Summary of a report on assessment of the iron nutritional status of the United States population\textsuperscript{1–3}

Expert Scientific Working Group

ABSTRACT This report summarizes the evaluations by an Expert Scientific Working Group of the iron nutritional status of the US population based on biochemical data for persons aged 1 through 74 yr in the second National Health and Nutrition Examination Survey, 1976–1980. Three approaches were used for estimating the prevalence of impaired iron status. The first (ferritin model) required that at least two of three indicators be abnormal: serum ferritin, transferrin saturation, and erythrocyte protoporphyrin. A second approach (MCV model), using mean corpuscular volume rather than ferritin, also required that at least two of the three indicators be abnormal. Finally, the change in median hemoglobin concentration (hemoglobin percentile shift) was determined after exclusion of individuals with one or more abnormal iron status values. The range of prevalence estimates was fairly low with the exception of children aged 1–2 yr, males aged 11–14 yr, and females aged 15–44 yr. The associations of impaired iron status with hemoglobin levels, inflammatory disease, and socioeconomic and demographic variables were examined. Data on iron overload were also assessed. \textit{Am J Clin Nutr} 1985;42:1318–1330.

KEY WORDS Nutrition survey, iron nutrition, iron deficiency, nutritional assessment

Introduction

Iron deficiency is generally acknowledged to be the most common, single nutritional deficiency in both developing and developed countries. Concern about high estimates of the prevalence of iron deficiency in the US (1, 2, 3) led the Food and Nutrition Board to recommend in 1974 that the level of iron fortification in cereal grain products be increased (4). The Food and Drug Administration (FDA) has responsibility for assessing the public health need for and safety of fortification of food products with nutrients. Accordingly, the FDA’s Center for Food Safety and Applied Nutrition requested that the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) assemble an ad hoc Expert Scientific Working Group\textsuperscript{4} (ESWG) to review the most recent, relevant, scientific data on the iron, zinc, and folate nutritional status of the US population. The principal data examined were derived from the hematological, biochemical, and clinical studies conducted

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during the second National Health and Nutrition Examination Survey (NHANES II), 1976–1980. This report summarizes the observations on the prevalence of iron deficiency and evidence of iron overload, and the analyses that undergird them. The full ESWG reports on iron (5), zinc (6), and folate (7) nutritional status have been published.

The NHANES II was designed to supply health and nutritional information for the civilian noninstitutionalized population of the US aged 6 mo through 74 yr. The survey protocol included questionnaires to elicit information on demographic variables, behavior, medical history, diet, and use of medications and dietary supplements; medical examination by a physician; special clinical procedures including anthropometry, spirometry trials, electrocardiograms, audiometry, speech recording, and allergy tests; x-ray examinations of cervical and lumbar spine and chest; urine tests; and tests on blood samples (8). Some hematological tests were performed on site at mobile examination centers, but the bulk of the samples were frozen and shipped to the Centers for Disease Control in Atlanta, GA for biochemical assessments. The sample design of NHANES II was a stratified, multistage, probability cluster of households throughout the US. Groups in the population thought to be at special risk for impaired nutritional status were oversampled to improve the statistical reliability of data for these groups. The sampling plan identified 27,803 subjects of whom 20,322 were examined at 64 locations throughout the US. Not every individual underwent all the tests and procedures, and some of the tests were restricted to subgroups of the survey population. Since each examined person represents a variable number of persons in the general population, a complex weighting procedure with consideration of design effects is required to ensure valid statistical treatment of NHANES II data (9). Applying calculations and statistical tests appropriate for a simple random sample to the NHANES II data can lead to serious misinterpretation.

Interpretation of the analyses of impaired iron status presented here is based on the theoretical model of iron deficiency in which the onset is usually gradual (10). When iron requirement is increased or intake declines, absorption of iron concomitantly increases, but most of the iron needed for hemoglobin synthesis and other functions must be mobilized from body stores. The decrease in tissue iron stores is accompanied by a parallel decrease in the amount of ferritin in plasma or serum. When stores are virtually depleted transferrin saturation is decreased, leading to a diminished supply of iron to the erythroid precursors, the development of features characteristic of iron-restricted erythropoiesis, and the accumulation of free protoporphyrin in the circulating red cells. At this stage, hemoglobin levels may decline slightly, usually remaining within normal limits although there is an increase in the number of microcytic cells present in the peripheral blood. Eventually, as normal cells are replaced, the hemoglobin levels drop to subnormal values and the number of microcytic erythrocytes is great enough to produce a decrease in the mean corpuscular volume (MCV).

Three stages of iron deficiency may be recognized. The first is a substantial reduction in the normal iron stores which average about 1000 mg in the adult male and 300 mg in the adult menstruating female (11). The plasma or serum ferritin has proved a useful assay of storage iron in normal subjects. When iron stores are depleted and before anemia may be identified by a decrease in hemoglobin below the normal limits, the second stage of iron deficiency, a state of iron-deficient erythropoiesis, exists. This moderate degree of iron deficiency may be recognized by assay of plasma iron supply to erythropoietic cells (reflected by decreases in transferrin saturation) and of iron availability for hemoglobin synthesis (reflected by increases in erythrocyte protoporphyrin). The third and most severe degree of iron deficiency involves overt microcytic anemia. This latter stage may be identified by a decrease in hemoglobin concentration (or hematocrit) and a decrease in MCV. Although the status of the iron stores is of obvious importance in relation to iron nutrition, the degree of iron depletion characterized above as iron-deficient erythropoiesis has usually been taken to indicate iron deficiency. Hence, it may be identified by that level of serum ferritin indicating depleted iron stores as well as by

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abnormal levels of transferrin saturation (low) and erythrocyte protoporphyrin (elevated).

No single biochemical indicator currently available is consistently diagnostic of iron deficiency. The use of several indicators of iron status provides a much better assessment. For example, in a study by Cook et al (12), the prevalence of anemia among individuals with only one abnormal index of iron metabolism (ferritin, transferrin saturation, or erythrocyte protoporphyrin) was found to be 10.9%, only slightly higher than the 8.3% found in the entire population studied. However, anemia was found in 28% of the individuals with two abnormal indicators and 63% of those with three abnormal indicators (12). For the analyses here, the presence of two or more abnormal values for iron status indicators was considered indicative of impaired iron status with acknowledgement that the indicators can be affected by inflammation as well as iron deficiency.

Two different models were used for the analyses of the prevalence of abnormal values indicative of impaired iron status in NHANES II. The first, termed the ferritin model, employed serum ferritin, transferrin saturation, and erythrocyte protoporphyrin as indicators. The second, termed the MCV model, employed MCV, transferrin saturation, and erythrocyte protoporphyrin as indicators. The sample size available for use with the ferritin model is relatively small, because ferritin was measured in a subsample (see below), but large enough to permit calculation of the national probability estimates for most age/sex groups. With the MCV model, the data from the entire NHANES II population may be used. Using the ferritin sample, a four-variable model could be constructed by adding the MCV determination but exploratory results with such a model suggested that it did not improve discrimination.

To assess the relative prevalence of anemia associated with or due to impaired iron status, the hemoglobin percentile shift model was used (13). Comparisons are then made of the prevalence estimates for impaired iron status and anemia obtained using the three different but interrelated models, and conclusions are drawn concerning the prevalence of iron deficiency (and iron overload) in the various segments of the US population.

Methods

Methods employed in determining iron status indicators

Venous blood samples from 18,693 persons and 292 capillary blood samples (from children <5 yr old if two attempts at venipuncture were unsuccessful) were collected. Data on hemoglobin, hematocrit, red blood cell count, white blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration for persons aged 6 mo through 74 yr, and erythrocyte protoporphyrin, serum iron, total iron-binding capacity, and transferrin saturation for persons aged 3 through 74 yr in NHANES II have been published (14). Data obtained from capillary blood samples have been eliminated from the present report.

Hemoglobin. Hemoglobin determinations were made for 18,981 persons aged 6 mo through 74 yr by the Coulter hemoglobinometer at the mobile examination centers. Various definitions of normal and low hemoglobin concentrations have been used to recognize the important differences associated with age and sex. One approach to generating cutoff values for hemoglobin employed in the present study was to exclude persons with evidence of iron deficiency and chronic disease to obtain the distribution of hemoglobin values of ostensibly normal persons as described by Dallman et al (13). Values below the lower limit of the 95 percent confidence interval (ie, below the 2.5th percentile) of this distribution were considered low and were used for some of the analyses recorded later. Because of the wide range of hemoglobin concentrations and the overlap of values in normal, nonanemic persons and iron-deficient individuals, any cutoff value chosen will necessarily result in some misclassifications. There is evidence that the number of iron-deficient individuals hidden within the normal range of hemoglobin concentrations is probably as great as the number of iron-deficient individuals who can be recognized as anemic (15). If such individuals had concentrations of hemoglobin that were unrestricted by lack of iron, those hemoglobin values might have fallen into a higher portion of the distribution curve. Hemoglobin levels may be reduced in conditions other than nutritional iron deficiency, such as infection and inflammation, hemorrhage, thalassemia and other hemoglobinopathies, folate or vitamin B12 deficiency, and pregnancy.

Mean corpuscular volume (MCV). Values for MCV were obtained for 18,801 persons aged 6 mo through 74 yr. The MCV was calculated by dividing the hematocrit (determined from the spun microhematocrit) by the red cell count (performed on the Coulter Model FN Counter). A cutoff of 80 fl is accepted as the lower limit of normal MCV in adults (16). The approach used in selecting the optimal cutoff values for low MCV in children of various ages is described later and use of age-specific cutoffs is supported by observations in the literature (17–19). A subnormal value of MCV is observed when iron deficiency becomes severe and is a fairly specific indicator of iron deficiency once thalassemia trait (which was expected to be rare in the NHANES II white population) and the anemia of chronic disease have been excluded.

Serum iron, total iron-binding capacity (TIBC), and transferrin saturation. Serum iron concentration was determined for 17,652 persons, TIBC was determined for 15,693 persons, and transferrin saturation was calculated.
for 15,661 persons aged 6 mo through 74 yr. Serum iron was assayed by a modification of the automated Technicon AAI-25 method (Technicon Instruments Corp., Tarrytown, NY), employing ferrozine as chromogen, and measured spectrophotometrically at 562 nm (20). The TIBC was determined by saturating serum with excess iron and adding magnesium carbonate to remove the iron not bound to serum transferrin. The bound iron remaining in the supernatant after centrifugation was then measured as above. Transferrin saturation was calculated as the ratio of serum iron to TIBC. If values for serum iron or TIBC were extremely high or extremely low, the samples were reasayed to confirm the extreme values. Because serum iron was given a higher priority for reassay, samples were sometimes exhausted before the TIBC value could be confirmed, accounting for a disproportionate number of missing TIBC values and missing transferrin saturation values for samples with high or low serum iron values. Serum iron is known to exhibit marked diurnal variations but does not peak at the same time of day in all individuals (21–26). Serum iron and TIBC also exhibit substantial day-to-day variations (27). Because of the constraints of the survey design, blood samples were drawn at various times of the day. Although transferrin saturation values of NHANES II samples collected in the morning tended to be higher than those of samples collected in the afternoon, the time of sample collection did not have a significant effect on the analyses of prevalence of abnormal values for iron status indicators. A transferrin saturation <16% in adults is widely regarded as indicative of iron-deficient erythropoiesis (28). Since children and infants seem to exhibit age-related differences in the normal level of transferrin saturation (18, 29, 30), cutoff values for children were determined by a procedure described later. Transferrin saturation declines rapidly in response to infection or inflammation as well as iron deficiency (31).

Erythrocyte protoporphyrin. Erythrocyte protoporphyrin levels were measured for 18,228 persons aged 6 mo through 74 yr. The protoporphyrin was measured fluorometrically in a Perkin Elmer model MFP-2A spectrophotometer (Norwalk, CT) at excitation and emission wavelengths of 404 and 655 nm, and expressed as units per dl of red blood cells (RBC) (20). The information provided by changes in erythrocyte protoporphyrin is somewhat comparable to that of the transferrin saturation, but erythrocyte protoporphyrin is more stable and responds more gradually to changes in iron supply to the marrow. Erythrocyte protoporphyrin is not useful in distinguishing iron deficiency from infection and is also elevated in response to lead toxicity (32–34). However, in NHANES II the inclusion of children aged 1–4 yr with elevated erythrocyte protoporphyrin values in association with high blood lead values did not affect the estimates of the prevalence of impaired iron status. In adults a protoporphyrin level greater than 70 μg/dl of RBC has been found to correspond to a transferrin saturation of less than 16% (35). The basis for the choice of cutoff values used for children in this report is described later.

Serum ferritin. Serum ferritin values were obtained for 5,157 persons aged 3 through 74 yr. This ferritin sample included a subgroup of NHANES II population selected as follows: i) all persons whose NHANES II numbers ended in 8, ii) persons with one or more abnormal hematologic values (RBC, hemoglobin, hematocrit, MCV, WBC), and iii) persons whose serum samples were randomly chosen at the end of the survey for ferritin analysis. In addition to serum ferritin determination, evaluations of blood smears and assessments of serum and RBC folate, and serum vitamin B12 were also performed for groups i and ii described above. Despite the unusual selection procedure for the ferritin sample, application of the appropriate weighting factors to the results yields serum ferritin data representative of the US population at the time of the survey. Serum ferritin values in the NHANES II samples were measured at the University of Kansas Medical Center employing the two-site immunoradiometric assay (36) run in triplicate. Standards were run in quadruplicate with each assay and appropriate dilutions were reasayed when sample values were >200 ng/ml or <20 ng/ml. In normal adults the concentration of serum ferritin parallels the total amount of storage iron such that, in the range of 20–300 ng/ml, 1 ng/ml serum ferritin is equivalent to approximately 10 mg of storage iron (37). A decline in serum ferritin is a very early indicator of developing iron deficiency. In adults values of <12 ng/ml are considered to indicate depletion of iron stores (38), and in children the cutoff value of 10 ng/ml has been suggested (18, 39). Serum ferritin values remain relatively stable day to day but tend to increase in response to infection, malignancy, and liver disease (37, 40). Extremely high serum ferritin concentrations are useful in assessing the possible presence of iron overload disorders.

Methods employed for data analysis in prevalence determinations

Reference data. For purposes of identifying indicators of impaired iron status, a reference sample was formed by excluding persons who had capillary blood samples collected by finger stick; pregnant women and women who reported they had been pregnant within the preceding yr; and persons with white cell counts <3.4 or >11.5 × 10⁹/l, protoporphyrin values >70 μg/dl, transferrin saturation <16%, or MCV <80 or >96 fl. These cutoff values used to form the reference sample are, of course, not appropriate for characterizing iron deficiency in children and more appropriate cutoff values for these have been presented elsewhere. Tabulated data on hemoglobin, MCV, erythrocyte protoporphyrin, transferrin saturation, and serum ferritin levels for the reference sample are given in the full report on iron status (5).

Choice of cutoffs for abnormal values of iron status indicators. Detection of nutritional iron deficiency when the deficiency may be mild has proved difficult and it is readily apparent that a single abnormal value for the indicators currently available may be misleading. In the literature, values associated with iron deficiency in adults include serum ferritin <12 ng/ml (38), transferrin saturation <16% (28), erythrocyte protoporphyrin >70 μg/dl RBC (35), and MCV <80 fl (16, 41). For the most part, normal values for iron status indicators have been derived from studies of healthy young men, a population with a demonstrably low prevalence of iron deficiency. The normal values found in young men have conventionally been regarded as also desirable for women and adults of all ages. Although there are physiologic differences in iron balance between men and women, there is no evidence that the availability of iron for erythropoiesis is reflected differently by the available indices of iron nutritional status. However, there is substantial evidence (6, 17, 19, 30, 42) of age-related changes in iron status indicators in infancy, child-
hood, and adolescence, based on physiologic differences in iron metabolism in children. Recognizing that for any given determination of nutritional status there exists an overlap between individuals who have the deficiency and individuals falsely identified, an appropriate cutoff can be chosen by knowing either how many individuals are correctly identified as deficient or how many individuals are falsely identified. The approach used to select cutoff values for children and adolescents was one that attempted to keep the percentage of falsely identified individuals similar to that of the young women, a group expected to have a fairly high prevalence of iron deficiency. Falsey identified individuals were defined as those who had an abnormal value for the indicator being examined but had normal values for all other indicators, that is, for three of the following: hemoglobin, MCV, protoporphyrin, transferrin saturation. The determination of normal values for the indicators used in this analysis was based on the reference population defined earlier and required a hemoglobin value greater than the tenth percentile, MCV greater than the fifth percentile, erythrocyte protoporphyrin less than the ninetieth percentile, and transferrin saturation greater than the tenth percentile. Values were smoothed across age/sex groups to give a graduation by age and the same values were used for males and females ages 1 through 14 yr. Cutoff values for serum ferritin for children were not selected in this fashion; rather, the previously reported value of less than 10 ng/ml was employed (18, 39). The cutoff values derived for children and adolescents, together with those selected for adults, are shown in Table 1 and were used for the prevalence analyses in this report.

Employing these cutoff values the ferritin and MCV models described above were used to determine prevalence values for two or three abnormal iron status indicators. Some difference between the two models might be expected because the first involved the serum ferritin measurement, taken to indicate depletion of iron stores (an early change), whereas the second model involved the MCV, taken to indicate impaired erythropoiesis (a late change). Because of the small number of subjects, prevalence analyses were not conducted for infants aged 6 mo–1 yr.

Hemoglobin percentile shift method used to assess relative prevalence of anemia

The change or shift in the median hemoglobin concentration (50th percentile) of a population after excluding individuals with one or more abnormal values for iron status indicators is called the hemoglobin percentile shift (13). The lower median value in the original population as compared to that of the reference population is attributed to the presence of subjects with depressed hemoglobin values resulting from iron deficiency or disease. The additional percentile value (over 50) which the median value of the reference population marks in the original distribution is taken as the relative prevalence of anemic subjects in the population. Although the excluded population contains many persons with relatively high concentrations of hemoglobin who are probably not iron deficient, these incorrectly identified persons are assumed to be distributed randomly in the excluded group and make little contribution to the shift in the distribution. The shift in distribution to a higher median hemoglobin value is produced by the exclusion of subjects with one or more abnormal values for iron status indicators, most of whom have hemoglobin values lower than the median of the original distribution. For the analysis reported here, the reference population was formed by excluding persons with transferrin saturation < 16% or erythrocyte protoporphyrin > 70 µg/dl RBC. Results of similar analyses using three criteria (including MCV) were not significantly different from those reported here using only two criteria for exclusion (13, 39). Although this procedure essentially categorizes persons as iron deficient who may have only one abnormal value for iron status indicators, it was more important for the hemoglobin percentile shift model to exclude all persons who might be iron deficient than to identify correctly the iron-deficient individuals as was done with the ferritin and MCV models. For the same reason, the use of a single cutoff value rather than age-specific cutoffs is possible. The ferritin model would be expected to give the highest prevalence because it depends on an indicator of early reduction in stores and the MCV model to give a lower value because it depends on late red cell changes. The hemoglobin percentile shift model would be expected to yield a value more similar to that given by the MCV model than by the ferritin model.

Results

Prevalence of two or three abnormal values for iron status indicators assessed by the two models

Results of the analysis of the prevalence of two or three abnormal values using the ferritin model (serum ferritin, transferrin saturation, and erythrocyte protoporphyrin) for persons aged 3–74 yr in the ferritin sample, and using the MCV model (MCV, transferrin saturation, and erythrocyte protoporphyrin) for persons

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Serum ferritin (ng/ml)</th>
<th>Transferrin saturation (%)</th>
<th>Erythrocyte protoporphyrin (µg/dl RBC)</th>
<th>MCV (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–2</td>
<td>—</td>
<td>&lt;12</td>
<td>&gt;80</td>
<td>&lt;73</td>
</tr>
<tr>
<td>3–4</td>
<td>&lt;10</td>
<td>&lt;14</td>
<td>&gt;75</td>
<td>&lt;75</td>
</tr>
<tr>
<td>5–10</td>
<td>&lt;10</td>
<td>&lt;15</td>
<td>&gt;70</td>
<td>&lt;76</td>
</tr>
<tr>
<td>11–14</td>
<td>&lt;10</td>
<td>&lt;16</td>
<td>&gt;70</td>
<td>&lt;78</td>
</tr>
<tr>
<td>15–74</td>
<td>&lt;12</td>
<td>&lt;16</td>
<td>&gt;70</td>
<td>&lt;80</td>
</tr>
</tbody>
</table>
aged 1-74 yr in the entire NHANES II pop-
ulation are shown in Figure 1. The highest
prevalences of abnormal values using the fer-
rinin model were found in nonpregnant
women aged 15-19 yr (14.2%), males aged 11-
14 yr (12.1%), and nonpregnant women aged
20-44 yr (9.6%). The prevalence was low
(<2%) in males of all age groups 15 yr and
older. The ferritin model could not be applied
to infants aged 1-2 yr because ferritin was not
measured in this age group. Using the MCV
model, the highest prevalence of abnormal
values was found in children aged 1-2 yr
(9.4%). Relatively high prevalences (4.5 to
5.4%) were observed also in women aged 15-
64 yr. In children <15 yr of age and in women
<65 yr of age, the prevalences of abnormal
values obtained using the ferritin model were
higher than those obtained using the MCV
model. The greatest differences were seen in
groups with relatively high prevalence esti-
mates of abnormal values and the greatest
numbers of low serum ferritin values (ie, males
aged 11-14 yr, females aged 15-19 yr, and
females aged 20-44 yr). The failure of the fer-
rinin model to yield greater prevalence esti-
mates than the MCV model in the elderly may
reflect increasing influences of factors other
than iron deficiency (such as inflammation
and chronic disease) in the older groups. The
number of pregnant women aged 15-19 yr was
not large enough to permit a reliable prevai-
ience estimate. For the 61 pregnant women
aged 20-44 yr in the ferritin sample, the pre-
valence of abnormal values was 25.5 ± 8.3%,
and for the 91 pregnant women aged 20-44
yr in the MCV sample, the prevalence of ab-
normal values was 10.7 ± 3.5%. These data
were obtained from women at varying stages
of pregnancy. Although the occurrence of two
or three abnormal values for iron status vari-
able in an individual provides presumptive
evidence of impaired iron status, such an oc-
currence cannot be taken as the basis for a
definitive diagnosis of iron deficiency. Erythro-
cytic protoporphyrin, transferrin saturation,
and to a lesser extent MCV, do not distinguish
between changes resulting from iron deficiency
and the effects of infection or inflammation.

Prevalence of anemia assessed by the
hemoglobin percentile shift model

The percentile shift method of evaluating
iron status is dependent on changes in he-
moglobin concentration after excluding per-
sons with abnormal transferrin saturation or
erthrocyte protoporphyrin. Therefore, this
method might be expected to be less sensitive
than the biochemical indicators previously
discussed.

The relative prevalence estimates of anemia
by the hemoglobin percentile shift (also shown
in Fig 1) are highest for the children aged 1-2
yr (9.2%), the males aged 11-14 yr (5.4%),
and the females aged 20-44 yr (4%); the pre-
valence is close to zero for the males aged 15-
44 yr and ranges from 2 to 3.8% for the re-
main ing age/sex groups.

Comparison of the prevalence estimates of
impaired iron status derived using the three
different models

Figure 1 illustrates the extent of agreement
in the various age/sex groups among the esti-
mates of the prevalence of impaired iron sta-
tus (resulting from iron deficiency and/or in-
fiammatory disease) given by the three models;
especially close are the estimates given by the
MCV model and the hemoglobin percentile
shift model. Relatively large differences are
seen, however, in the males aged 11-14 yr and
the females aged 15-19 yr. Both these groups
have a high prevalence of low ferritin values
and relatively small sample size compared to
other sex/age groups.

Association of low hemoglobin
concentrations with abnormal values for iron
status indicators

The association of low hemoglobin concen-
trations with zero, only one, and two or three
abnormal values for iron status indicators in
groups of subjects was determined using both
the ferritin and MCV models (Tables 2 and
3). The operational definition of low hemo-
globin used for the present analyses was a value
below the 95% confidence interval for each
age/sex group in the reference sample formed
by excluding persons with evidence of iron de-
iciency. These analyses were restricted to
whites because the question of whether sepa-
rate hemoglobin criteria are required for blacks
and whites has not yet been resolved and be-
cause the number of black subjects included
in the survey was too small for reliable deter-
mation of the 95% confidence interval. In
some instances, age/sex groups had to be
combined to obtain enough persons with ab-

normal values to permit determination of the prevalence of low hemoglobin concentrations. The increase in the prevalence of low hemoglobin values with increasing numbers of abnormal values for iron status indicators supports the usefulness of the ferritin and MCV
TABLE 2
Percent* of white persons with low hemoglobin concentrations† in iron status groups as defined by two models:

<table>
<thead>
<tr>
<th>Sex and Age</th>
<th>Ferritin model‡</th>
<th>MCV model§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 Normal values</td>
<td>1 Abnormal value</td>
</tr>
<tr>
<td>Males and females</td>
<td>2.5 (361)*</td>
<td>2.8 (222)</td>
</tr>
<tr>
<td>Females 20–44**</td>
<td>2.2 (434)</td>
<td>17.6 (84)</td>
</tr>
<tr>
<td>Males 45–74</td>
<td>2.0 (613)</td>
<td>7.4 (126)</td>
</tr>
<tr>
<td>Females 45–74</td>
<td>2.0 (598)</td>
<td>3.7 (183)</td>
</tr>
</tbody>
</table>

* All statistics are weighted to represent the US population at the midpoint of the survey (March 1, 1978) by a method that accounts for the complex survey design.
† See text for criteria for low hemoglobin concentrations.
‡ The ferritin model employs serum ferritin, transferrin saturation, and erythrocyte protoporphyrin as variables. See Table 1 for age-specific cutoffs used in the analysis.
§ The MCV model employs MCV, transferrin saturation, and erythrocyte protoporphyrin as variables. See Table 1 for age-specific cutoffs used in the analysis.
‖ Indicates a statistic which may be unreliable because of small sample size.
¶ The value in parentheses is the number of examined persons.
** Pregnant women are excluded.

models for identifying populations with impaired iron status (Table 2). Most persons in the populations so identified do not have an impairment in iron status severe enough to produce low hemoglobin levels. In addition, the data in Table 3 illustrate that in many women low hemoglobin values were not associated with abnormal values for iron status indicators. This finding is occasioned by the fact that the definition of low hemoglobin required classifying 2.5% of an ostensibly normal population as having low values. Of the women with hemoglobin values ≥ 11.9 g/dl only 8% and 3%, for the ferritin and MCV

TABLE 3
Number and percent* of white females† aged 20–44 yr with low and normal hemoglobin concentrations‡§, by iron status as defined by two models: NHANES II, 1976–1980

<table>
<thead>
<tr>
<th>Hemoglobin &lt; 11.9 g/dl</th>
<th>Hemoglobin ≥ 11.9 g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron status</td>
<td>Ferritin model‡</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>3 Normal values</td>
<td>23</td>
</tr>
<tr>
<td>1 Abnormal values</td>
<td>22</td>
</tr>
<tr>
<td>2 or 3 Abnormal values</td>
<td>33</td>
</tr>
<tr>
<td>Totals</td>
<td>78</td>
</tr>
</tbody>
</table>

* The percent is weighted to represent the US population at the midpoint of the survey (March 1, 1978) by a method that accounts for the complex survey design.
† Pregnant women are excluded.
‡ Low hemoglobin concentration is defined as <11.9 g/dl (see text); normal hemoglobin concentration is defined as ≥11.9 g/dl.
§ The ferritin model employs serum ferritin, transferrin saturation, and erythrocyte protoporphyrin as variables. See Table 1 for age-specific cutoffs used in the analysis.
¶ The MCV model employs MCV, transferrin saturation, and erythrocyte protoporphyrin as variables. See Table 1 for age-specific cutoffs used in the analysis.
models, respectively, had two or three abnormal values for iron status indicators.

If persons having both low hemoglobin concentration, as defined above, and two or three abnormal values for iron status indicators may be considered to have iron-deficiency anemia, the prevalence of iron-deficiency anemia in white, nonpregnant women aged 20–44 yr can be calculated to be 1.6% when the ferritin model is used and 1.7% when the MCV model is used.

Contribution of inflammatory disease to estimates of prevalence of abnormal values for iron status indicators

The contribution of inflammatory disease to the estimates of prevalence of abnormal values for iron variables was assessed by determining the percentages of persons classified as having two or three abnormal values using the MCV model who also had serum ferritin > 50 ng/ml. Serum ferritin levels have been reported to increase somewhat (>50 ng/ml) in patients with inflammation alone (43, 44) but usually remain <50 ng/ml in patients with both iron deficiency and inflammation (40). This analysis must be interpreted with caution because of the small sample size. Less than 10% of the females aged 20–44 yr identified as having impaired iron status using the MCV model had a serum ferritin > 50 ng/ml; 67% had serum ferritin values < 12 ng/ml. The proportion of serum ferritin values > 50 ng/ml (presumably representing an inflammatory state) increased to 50% in males and 34% in females aged 45–74 yr; only 34% of males and 43% of females aged 45–74 yr with two or three abnormal values had serum ferritin values < 12 ng/ml. These results suggest that inflammation may have contributed more to the prevalence of abnormal values for iron status indicators in groups over 45 yr than in the younger group.

Prevalence of abnormal values for iron status indicators in groups with selected characteristics

Using the ferritin model for persons aged 15–74 yr and the MCV model for all age groups, variations in the prevalence of two or three abnormal values for iron status indicators associated with race, poverty status, level of education, or parity were determined. Because of large differences in sample size, interpretations and comparisons must be made with caution.

Overall, blacks tended to have a higher prevalence of abnormal values than did whites. Using the ferritin model, the difference was statistically significant (p < 0.05) for females aged 15–19 yr and approached significance (p < 0.10) for persons aged 45–74 yr. Using the MCV model, the difference was significant (p < 0.05) for persons aged 45–74 yr and for children aged 3–4 yr, and approached significance (p < 0.10) for females aged 15–19 yr.

Little association of the poverty index ratio with iron status was detected using the ferritin model, although the prevalence of abnormal values was 14% higher for females aged 15–19 yr below the poverty level than for those above the poverty level, and this difference approached statistical significance (p < 0.10). Using the MCV model, a higher prevalence of abnormal values was associated with poverty. The differences were marked and statistically significant (p < 0.05) for the children aged 1–2 and 3–4 yr, females aged 15–19 yr, and persons aged 45–74 yr, and approached significance (p < 0.10) for females aged 20–44 yr.

Some association between higher prevalence of abnormal values and lower levels of education was seen for females aged 20–44 yr using both the ferritin and MCV models, and for persons aged 45–74 yr using the MCV model. For males aged 20–44 yr the prevalence of abnormal values was very low and showed no differences associated with level of education.

The prevalence of abnormal values was higher for women with three or more children than for women with fewer than three children.

Multivariate analyses should be conducted to elucidate further these and possibly other relationships.

Iron overload

Of the data available from NHANES II, transferrin saturation and serum ferritin are considered the two best variables for assessing iron overload (45–51). This analysis was therefore restricted to the sample in which ferritin determinations were made. Serum ferritin levels shown in Table 4 and transferrin saturation > 70% (49) were used as criteria for
determining iron overload in adults. If the TIBC value was missing, as it was in many samples
with high serum iron (for reasons previously stated), serum iron level > 250 μg/dl was used
as a criterion in place of the transferrin saturation > 70%. Some persons with elevated
transferrin saturations may have been missed when serum iron was used as a criterion.

Among the persons aged 20–74 yr in the ferritin subsample (n = 3,540), there were 22
persons with high transferrin saturation (or high serum iron), 219 persons with high serum
ferritin, and 9 persons with high values for both transferrin saturation (or serum iron) and
serum ferritin. Examination of the available medical history and other data of these nine
individuals led to the conclusion that the data in five were consistent with the diagnosis of
uncomplicated idiopathic hemochromatosis. The condition of the remaining four persons
with indicators characteristic of iron overload was complicated by the presence of high MCV
values and low folate status, possibly indicative of erythropoietic abnormalities. Follow-up
studies on persons identified as having characteristics of iron overload were recommended
because the consequences of the disease are treatable.

NHANES II was not designed to yield statistically valid estimates of the prevalence of
conditions such as iron overload which occur at low frequency in the population. However,
the number of persons with laboratory evidence of hemochromatosis in the NHANES II ferritin
sample is quite close to the number that would be predicted on the basis of published
estimates of the prevalence of hemochromatosis in this country (48, 52, 53). Calcu-
lation of the gene frequency from the unweighted NHANES II data and the minimal estimate
of the frequency of the homozygous allele yielded a value 0.038 which is quite similar to the
frequencies reported for selected populations in the US (0.056 ± 0.02) (54), central Sweden
(0.069) (55), Canada (0.056) (56), Scotland (0.045) (57), and Britain (0.032–0.063) (45). Since these reports
may come from geographic hotspots of idiopathic hemochromatosis, the general prevalence
may be somewhat lower. Despite the acknowledged limitations of the data, the apparent
prevalence of uncomplicated overt iron overload was not higher than expected.

Table 4

<table>
<thead>
<tr>
<th>Age group</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–44 yr</td>
<td>&gt;200</td>
<td>&gt;150</td>
</tr>
<tr>
<td>45–64 yr</td>
<td>&gt;300</td>
<td>&gt;200</td>
</tr>
<tr>
<td>65–74 yr</td>
<td>&gt;400</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

Discussion and conclusions regarding the iron status of the US population

The ranges of prevalence estimates obtained from these analyses of the NHANES II data
tend to be lower than some of those derived in other recent surveys of North American
populations: the Ten-State Nutrition Survey (1968–70) (1), Nutrition Canada National
74) (2). Comparison of absolute values among these surveys, however, is nearly impossible.
Assessments in earlier surveys were usually based on determinations of the prevalence of
deficient or low values of hemoglobin and/or transferrin saturation whereas NHANES II
also included erythrocyte protoporphyrin, MCV, and (in a subsample) serum ferritin. In
addition to differences in the use of single vs multiple variables, there are also differences
in the choice of cutoff values, the survey and the laboratory analytical methodologies,
the sample population surveyed, and the age groups in which the data were reported. De-
spite these differences, the trends in the relative prevalences of impaired iron status among
the various age/sex groups in the NHANES II group were generally similar to those in the
earlier surveys. The NHANES II data show a relatively high prevalence of impaired iron
status in the children aged 1–2 yr. The prevalence was relatively lower in subjects aged 3–
10 yr than in the younger children. The males aged 11–14 yr in NHANES II showed rather
high prevalence estimates compared to all other age/sex groups (including the females
aged 11–14 yr), and a great discrepancy was evident in the estimates given by the three dif-
f erent models for this group. This finding was
somewhat unexpected, but the highest prevalence estimate for males aged 11-14 yr was obtained with the most sensitive model (the ferritin model). In addition, the males and females aged 11-14 yr had the smallest sample size of the age/sex groups used in analyses, thus, their prevalence estimates had relatively large confidence intervals. In the earlier Ten-State Nutrition Survey and NHANES I, young adolescent males exhibited a higher prevalence of anemia but a lower prevalence of iron deficiency than the young adolescent females. In NHANES II, the prevalence results were reversed in the groups aged 15-19 yr (much higher prevalence estimates for females than males), and this finding is quite similar to the results of earlier surveys. In agreement with results of previous surveys, the NHANES II women aged 20-44 yr had a relatively high prevalence of impaired iron status which declined in the women aged 45-64 yr. Men aged 20-64 yr in NHANES II showed a very low prevalence of impaired iron status. The NHANES II prevalence estimates increased in men aged 65-74 yr, and values were similar to those of women aged 65-74 yr. The evidence suggested that a large number of abnormal values in the iron status indicators among older persons aged 45-74 yr in NHANES II may have been related to inflammation rather than to iron deficiency. Factors contributing to the fairly low prevalence of impaired iron status for most age/sex groups cannot be identified with certainty, but may be related to the availability of high quality food through feeding programs, bioavailability of the forms of iron fortification used more recently in food, use of iron and ascorbic acid supplements or dietary intake of ascorbic acid, and use of oral contraceptive agents which decreased menstrual iron loss (59).

Blacks tended to have slightly higher prevalences of abnormal values suggesting impaired iron status than did whites, but differences were statistically significant only for children aged 3-4 yr, females aged 15-19 yr, and persons aged 45-74 yr. Status below the poverty level was associated with higher prevalence estimates, most notably in preschool children aged 1-4 yr, females aged 15-19 yr, and older persons aged 45-74 yr. In women aged 20-44 yr and persons aged 45-74 yr, the prevalence of abnormal values tended to decrease with increasing levels of education and greater parity in women was associated with evidence of poor iron status. Although the data available for the evaluation of the extent of iron overload in the US population were limited, the prevalence was not higher than expected from previous estimates.

In conclusion, the prevalence estimates of impaired iron status did not exceed 2% for males aged 15-64 yr or 6.1% for children aged 3-10 yr, females aged 11-14 and 45-74 yr, and males aged 65-74 yr. Several groups in the population exhibited relatively high prevalences of impaired iron status: children aged 1-2 yr (9.2-9.4%), males aged 11-14 yr (3.5-12.1%), and females aged 15-44 yr (2.5-14.2%). The NHANES II data on iron nutritional status should serve as a useful baseline against which the results of future surveys may be compared.

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