IRON OVERLOAD IN AFRICA

Interaction between a Gene and Dietary Iron Content

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Abstract *Background and Methods.* In contrast to hemochromatosis, which in white populations is inherited through a gene linked to the HLA locus, iron overload in sub-Saharan Africa is believed to result solely from increased dietary iron derived from traditional home-brewed beer. To examine the hypothesis that African iron overload also involves a genetic factor, we used likelihood analysis to test for an interaction between a gene (the hypothesized iron-loading locus) and an environmental factor (increased dietary iron) that determines transferrin saturation and unsaturated iron-binding capacity. We studied 236 members of 36 African families chosen because they contained index subjects with iron overload. Linkage to the HLA region was tested with use of lod scores.

Results. In the index subjects, increased iron was present in both hepatocytes and cells of the mononuclear-phagocyte system. Among family members with increased dietary iron due to the consumption of traditional beer, transferrin saturation in serum was distributed bimodally, with 56 normal values (<60 percent saturation) and 44 elevated values; the mean serum ferritin concentration was five times higher in the subjects with elevated transferrin saturation ($P<0.005$). The pedigree analysis provided evidence of both a genetic effect ($P<0.005$) and an effect of increased dietary iron ($P<0.005$) on transferrin saturation and unsaturated iron-binding capacity. In the most likely model, increased dietary iron raised the mean transferrin saturation from 30 to 81 percent and lowered the mean unsaturated iron-binding capacity from 38 to 13 μmol per liter in subjects heterozygous for the iron-loading locus. The hypothesis of tight linkage to HLA was rejected.

Conclusions. Iron overload in Africa may be caused by an interaction between the amount of dietary iron and a gene distinct from any HLA-linked gene. (N Engl J Med 1992;326:95-100.)

IRON overload in the absence of hyperplastic refractory anemia or blood transfusions is found in two epidemiologically important conditions: hereditary hemochromatosis and African dietary iron overload.[1] In hereditary hemochromatosis, an inborn error of metabolism leads to the absorption of excess iron from diets with normal iron content; it has been reported predominantly in white populations.[2-5] The gene for this condition is tightly linked to the HLA locus on chromosome 6, but its exact site and normal gene product are not known.[5] In the African form of iron overload, increased absorption is believed to result from an environmental factor — namely, increased amounts of bioavailable iron in the diet. The source of this dietary iron is a traditional fermented beer that is home-brewed from local crops in steel drums.[6] A genetic defect has not been identified. In a recent community survey in Zimbabwe, we observed serologic evidence of toxic iron overload in only a minority of drinkers of traditional beer.[7] This finding led us to hypothesize that in addition to increased amounts of dietary iron, an inborn error of metabolism also may be involved in the excessive iron absorption seen in African dietary iron overload. To test for an interaction between genotype and environment, we conducted a study of African families selected because they had members with evidence of iron overload.

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METHODS

Informed consent was obtained from all the study subjects, who were predominantly rural dwellers from four ethnic groups of southern and central Africa.

Identification of Index Subjects

Thirty-six index subjects were selected as follows: 28 had had diagnostic liver biopsies showing excess hepatocellular iron, 4 presented clinically with hepatomegaly and a history of consuming traditional beer, and 4 were discovered in population surveys to have elevated serum ferritin levels and transferrin saturation greater than 60 percent. Two of these index subjects, included as a result of clerical error or mistaken identity, were later found not to have iron overload; they and their family members were included in the genetic analyses but were excluded from the description of index subjects (Tables 1 and 2).

Histologic Analysis of Liver-Biopsy Specimens

The deposition of iron in hepatocytes was graded on a scale of 0 to 4,[8,9] the presence or absence of iron was noted in cells of the mononuclear-phagocyte system, and the specimens were evaluated for pathologic changes (Table 2).

Study of Family Members

We visited the villages of the index subjects and determined their precise relationships to other family members in interviews. Two hundred eight family members of the 36 index subjects were studied, including 10 parents, 54 full siblings, 64 children, 21 half siblings, 41 other relatives, and 10 spouses and 8 other people who were part of the kindreds through marriage but not genetically related to the index subjects. Eight of the index subjects died before their villages were visited. Blood samples were collected by venipuncture

Table 1. Clinical and Demographic Characteristics of the Index Subjects Alive at the Time of the Study.*

Identification of Subjects	No.	Sex	Age	Lifetime Beer Consumption†	Hemoglobin	Transferrin Saturation	Unsaturated Iron-Binding Capacity	Serum Ferritin†
		M/F	*yr*	*liters*	*g/liter*	*%*	*μmol/liter*	*μg/liter*
By liver biopsy								
Grade 4 hepatocellular iron	7	5/2	63±9	7,826 (2,155–28,415)	149±11‡	87±23	6±11	4415 (3507–5495)
Grade 3 hepatocellular iron	9	7/2	60±9	15,854 (7,247–34,685)	147±19§	79±24	9±10	3074 (1541–6133)
Grade 2 hepatocellular iron	3	3/0	59±8	24,468 (10,930–54,778)	127±27	43±9	26±3	3042 (2255–4104)
By clinical presentation	4	4/0	66±17	8,472 (2,262–31,740)	118±15	73±27	8±8	2138 (1380–3311)
By community screening	3	3/0	44±12	9,288 (4,958–17,398)	151¶	84±28	9±16	891 (501–1585)

*Data on two index subjects included by error and later found not to have evidence of iron overload were excluded. Plus–minus values are means ±SD.
†Values shown are geometric means and ranges of standard deviations. ‡N = 6. §N = 8. ¶N = 1.

at various times of day so that, in general, the subjects were not fasting. The subjects were asked to estimate their consumption of traditional beer: the amount ingested on a typical day, the number of days the beverage was consumed in a typical month, the year drinking started, and if they were no longer drinking, the year they stopped. Traditional beer was taken to be a beverage brewed at home in nongalvanized steel drums that is known to have a high content of bioavailable iron.[6] We estimated total lifetime consumption by multiplying the amount ingested on a typical day times the number of days of drinking per month times 12 times the number of years of drinking. The information derived from this equation provided only broad approximations, because consumption was probably not uniform over time and information was obtained by recollection.

Analysis of Blood Samples

Serum iron levels and total iron-binding capacity were determined for all 208 family members and 28 living index subjects.[10,11] Transferrin saturation was calculated by dividing the serum iron level by the total iron-binding capacity and multiplying by 100, with the result not to exceed 100 percent; unsaturated iron-binding capacity was calculated by subtracting the serum iron level from total iron-binding capacity, with the difference not to be less than zero.[12] Serum ferritin concentrations were measured in 235 subjects by radioimmunometric assay. Hemoglobin concentrations were measured in 182 subjects with a Coulter Counter S Plus 100 (Coulter, Hialeah, Fla.). Liver function was tested[13,14] in 177 subjects, and hepatitis B markers were screened by radioimmunoassay in 168. In 167 participants, HLA antigens were assayed on lymphocytes with Terasaki HLA-ABC Sup-Black Trays (One Lambda, Los Angeles) and Biotest Lymphocyte ABC Trays (Biotest Diagnostics, Frankfurt, Germany), and haplotypes were determined.

Definition of a Group with Increased Dietary Iron

Above an estimated lifetime consumption of 350 liters of traditional beer, there is a significant increase in the prevalence of elevated (≥60 percent) transferrin saturation, decreased (less than 18 μmol per liter) unsaturated iron-binding capacity, and increased (more than 300 μg per liter) serum ferritin concentration ($P<0.005$ by the chi-square test; data not shown). Twenty-six index subjects and 100 family members with estimated lifetime consumptions of more than 350 liters of traditional beer were assigned to a group considered to have increased dietary iron; 2 index subjects and 108 family members were assigned to a group considered not to have increased dietary iron.

Table 2. Liver-Biopsy Results in 25 Index Subjects.

Variable	No. (%) of Subjects
Iron	
Hepatocellular	
Grade 4	9 (36)
Grade 3	11 (44)
Grade 2	5 (20)
Mononuclear phagocyte	25 (100)
Histologic features	
Fibrosis	23 (92)
Hepatocyte necrosis	10 (40)
Inflammation	9 (36)
Cirrhosis	7 (28)
Hepatoma	5 (20)
Steatosis	2 (8)

Statistical Analysis

Variables were compared between categories of dietary iron and of transferrin saturation by t-tests and chi-square tests. The relation between certain variables was examined with Pearson's correlation. HLA-antigen frequencies in the index subjects with evidence of iron overload were compared with those for the population of Zimbabwe[15] by the chi-square test. In Table 3, measures of iron status were adjusted for age and sex by least-squares analysis. In Table 4, measures of iron status were adjusted for age, sex, and category of dietary iron in a similar manner.

Genetic Analysis of Families

Two genetic analyses of the study families were performed, one based on transferrin saturation and one on unsaturated iron-binding capacity. Transferrin saturation was used for genetic analysis because in our data this measure had a bimodal distribution in the group with increased dietary iron (Fig. 1) and because in HLA-linked hemochromatosis, the only genetic iron-loading disorder now recognized, this value is considered to be the single best marker for the presence of the iron-loading defect.[16] Unsaturated iron-binding capacity was used for genetic analysis because it also had a bimodal distribution among family members with increased dietary iron (Fig. 1). Transferrin saturation reflects the proportion of iron-binding sites on serum transferrin molecules that are bound to iron. Unsaturated iron-binding capacity generally parallels transferrin saturation inversely, reflecting the additional amount of iron that serum transferrin molecules could bind before becoming fully saturated. Transferrin production in the liver appears to be regulated by the amount of iron in hepatocytes[17]; as the organ becomes loaded with iron, transferrin synthesis diminishes, which may contribute to increased transferrin saturation and reduced unsaturated iron-binding capacity. Seven index subjects in whom measurements were not made were assigned the mean transferrin saturation and unsaturated iron-binding capacity measured in the other index subjects with the same grade of hepatocellular iron (Table 1). Families of two unaffected index subjects were included, since even unaffected persons contributed to the estimates. Values for transferrin saturation and unsaturated iron-binding capacity were adjusted for age and sex by regression.

We used likelihood analysis to test the hypothesis that the interaction of an iron-loading gene with dietary intake of iron determines transferrin saturation and unsaturated iron-binding capacity. In addition to chi-square statistics to test for the presence of each effect, the analysis produced parameter estimates.[18-25] Linkage to HLA was tested with use of lod scores; lod scores of 3 or more

Table 3. Iron Tests in Family Members, According to Category of Dietary Iron.*

Dietary Iron	No. of Subjects	Sex	Age	Lifetime Beer Consumption†	Transferrin Saturation	Unsaturated Iron-Binding Capacity	Serum Ferritin†
		M/F	*yr*	*liters*	*%*	*μmol/liter*	*μg/liter*
Increased	100	72/28	54±15	5970 (1422–25,061)	52±29	27±17	565 (178–1795)
Not increased	108	51/57	33±16	1 (0.5–4)	36±13	35±12	118 (47–299)‡
Normal values					20–60	18–63	20–300

*Plus–minus values are means ±SD. Values for transferrin saturation, unsaturated iron-binding capacity, and serum ferritin were adjusted for age and sex. P<0.005 for all comparisons between groups.

†Values shown are geometric means and ranges of standard deviations.

‡N = 117.

Table 4. Iron Tests in Family Members, According to Relationship to Index Subjects.*

Relationship	No. of Subjects	Sex	Age	Lifetime Beer Consumption†	Transferrin Saturation	Unsaturated Iron-Binding Capacity	Serum Ferritin†
		M/F	*yr*	*liters*	*%*	*μmol/liter*	*μg/liter*
First-degree relatives	122	65/57	41±20	40 (0.6–2,831)	47±26	29±17	281 (56–1403)
Other family members‡	88	59/29	46±17	175 (2–14,757)	39±25	33±16	212 (46–979)§
P value		0.045	0.055	0.017	0.012	0.037	0.042

*Plus–minus values are means ±SD. Values for transferrin saturation, unsaturated iron-binding capacity, and serum ferritin were adjusted for age, sex, and category of dietary iron.

†Values shown are geometric means and ranges of standard deviations.

‡All members of the two families whose index subjects were later found not to have iron overload are included.

§N = 87.

support linkage, whereas lod scores below −2 exclude linkage.[26] Details of the methods of analysis are available elsewhere.*

Results

Index Subjects

The 34 index subjects with evidence of iron overload included 6 women and 28 men, all of whom reported the consumption of traditional beer. The clinical characteristics of the 26 who were alive when their villages were visited are shown in Table 1, grouped according to method of selection and grade of hepatocellular iron. Hemoglobin concentrations tended to be in the normal range, making iron-loading dyserythropoiesis an unlikely cause of iron overload in these subjects.

Liver-biopsy specimens from 25 index subjects were available for review (Table 2). Parenchymal-cell iron was predominantly of grades 3 and 4. In all the specimens there was iron in Kupffer cells and in macrophages in portal triads or in areas of periportal fibrosis or fibrous septae. There were occasional iron deposits in bile-duct epithelium, but this was not a prominent finding. No gradient of parenchymal-cell iron, maximal in periportal hepatocytes and decreasing toward the terminal hepatic venule, was apparent in these specimens. Most specimens showed varying degrees of fibrosis. Steatosis was an infrequent finding, and in only one case was it associated with Mallory's bodies and pericellular neutrophils, the morphologic findings typical of alcoholic liver disease.

The identity of HLA antigens was determined or inferred in 21 of the 34 index subjects who had evidence of iron overload. None of these subjects were found to have the A3 or B14 antigens that are associated with HLA-linked hemochromatosis in white populations, but six had B7. The antigen frequencies in this group of subjects did not differ significantly from those previously reported for Zimbabweans.[15]

Family Members

The results of iron tests in the family members according to the presence or absence of increased dietary iron are shown in Table 3. Mean values for transferrin saturation and serum ferritin were significantly higher in the group with increased dietary iron; mean values for unsaturated iron-binding capacity were significantly lower. Mean ages were also significantly higher among those with increased dietary iron, reflecting both general custom for the consumption of traditional beer to begin after men have reached adulthood and after women have completed childbearing and the fact that a number of years are required to reach the threshold of 350 liters we defined as indicating increased dietary iron.

Results according to whether study subjects were first-degree relatives of index subjects or were other family members are shown in Table 4. Mean values for transferrin saturation and serum ferritin were significantly higher in first-degree relatives than in other family members, and mean unsaturated iron-binding capacity was significantly lower.

Figure 1 shows the bimodal distribution of transferrin saturation and unsaturated iron-binding capacity in the subjects with increased dietary iron. There were no significant differences in mean beer consumption or the frequency of positivity for hepatitis B surface antigen and no consistent differences in liver-function tests between the subjects with transferrin saturation ≥60 percent and those with lower values. However, the 44 subjects with the higher values were older than the 56 with the lower values (58±14 vs. 51±16 years, P<0.05), and their geometric mean serum ferritin concentration was markedly higher (1936 μg per liter [range of standard deviation, 1114 to 3364] vs. 422 μg per liter [142 to 1218], P<0.005). In contrast to the

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bimodal distribution of transferrin saturation, no bimodal distribution of serum ferritin concentration was obvious in the group with increased dietary iron (Fig. 2), but there was a strong correlation between transferrin saturation and log serum ferritin ($r = 0.70$, $P<0.005$).

There were low but significant correlations between age and transferrin saturation ($r = 0.27$) and age and log serum ferritin ($r = 0.28$) in the group with increased dietary iron. There was no significant correlation between log estimated lifetime consumption of traditional beer and transferrin saturation, but there was a significant correlation between log beer consumption and log serum ferritin ($r = 0.25$).

Genetic Analysis

The genetic analysis supported the hypothesis that the interaction of an iron-loading gene with dietary iron intake determines transferrin saturation and unsaturated iron-binding capacity. The analysis provided evidence of both a major-locus effect ($\chi^2_6 = 53.59$, $P<0.005$ for transferrin saturation, and $\chi^2_6 = 13.40$, $P<0.05$ for unsaturated iron-binding capacity) and an effect of increased dietary iron ($\chi^2_3 = 56.11$, $P<0.005$ for transferrin saturation, and $\chi^2_3 = 39.14$, $P<0.005$ for unsaturated iron-binding capacity). We inferred recessive inheritance of the iron-loading gene in the absence of increased dietary iron and dominant inheritance in the presence of increased dietary iron. That is, iron loading occurs in people heterozygous for the allele only when their iron intake is excessive (details of this analysis are available elsewhere*).

Table 5 presents parameter estimates of transferrin saturation and unsaturated iron-binding capacity for the inferred mode of inheritance. Increased dietary iron raised the mean transferrin saturation from 30 to 33 percent in subjects without the allele determining increased transferrin saturation, from 30 to 81 percent in subjects heterozygous at the locus, and from 69 to 81 percent in subjects homozygous at the locus. Similar estimates of allele frequency in the two analyses ($q = 0.16$ for transferrin saturation, and $q = 0.20$ for unsaturated iron-binding capacity) corresponded to a frequency of homozygosity of about 3 to 4 percent and a frequency of heterozygosity of 28 to 33 percent. Polygenic inheritance accounted for 19 percent of the variance in transferrin saturation in the genotype and 29 percent of the variance in unsaturated iron-binding capacity. Lod scores for linkage between the major locus and HLA were less than -2 for recombination frequencies of 0.19 or less for transferrin saturation and of 0.10 or less for unsaturated iron-binding capacity, indicating that the proposed iron-loading locus in these African pedigrees is not tightly linked to the HLA region.

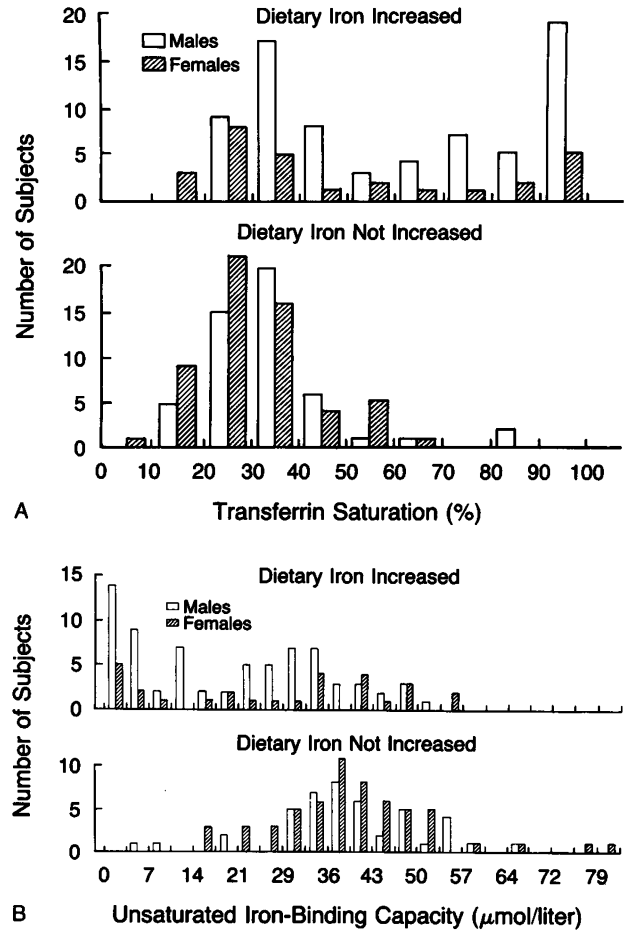

Figure 1. Transferrin Saturation (Panel A) and Unsaturated Iron-Binding Capacity (Panel B) in Male and Female Family Members, According to Category of Dietary Iron.

Unsaturated iron-binding capacity was less than 18 μmol per liter in 44 of 48 subjects with transferrin saturation ≥60 percent; transferrin saturation was ≥60 percent in 44 of 47 subjects with unsaturated iron-binding capacity of less than 18 μmol per liter.

Discussion

Sub-Saharan Africa is the only part of the world in which iron overload due to increased dietary iron has been recognized. One possible exception is central Asia, where it has been proposed that an arthropathy known as Kashin–Beck or Urov disease is related to a diet containing excess amounts of iron or other metals.[27,28] The traditional beer from which the excess dietary iron is derived in sub-Saharan Africa has an average iron content of about 80 mg per liter,[6,29] and some people commonly drink several liters a day on weekends. For comparison, the typical Western diet contains a total of 15 to 20 mg of iron per day.[30]

The genetic analysis presented here provides evidence of a major locus in the African pedigrees that results in elevated transferrin saturation, reflecting iron loading in homozygous subjects, and that con-

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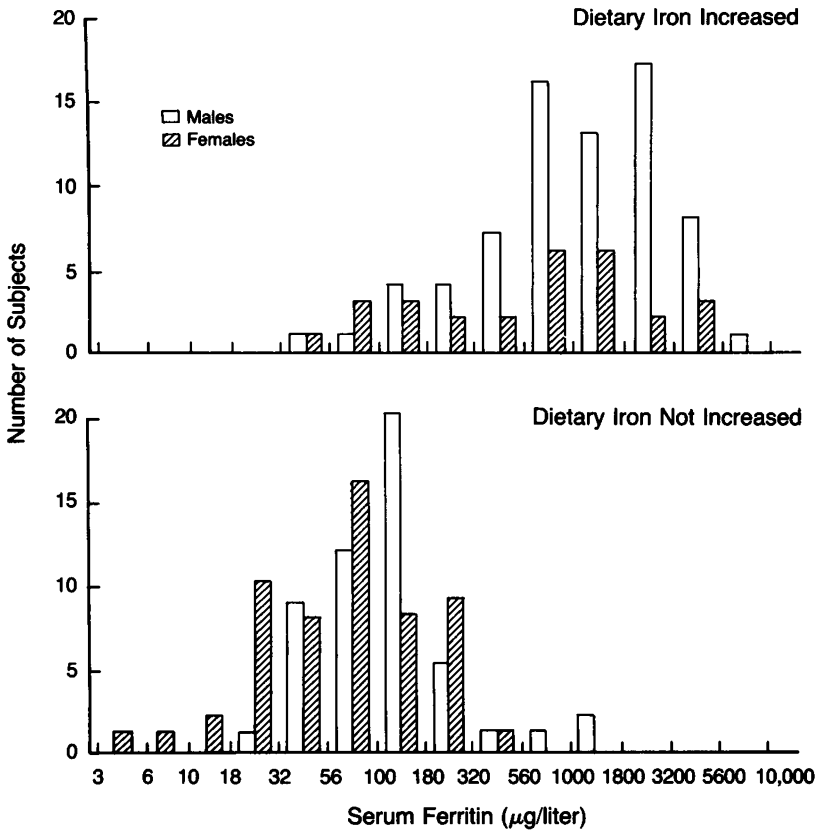

Figure 2. Serum Ferritin Concentrations in Male and Female Family Members, According to Category of Dietary Iron.

tributes to a bimodal distribution of transferrin saturation in subjects with increased dietary iron. Similar findings in an independent analysis of unsaturated iron-binding capacity support this result. The analysis suggests an interaction between genotype and environment in which the three genotypes respond differently to elevated levels of dietary iron (Table 5). According to this model, exposure to a diet containing increased levels of iron raises transferrin saturation minimally (from 30 to 33 percent) in normal homozygous subjects, but substantially in subjects heterozygous (from 30 to 81 percent) or homozygous (from 69 to 81 percent) for the proposed iron-loading locus. Although we may have overestimated the allele frequency because of the small number of independent members of the study pedigrees and the inexactness of the ascertainment correction, the present analysis indicates that up to one third of the African population may be at risk for substantial iron loading because of this interaction. Our previous population survey found that 18.5 percent of male drinkers of traditional beer had increased transferrin saturation.[7] In interpreting these estimates, a single measurement in each subject did not allow us to eliminate cases of sporadic elevation of transferrin saturation, and we may have misclassified some subjects according to environmental iron exposure. Also, it is possible that persons homozygous for the proposed iron-loading locus were underrepresented in the group of family members with increased dietary iron, since such people could be expected to die young.

Several considerations suggest that the proposed iron-loading locus in these families is different from the HLA-linked gene that leads to iron overload in white populations. First, the frequencies of the HLA Class I antigen in the index subjects did not differ significantly from those of the Zimbabwean population. Second, in the pedigree analysis, the hypothesis of tight linkage of the locus determining increased transferrin saturation and reduced unsaturated iron-binding capacity to the HLA haplotype was rejected. Third, the histologic pattern of hepatic iron deposition in the index subjects differed from that in patients with HLA-linked hemochromatosis, in that iron in cells of the mononuclear-phagocyte system was more prominent. The reason for this difference in the distribution of excess iron, which has been described previously,[31] is unclear, but the distribution in both parenchymal cells and cells of the mononuclear-phagocyte system was similar to that observed in iron-loading anemia. Since reticulocyte counts were not determined in this study, a compensated hemolytic state cannot be excluded as a possible stimulus to increased gastrointestinal absorption of iron, but it seems unlikely.

Iron overload has been implicated as an etiologic factor in hepatic portal fibrosis and cirrhosis,[32] scurvy and osteoporosis,[33] diabetes mellitus,[34] congestive heart failure,[35] esophageal carcinoma,[35] and infections[36-38] in Africa. Discovery of the possible genetic defect might make it possible to identify people at risk for iron overload and increase our understanding of

Table 5. Parameter Estimates (±SE) of Transferrin Saturation and Unsaturated Iron-Binding Capacity for the Selected Model.*

CATEGORY	μ_1	μ_2	μ_3	σ
Transferrin saturation (%)				
Dietary iron not increased	30±1	30±1	69±7	10±1
Increased dietary iron	33±2	81±3	81±3	13±1
Unsaturated iron-binding capacity (μmol/liter)				
Dietary iron not increased	38±1	38±1	18±9	12±1
Increased dietary iron	38±2	13±2	13±2	8±1

*The parameter estimates are indicated as follows: μ_1 is the estimate for a homozygous subject with lower mean transferrin saturation and higher mean unsaturated iron-binding capacity, μ_2 the estimate for a heterozygous subject, and μ_3 the estimate for a homozygous subject with higher mean transferrin saturation and lower mean unsaturated iron-binding capacity; σ denotes the phenotypic standard deviation.

iron metabolism. Furthermore, public health strategies designed to educate the community about the adverse effects of a diet with a high iron content and to alter the way in which traditional beer is prepared or consumed would help prevent the clinical consequences of this form of iron overload.

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