liter			
NHANES II:						
Non-Hispanic white	153	138	147	154	160	170
HHANES:						
Mexican-American	158	142	151	158	165	173
Puerto Rican	153	136	147	153	158	169
Cuban	158	145	152	158	165	174
Hematocrit:			Percent			
NHANES II:						
Non-Hispanic white	0.44	0.40	0.43	0.45	0.46	0.49
HHANES:						
Mexican-American	0.45	0.41	0.44	0.45	0.47	0.49
Puerto Rican	0.45	0.40	0.43	0.45	0.46	0.49
Cuban	0.45	0.41	0.44	0.45	0.47	0.49
NHANES III pilot studies:						
All persons	0.44	0.40	0.43	0.44	0.46	0.49
White	0.44	*	0.42	0.44	0.46	*
Female						
Erythrocyte protoporphyrin:			Micromoles per liter red blood cells			
NHANES II:						
Non-Hispanic white	0.98	0.66	0.80	0 91	1.07	1.44
HHANES:						
Mexican-American	1.03	0.57	0.75	0.91	1.12	1 80
Puerto Rican	0.94	0.57	0.75	0 87	1 01	1.44
Cuban	0.98	0.59	0.71	0 87	1.07	1.62
NHANES III pilot studies:						
All persons	0.91	0.54	0 69	0.82	0.97	1.61
White	*	*	*	*	*	*
Transferrin saturation:			Percent			
NHANES II:						
Non-Hispanic white	27	12	20	26	34	48
HHANES:						
Mexican-American	25	9	17	23	31	44
Puerto Rican	25	11	18	24	30	43
Cuban	26	10	20	25	31	45
NHANES III pilot studies:						
All persons	25	9	18	24	33	44
White	*	*	*	*	*	*

See notes at end of table.

Table 3. Levels of selected iron status indicators of persons 20–44 years of age, by sex and national origin: NHANES II, HHANES, and NHANES III pilot studies—Con.

Characteristic	Mean	Percentile				
		5th	25th	50th	75th	95th
Female—Con.						
Mean corpuscular volume:			Femtoliters			
NHANES II:						
Non-Hispanic white	90	82	87	90	94	99
HHANES:						
Mexican-American	87	78	84	87	90	95
Puerto Rican	87	78	84	88	91	95
Cuban	87	79	84	88	91	95
Hemoglobin:			Grams per liter			
NHANES II						
Non-Hispanic white	135	119	128	135	142	151
HHANES:						
Mexican-American	134	116	128	135	141	151
Puerto Rican	133	116	126	133	140	150
Cuban	136	118	131	136	142	154
Hematocrit:			Percent			
NHANES II:						
Non-Hispanic white	0.40	0.36	0.38	0.40	0.42	0.45
HHANES:						
Mexican-American	0.40	0.35	0.38	0.40	0.42	0.44
Puerto Rican	0.40	0.35	0.38	0.40	0.42	0.45
Cuban	0.40	0.35	0.39	0.40	0.41	0.44
NHANES III pilot studies:						
All persons	0.39	0.34	0.37	0.39	0.41	0.44
White	0.38	*	0.37	0.38	0.41	*

NOTES: NHANES II is the second National Health and Nutrition Examination Survey, conducted in 1976–80; HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84; the NHANES III (third National Health and Nutrition Examination Survey) pilot studies were conducted in 1987–88. Data from NHANES II and HHANES are weighted; data from the NHANES III pilot studies are unweighted.

Table 4. Serum ferritin values of females 20–44 years of age with low hemoglobin or impaired iron status, by national origin: NHANES II and HHANES

Indicator, survey, and national origin	Number in sample	Mean micrograms per liter	Percent with SF less than 12 micrograms per liter
Low hemoglobin[1]			
NHANES II:			
Non-Hispanic white	66	32.7	45
HHANES:			
Mexican-American	69	16.4	65
Puerto Rican	30	33.5	50
Cuban	10	*	*
Impaired iron status[2]			
NHANES II:			
Non-Hispanic white	45	17.9	76
HHANES:			
Mexican-American	91	14.6	70
Puerto Rican	24	25.5	58
Cuban	15	*	*

[1]Less than 119 grams per liter.
[2]According to MCV model, which is based on mean corpuscular volume, transferrin saturation, and erythrocyte protoporphyrin.

NOTES: SF is serum ferritin. NHANES II is the second National Health and Nutrition Examination Survey, conducted in 1976–80; HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84. Data are unweighted.

Table 5. Percent of females 20–44 years of age with low serum ferritin or impaired iron status who have low hemoglobin, by national origin: NHANES II and HHANES

Indicator, survey, and national origin	Number in sample	Percent with low hemoglobin[1]
Low serum ferritin[2]		
NHANES II:		
Non-Hispanic white	127	24
HHANES:		
Mexican-American	234	22
Puerto Rican	59	29
Cuban	30	27
Impaired iron status[3]		
NHANES II:		
Non-Hispanic white	72	36
HHANES:		
Mexican-American	145	30
Puerto Rican	32	41
Cuban	21	*

[1]Less than 119 grams per liter.
[2]Less than 12 micrograms per liter.
[3]According to ferritin model, which is based on serum ferritin, transferrin saturation, and erythrocyte protoporphyrin.

NOTES: NHANES II is the second National Health and Nutrition Examination Survey, conducted in 1976–80; HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84. Data are unweighted.

Table 6. Median serum ferritin of Hispanic males 20–74 years of age, by serum bilirubin or serum aspartate aminotransferase, age, and national origin: HHANES

Serum analyte, age, and national origin	All persons	Total minus persons with analyte greater than manufacturer's normal range[1]	Total minus persons with analyte greater than NHANES I 95th percentile[2]	Total minus persons with analyte greater than NHANES I 75th percentile[3]
Total bilirubin		Micrograms per liter		
20–44 years:				
Mexican-American	126.3	126	124	114
Puerto Rican	146.5	149	149	147
Cuban	153.2	159	159	134
45–64 years:				
Mexican-American	158.8	149	146	133
Puerto Rican	196.2	204	204	192
Cuban	157.8	157	157	139
65–74 years:				
Mexican-American	116.9	116	116	117
Puerto Rican	187.6	187	214	214
Cuban	193.4	193	188	117
Aspartate aminotransferase				
20–44 years:				
Mexican-American	126.3	120	120	99
Puerto Rican	146.5	121	124	124
Cuban	153.2	159	136	112
45–64 years:				
Mexican-American	158.8	149	149	119
Puerto Rican	196.2	192	192	217
Cuban	157.8	146	151	142
65–74 years:				
Mexican-American	116.9	100	100	74
Puerto Rican	187.6	187	187	227
Cuban	193.4	186	155	186

[1]22.23 micromoles per liter total bilirubin and 22 units per liter aspartate aminotransferase.
[2]18.8 micromoles per liter total bilirubin and 21 units per liter aspartate aminotransferase.
[3]12.0 micromoles per liter total bilirubin and 14 units per liter aspartate aminotransferase.

NOTES: HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84; NHANES I is the first National Health and Nutrition Examination Survey, conducted in 1971–75. Data are weighted.

Table 7. Serum ferritin levels of persons 20–74 years of age, by poverty status, sex, age, and national origin: NHANES II and HHANES

Characteristic	Poor				Nonpoor			
	Number of examined persons	Mean	Geometric mean	Median	Number of examined persons	Mean	Geometric mean	Median
				Micrograms per liter				
Male								
20–44 years:								
NHANES II:								
Non-Hispanic white	44	86.0	67.0	66	495	102.3	81.4	89
HHANES:								
Mexican-American	163	137.2	110.8	107	551	151.0	121.9	131
Puerto Rican	63	145.8	118.0	129	137	167.6	138.6	150
Cuban	18	*	*	141	107	177.7	152.0	164
45–64 years:								
NHANES II:								
Non-Hispanic white	34	92.2	71.3	72	491	129.4	96.1	103
HHANES:								
Mexican-American	86	181.7	133.7	134	262	207.6	148.9	171
Puerto Rican	54	182.6	143.3	162	106	215.6	178.1	207
Cuban	24	*	*	135	143	212.2	159.1	170
65–74 years:								
NHANES II:								
Non-Hispanic white	44	128.3	95.6	93	271	134.3	90.4	96
HHANES:								
Mexican-American	28	197.5	123.9	110	36	189.4	115.8	114
Puerto Rican	6	*	*	*	13	*	*	187
Cuban	12	*	*	156	25	240.5	186.6	234
Female								
20–44 years:								
NHANES II:								
Non-Hispanic white	82	32.7	23.1	27	524	39.0	24.9	27
HHANES:								
Mexican-American	256	38.9	21.0	24	589	42.9	23.9	27
Puerto Rican	168	48.8	27.6	30	188	52.3	35.5	37
Cuban	43	35.2	21.1	30	124	48.9	32.5	38
45–64 years:								
NHANES II:								
Non-Hispanic white	44	63.9	45.9	57	401	76.4	54.7	62
HHANES:								
Mexican-American	136	103.7	64.9	81	295	103.3	62.9	81
Puerto Rican	127	102.7	59.8	72	109	108.1	68.3	82
Cuban	32	94.4	69.1	72	149	84.6	56.2	72
65–74 years:								
NHANES II:								
Non-Hispanic white	63	80.0	61.4	64	257	101.1	72.2	80
HHANES:								
Mexican-American	49	150.2	90.8	98	37	135.1	87.7	75
Puerto Rican	28	127.3	89.2	104	17	*	*	151
Cuban	18	*	*	96	28	132.3	95.2	111

NOTES: NHANES II is the second National Health and Nutrition Examination Survey, conducted in 1976–80; HHANES is the Hispanic Health and Nutrition Examination Survey, conducted in 1982–84. Data are weighted.

Appendix
Glossary of abbreviations used in this report

AST—aspartate aminotransferase
EIA—enzyme-linked immunoassay
EP—erythrocyte protoporphyrin
HANES—Health and Nutrition Examination Surveys
HB—hemoglobin
HCT—hematocrit
HHANES—Hispanic Health and Nutrition Examination Survey
ICSH—International Committee for Standardization in Hematology
IRMA—immunoradiometric assay
MCV—mean corpuscular volume
NHANES I—first National Health and Nutrition Examination Survey
NHANES II—second National Health and Nutrition Examination Survey
NHANES III—third National Health and Nutrition Examination Survey
RBC—red blood cell count
SF—serum ferritin
SI—serum iron
TIBC—total iron-binding capacity
TS—transferrin saturation
UK—University of Kansas
μg/L—micrograms per liter

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