

## Iron overload in urban Africans in the 1990s

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### Abstract

**Background**—In a previously described model, heterozygotes for an African iron loading locus develop iron overload only when dietary iron is high, but homozygotes may do so with normal dietary iron. If an iron loading gene is common, then homozygotes with iron overload will be found even in an urban population where traditional beer, the source of iron, is uncommon.

**Aims**—To determine whether iron overload and the C282Y mutation characteristic of hereditary haemochromatosis are readily identifiable in an urban African population.

**Methods**—Histological assessment, hepatocellular iron grading, and dry weight non-haem iron concentration were determined in post mortem tissue from liver, spleen, heart, lungs, and skin. DNA of subjects with elevated hepatic iron indexes was analysed for the C282Y mutation. Iron concentrations in other tissues were compared.

**Results**—A moderate increase (>30  $\mu\text{mol/g}$ ) in hepatic iron concentrations was found in 31 subjects (23%; 95% confidence interval 15.9 to 30.1%), and they were considerably elevated (>180  $\mu\text{mol/g}$ ) in seven subjects (5.2%; 95% confidence interval 1.5 to 8.9%). Appreciably elevated hepatic iron concentrations were associated with heavy iron deposition in both hepatocytes and macrophages, and either portal fibrosis or cirrhosis. All were negative for the C282Y mutation. Very high concentrations were uncommon in subjects dying in hospital. Concentrations of iron in spleen, heart, lung, and skin were significantly higher in subjects with elevated hepatic iron.

**Conclusions**—Iron overload is readily identified among urban Africans and is associated with hepatic damage and iron loading of several tissues. The condition is unrelated to the genetic mutation found in hereditary haemochromatosis.

(Gut 1999;45:278-283)

Keywords: haemosiderosis; cirrhosis; iron; liver; Africa; haemochromatosis

In Western countries, iron overload is regarded as predominantly a problem that affects the white population in the form of autosomal recessive HLA linked haemochromatosis, with a prevalence of up to 0.45%.<sup>1</sup> In sub-Saharan Africa, iron overload is related to a diet high in iron and affects 10% or more of some rural

populations.<sup>2</sup> The source of the excess dietary iron in Africa is a traditional fermented beverage prepared in non-galvanised steel drums.<sup>3</sup> Several studies have provided convincing evidence that African iron overload causes cirrhosis,<sup>4,5</sup> and there may also be aetiological associations with hepatocellular carcinoma,<sup>6-9</sup> tuberculosis,<sup>6</sup> and other infections.<sup>10,11</sup> Despite the indications that iron overload in Africans is a cause of serious disease, the condition is under-recognised by clinicians. The prevalence of iron overload in urban areas is not known, although there was a high prevalence in Johannesburg in the 1970s.<sup>2,10</sup>

A recent study suggested that a non-HLA-linked iron loading gene may be a factor in the development of iron overload in Africa, interacting with dietary iron content.<sup>12</sup> According to the model presented in that study, heterozygotes for an African iron loading locus develop iron overload only when dietary iron is high, but homozygotes may do so with normal dietary iron. The brewing and consumption of traditional beer is now a practice almost exclusive to the rural areas. In this study, we postulate that if an iron loading gene is common in the African population, then homozygotes with primary iron overload will be found even in an urban population that does not have high dietary iron intake. The present study was undertaken to determine the prevalence of iron overload in the capital city of Zimbabwe in the 1990s by prospectively measuring tissue iron concentrations in victims of accidental or traumatic deaths.

### Methods and materials

This cross sectional post mortem study included 135 community subjects over the age of 16 years who experienced traumatic, accidental, or suicidal death in Harare, Zimbabwe, during the period November 1993 to November 1995, and who underwent autopsy. The study was approved by the hospital ethical review board. The subjects were enrolled in the study in an unselected manner, but not all subjects undergoing post mortem examination were included. In addition, 59 patients who died in Parirenyatwa Hospital in Harare and underwent post mortem examination during the same period were studied. In Zimbabwe, post mortems are performed after only a small proportion of deaths because of cultural reluctance to accept the procedure. Whether a patient underwent post mortem examination after a hospital death was largely determined by the relatives' willingness to give consent to the

**Abbreviation used in this paper:** PCR, polymerase chain reaction.

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Accepted for publication  
17 February 1999

procedure irrespective of the doctor's keenness to have the procedure performed.

At the time of autopsy, pieces of tissue were collected from the liver, spleen, heart, lungs, and skin and stored separately in formalin until analysis of tissue iron concentrations. The dry weight non-haem iron concentration was determined using the method of Torrance and Bothwell.<sup>13</sup> A piece of diced tissue weighing about 1 g was incubated at 80°C for 30 hours to dry the tissue completely. Then 10 ml of acid solution (0.6 M trichloroacetic acid and 3 M hydrochloric acid) was added to the specimen and the mixture was incubated at 65°C for 20 hours to extract the iron. A chromagen solution containing 0.1% bathophenanthroline disulphonate and 1% thioglycolic acid was added to an aliquot of the acid digestion mixture and the absorbance was measured spectrophotometrically at 535 nm. The standard curve was constructed using ferric chloride solutions. Control samples of ox liver were analysed with each assay to ensure consistency of results. The methodology was verified using standard liver tissue obtained from the International Committee for Standardization in Haematology. An hepatic iron index,<sup>14</sup> calculated as the hepatic iron concentration in  $\mu\text{mol/g}$  dry weight divided by the age in years, was used to determine how many subjects had iron loading of a degree that could not be explained by the possible effects of alcohol.

For subjects with the highest hepatic iron concentrations, histological sections of liver tissues were stained with haematoxylin and eosin, with Perls' stain for iron,<sup>15</sup> and with Gordon and Sweet's stain for reticulin.<sup>16</sup> An histological assessment was performed independently by at least two investigators. The hepatocellular iron was graded in sections stained with Perls' reagent on a scale of 0 to 4+ using Scheuer's method.<sup>17, 18</sup> Grade 0 was assigned to sections with no hepatocellular iron visible by light microscopy. In grade 1+ sections, microscopic iron was visible in <5% of hepatocytes. Grade 2+ was assigned to slides with a faint purple colour on gross inspection and with microscopic granules of iron present in 5–10% of hepatocytes. Grade 3+ sections were grossly purple and 40% of the hepatocytes were heavily laden with iron granules. Grade 4+ slides were deep blue by gross inspection and most of the hepatocytes were heavily laden with large granules of iron. Macrophage iron was also assessed according to whether iron was absent, present, or present and prominent. Hepatocellular iron grades of

3+ or 4+ are suggestive of iron overload.<sup>19, 20</sup> Portal fibrosis was assessed in sections stained with Gordon and Sweet's reagent. Moderate to prominent thickening of the portal tracts was taken to indicate the presence of portal fibrosis. Marked thickening and linkage of adjacent portal tracts, distortion of the lobular pattern, and areas of nodular hyperplasia were taken to indicate the presence of cirrhosis.<sup>4, 21</sup>

For subjects that proved to have elevated hepatic iron indexes, DNA was isolated from spleen samples and analysed for the C282Y mutation which is typical of hereditary haemochromatosis in whites.<sup>22</sup> Spleen samples (2–3 mg) that had been stored in formalin were sliced with a blade and placed in an Eppendorf tube. The slices were washed once with 1 ml of a buffer containing 40 mM Tris base (pH 11.0), 1 mM desferrioxamine B, and 0.1% Tween 20, and then centrifuged at 14 000 *g* for three minutes. Afterwards, 200  $\mu\text{l}$  of the same buffer was added to the tubes and the slices were homogenised with a Kontes motor cordless homogeniser (Vineland, New Jersey, USA) for five minutes. This was followed by centrifugation and a second homogenisation in 200  $\mu\text{l}$  buffer. The homogenate was washed twice more with 1 ml of the same buffer. The final pellet of cells (20–30  $\mu\text{l}$ ) was resuspended in an equal volume of phosphate buffered saline (pH 7.4). A 5  $\mu\text{l}$  portion of this suspension was used for the extraction of DNA by the addition of 100  $\mu\text{l}$  of a lysis buffer (10 mM Tris/HCl, pH 8.0, 1 mM EDTA, 1% Tween 20). Each tube was supplemented with 20  $\mu\text{l}$  proteinase K (20 mg/ml) and 1 mM  $\text{MgCl}_2$ . The tubes were incubated at 56°C for 96 hours, and 10  $\mu\text{l}$  proteinase K was added after four and 24 hours. The tubes were then heated at 95°C for 10 minutes to inactivate proteinase K, and the insoluble debris was precipitated by centrifugation at 14 000 *g* for two minutes. From the supernatant, 3  $\mu\text{l}$  was used as the DNA template for the polymerase chain reaction (PCR). The following primers encompassing the critical region of the human HFE gene (Cys-282) were constructed: 5' HFE/GT, GATAAGCAGCCAATGGATGCC; 3' HFE/GT, CACAATGATGGGCTGATCCAG. These primers produced a 160 bp PCR product which contained the Cys-282 site. In case of the C282Y mutation, a new cleavage site for the restriction enzyme, *Sna*BI (Promega, Madison, Wisconsin, USA), appeared giving rise to two smaller DNA fragments of 120 and 40 bp. The PCR was performed in a 50  $\mu\text{l}$  volume. The PCR mixture consisted of 3  $\mu\text{l}$  DNA

Table 1 Ages and tissue iron concentrations according to sex and category of death

	Traumatic and suicidal deaths		Hospital deaths	
	Men (n=107)	Women (n=28)	Men (n=46)	Women (n=13)
Age (years)	36 (13)	37 (13)	38 (13)	37 (15)
Hepatic iron ( $\mu\text{mol/g}$ dry weight)	20.4 (8.8–47.3)	17.3 (5.5–54.3)	19.1 (9.6–38.3)	13.6 (8.0–23.2)
Splenic iron ( $\mu\text{mol/g}$ dry weight)	20.2 (8.5–47.0)	18.5 (5.5–62.1)	25.8 (9.4–70.6)	15.4 (8.2–28.8)
Heart iron ( $\mu\text{mol/g}$ dry weight)	4.7 (3.4–6.5)	5.0 (3.1–7.9)	4.6 (2.5–8.5)	4.9 (3.4–7.1)
Lung iron ( $\mu\text{mol/g}$ dry weight)	12.4 (6.5–23.7)	11.4 (4.7–27.5)	11.8 (6.6–21.0)	9.3 (5.9–14.6)
Skin iron ( $\mu\text{mol/g}$ dry weight)	1.9 (1.1–3.4)	2.0 (0.8–5.0)	1.9 (1.1–3.2)	1.8 (1.1–3.0)

Results are shown as mean and SD for age and as geometric mean and SD range for tissue iron concentration. Ages were unavailable for three traumatic deaths and one hospital death. Iron concentrations were unavailable for one or more of the non-hepatic tissues in 20 of the traumatic deaths and eight of the hospital deaths.

Table 2 Prevalence of iron overload: categories of non-haem hepatic iron concentration and the hepatic iron index classified according to sex and mode of death

	Traumatic/suicidal deaths (n=135)		Hospital deaths (n=59)	
	Men (n=107)	Women (n=28)	Men (n=46)	Women (n=13)
Hepatic iron concentration				
<30 µmol/g	82 (77)	23 (82)	34 (74)	12 (92)
30–180 µmol/g	22 (21)	2 (7)	12 (26)	1 (8)
180–360 µmol/g	4 (3)	3 (11)	0	0
Hepatic iron index				
<1.0	85 (79)	24 (86)	38 (83)	12 (92)
1.0–1.9	12 (11)	1 (4)	7 (15)	1 (8)
>1.9	10 (9)	3 (11)	1 (2)	0

extract, 1 µl each primer, and 45 µl PCR SUPERMix (Gibco BRL, Bethesda, Maryland, USA). The following PCR regimen was used: first step, 95°C for three minutes; second step, 95°C for one minute; third step, 54°C for two minutes; fourth step, 72°C for one minute; fifth step, five cycles of steps 2–4; sixth step, 94°C for one minute; seventh step, 54°C for two minutes; eighth step, 72°C for one minute; ninth step, 30 cycles of steps 6–9; tenth step, 72°C for 10 minutes; eleventh step, 4°C. A 10 µl portion of the PCR products was used for digestion in the presence and absence of *Sna*BI, at 37°C for one hour. The enzyme (or distilled water) was added in 10 µl containing 2 µl 10 × buffer, 0.2 µl bovine serum albumin, 6.8 µl water, and 1 µl *Sna*BI. Then 20 µl aliquots (in 5 µl 5 × DNA buffer) were electrophoresed on 3% agarose gel at 110 V. The homozygous pattern of DNA separation for the C282Y discloses the disappearance of a larger band (160 bp) and the appearance of two smaller bands. The absence of the smaller bands shows that the C282Y mutation did not occur.

Because the values for tissue iron concentration followed a skewed distribution, they were log transformed for statistical analysis. The concentrations of iron in tissues other than the liver were compared according to three categories of liver iron concentration using analysis of variance and linear regression modelling (table 4).

Table 3 Light microscopy findings in six of seven subjects with a hepatic iron concentration >180 µmol/g

Age	Sex	Hepatic iron (µmol/g)	Hepatic iron index	Hepatocellular iron grade	Portal macrophage iron	Kupffer cell iron	Histology
Not known	M	503	>5.0	3 to 4	Prominent	Prominent	Cirrhosis
55	F	288	5.2	3 to 4	Prominent	Prominent	Normal
51	M	190	3.7	3 to 4	Prominent	Prominent	Normal
84	F	214	2.5	3 to 4	Prominent	Prominent	Portal fibrosis
56	M	181	3.2	3 to 4	Prominent	Prominent	Cirrhosis
55	M	195	3.5	3 to 4	Prominent	Prominent	Portal fibrosis

Table 4 Relations of iron concentrations in non-hepatic tissues to category of hepatic iron concentration, traumatic and hospital deaths combined

Tissue	Category of hepatic iron concentration (µmol/g)			p Value
	<30 (n=151)	30–180 (n=36)	180–360 (n=6)	
Spleen (µmol/g dry weight)	15.9 (6.8–12.0)	47.0 (17.4–127.1)	120.8 (41.0–355.6)	<0.001
Heart* (µmol/g dry weight)	4.6 (3.0–7.1)	5.2 (3.4–8.0)	6.3 (4.9–8.3)	<0.001
Lung (µmol/g dry weight)	10.8 (6.0–19.6)	15.5 (7.8–30.9)	27.8 (8.7–88.5)	<0.001
Skin (µmol/g dry weight)	1.8 (1.0–3.3)	2.2 (1.3–4.0)	3.5 (1.3–9.7)	<0.001

Values are expressed as geometric means and SD ranges. The p value represents the level of significance for comparing iron concentrations for a given tissue according to category of hepatic iron by analysis of variance.

\*n=131, 31, 5 for the three hepatic iron categories.

## Results

A total of 135 accident, trauma, and suicide victims and a total of 59 hospital patients were studied. Table 1 presents demographic features of the subjects and tissue iron concentrations. Men outnumbered women by about 4 to 1. Mean tissue iron concentrations were similar in men and women and between accident, trauma, and suicide victims and hospital patients. Of the 59 hospital deaths, 25 (42%) were the result of AIDS related illnesses, 11 (19%) from congestive heart failure or complications of hypertension, and nine (15%) from malignancies of the colon, liver, and haematopoietic tissue. A minority of patients died from common medical conditions such as cerebrovascular accidents (5%) and gastrointestinal bleeding (5%), and only one died as a result of acute liver failure secondary to advanced cirrhosis.

Table 2 shows that non-haem hepatic iron concentrations were normal (<30 µmol/g dry weight) in most subjects. Elevated hepatic iron concentrations in excess of 30 µmol/g were found in 23% of men and 18% of women experiencing traumatic deaths. Substantial iron overload (hepatic iron concentration in excess of 180 µmol/g dry weight) was found in 3% of men and 11% of women suffering traumatic deaths. When subjects were classified according to the hepatic iron index, over 75% of both men and women had normal indexes of <1.0. In all, 9% of the men and 11% of the women suffering accidental death had an index equal to or above 1.9, a level comparable with that found in white homozygotes for HLA linked haemochromatosis and higher than can be explained by the effects of alcohol alone.<sup>14 23 24</sup>

Table 3 presents the histological findings for six of the seven accident victims with hepatic iron concentrations higher than 180 µmol/g dry weight. Substantial iron overload was confirmed by light microscopy, and no changes related to alcohol consumption were present. The findings were in keeping with the accepted pattern of African dietary iron overload, in that iron

deposition was present in both parenchymal and Kupffer cells and was associated with portal fibrosis and cirrhosis.

Table 4 shows that, in the combined data set of traumatic/suicidal and hospital deaths, there were significant correlations between the three hepatic iron levels on the one hand and the concentrations of iron in the spleen, heart, lungs, and skin on the other. By regression analysis, the hepatic iron concentration correlated best with the spleen iron concentration ( $r = 0.7$ ), followed by the lung iron concentration ( $r = 0.4$ ), and the heart and skin iron concentrations ( $r = 0.3$ ); a significance level of  $p < 0.001$  was found in each case.

DNA was isolated from spleen specimens from 32 of the 35 subjects with hepatic iron indexes  $> 1.0$ . None of these subjects had the C282Y mutation.

### Discussion

This study gives a significant level of the estimated prevalence of iron overload among individuals suffering traumatic or suicidal deaths in Harare, Zimbabwe. In all, 23% of the study subjects had hepatic iron concentrations above the normal range and 5% had concentrations above  $180 \mu\text{mol/g}$  dry weight, or more than five times the upper limit of normal. Histological evaluation of liver tissue in subjects with hepatic iron concentrations  $> 180 \mu\text{mol/g}$  showed prominent iron deposition in both parenchymal cells and macrophages in the liver. This observation is consistent with previous studies of African iron overload and confirms that the condition is distinct from HLA linked haemochromatosis in the white population in which iron deposition occurs predominantly in parenchymal cells.<sup>25</sup> Like haemochromatosis in whites, iron overload in this study was associated with portal fibrosis and cirrhosis.<sup>25</sup>

Only one of 59 hospital patients had a noticeably elevated hepatic iron index of more than 1.9 compared with 13 of 135 subjects suffering traumatic death ( $p = 0.07$ ) (table 2). Although the difference is not statistically significant, there is a trend towards a higher prevalence of iron overload in the traumatic death group. We do not have an explanation for this finding, but the sample size was considerably smaller for the hospital patients. Of note, the overall prevalence of increased iron stores of both moderate and marked degree as evidenced by an hepatic iron index of greater than 1.0 was similar in the hospital and trauma subjects. Analysis for a possible selection bias shows that there is a broad spectrum of cause of death in the hospital patients and that there is no evidence of selection of a particular condition or group of conditions.

None of the 32 subjects with elevated hepatic iron indexes tested in this study was positive for the C282Y mutation associated with HLA linked haemochromatosis in whites. According to the model presented in a recent study,<sup>12</sup> heterozygotes for a non-HLA-linked African iron loading locus develop iron overload only in the face of excessive dietary iron, but homozygotes may do so with normal dietary iron. If African

iron overload is purely environmental in aetiology, then the condition should be uncommon or absent in urban areas, where the brewing and consumption of traditional beer are not commonplace. The high prevalence of iron overload in this study population is compatible with the presence of homozygotes for a non-HFE related iron loading genetic defect and/or the continuing practice of drinking traditional iron laden beer among urban dwellers.

The relation between iron concentrations in the liver and other organs may provide insights into the systemic pattern of African iron overload and the susceptibility of different organs to iron loading. The results of this study (table 4) indicate that African iron overload may not just be a condition of hepatic iron loading but may be associated with a general accumulation of iron throughout the body. Like HLA linked haemochromatosis in whites, iron concentrations in the heart increased in parallel with the loading of the liver. In contrast with haemochromatosis in the white population, splenic iron concentrations also increased in parallel with hepatic iron deposition.<sup>25</sup> The significant correlation between hepatic and lung iron is interesting in view of the report that African iron overload may be a risk factor for pulmonary tuberculosis.<sup>6</sup> The significant correlation of skin iron with hepatic iron may suggest skin biopsy as an alternative to liver biopsy in the diagnosis of African iron overload, but the considerable overlap in the skin iron levels between subjects with normal and elevated hepatic iron concentrations indicates that the skin iron concentration would be a poor predictor of hepatic iron concentration for clinical purposes.

A drawback to the present study is that it was not a random sampling of the community, but such an investigation would probably have to be performed by non-invasive measures of iron status. Determination of iron status by serum levels of iron, transferrin saturation, and ferritin is limited because these tests are influenced by hepatitis,<sup>26</sup> the presence of inflammation, and alcohol consumption.<sup>27</sup> In addition, serum iron and transferrin saturation may be affected by vitamin C deficiency,<sup>28</sup> recent ingestion of iron preparations, and diurnal variation. Estimation of bone marrow iron stores is accurate in the diagnosis of iron deficiency, but is poor in distinguishing between normal iron status and iron overload states.<sup>29</sup> Non-invasive tests for hepatic iron overload include magnetic susceptometry,<sup>30</sup> computed tomography,<sup>31</sup> and nuclear magnetic resonance.<sup>32</sup> The necessary instrumentation for magnetic susceptometry is expensive and not widely available. Computed tomography and nuclear magnetic resonance can identify heavy iron overload but may not pick up iron loading in young subjects with moderately elevated hepatic iron concentrations. The chemical iron concentration of liver tissue is considered to be the definitive indication of the body's iron stores.<sup>33</sup> Randomised hepatic tissue sampling of the healthy living population was not possible because of the invasive nature of liver biopsy, but the present post mortem series comprised

otherwise healthy non-hospitalised subjects dying from trauma and suicide seemed to offer an alternative approach to estimating the population prevalence of iron overload.

Other drawbacks to this study are that we were not able to determine the history of alcohol consumption, or the hepatitis C, HIV, or haematological status of the study subjects. Alcoholic liver disease may be associated with moderate iron deposition in a minority of subjects. An hepatic iron index that adjusts the hepatic iron concentration for age has been used to distinguish homozygotes for HLA linked haemochromatosis with substantial iron overload for age from heterozygotes for HLA linked haemochromatosis and from subjects with siderosis due to alcoholic liver disease.<sup>14 24</sup> The normal hepatic iron index does not exceed 1.0, while alcoholic liver disease alone does not cause the hepatic iron index to exceed 1.7. An hepatic iron index of  $>1.9$  indicates a degree of iron overload comparable with that found in homozygotes for HLA linked haemochromatosis and is higher than the moderate degrees of iron overload that may be seen in patients with alcoholic liver disease and heterozygotes for HLA linked haemochromatosis. This hepatic iron index was calculated for each subject in our series, and 9% of men and 11% of women had an index of  $\geq 1.9$ . For comparison, we found evidence for iron overload in 11% of men in a previous population survey conducted in rural Zimbabwe using indirect measures of iron status.<sup>34</sup>

Two of the six patients with iron overload had cirrhosis. Caution is required in determining the cause of iron overload in subjects with a hepatic iron index  $>1.9$  as well as cirrhosis. In a study of 447 cirrhotic livers of patients presenting to the Mayo Clinic liver transplant programme, the investigators found that in only five of 38 patients with an hepatic iron index  $>1.9$  was haemochromatosis the cause of cirrhosis and iron overload.<sup>35</sup> The authors concluded that haemochromatosis of affected livers seems to be acquired and they hypothesise that end stage cirrhosis alone may cause heavy iron accumulation. They, however, stress that the hepatic iron index reliably distinguishes most patients with haemochromatosis from heterozygotes for the haemochromatosis gene<sup>23</sup> and from controls,<sup>14 24</sup> in particular patients with alcoholic liver disease. In explaining the differences between many earlier studies<sup>23 24 27</sup> and their own study, Ludwig and colleagues<sup>35</sup> point out that they studied iron overload in end stage liver disease, whereas other studies evaluated primarily early stage haemochromatosis. Whether or not this postulate is correct, the finding of notable iron overload in non-cirrhotic livers associated with four of 135 traumatic deaths in our study suggests that the substantial presence of iron overload is not secondary to cirrhosis in the Harare population.

The prevalence of iron overload in our study subjects cannot be taken to reflect the true prevalence in the general population because of possible sources of bias. Rather, the findings support the argument that iron overload does occur in urban areas despite the fact that the

practice of drinking traditional beer is found almost exclusively in rural areas.

In summary, our study suggests that iron overload remains an important contemporary problem in urban Zimbabwe, and that the condition is associated with serious morbidity. It is distinct from HLA linked haemochromatosis. Both gastroenterologists and medical practitioners in general should devote more attention to diagnosing, treating, and preventing this condition which is largely ignored at the present time.

This work was supported in part by a grant from the Office of Minority Health to the Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, by contract no 1HD 3-3196 from the National Institute of Child Health and Human Development, by a grant from the J F Kapnek Charitable Trust, and by a grant from the Research Board of the University of Zimbabwe.

- 1 Edwards CQ, Griffen LM, Goldgar D, et al. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med* 1988;**318**:1355-62.
- 2 Gordeuk VR. Hereditary and nutritional iron overload. *Bal-lie's Clin Haematol* 1992;**5**:169-86.
- 3 Bothwell TH, Seftel H, Jacobs P. Iron overload in Bantu subjects. Studies on the availability of iron in Bantu beer. *Am J Clin Nutr* 1964;**14**:47-51.
- 4 Bothwell TH, Bradlow BA. Siderosis in the Bantu. A combined histopathological and chemical study. *Arch Pathol* 1960;**70**:279-92.
- 5 Isaacson C, Seftel HC, Keeley KJ, et al. Siderosis in the Bantu: the relationship between iron overload and cirrhosis. *J Lab Clin Med* 1961;**58**:845-53.
- 6 Gordeuk VR, MacPhail AP, MacLaren CE, et al. Associations of iron overload in Africa with hepatocellular carcinoma and tuberculosis: Strachan's 1929 thesis revisited. *Blood* 1996;**87**:3470-6.
- 7 Gangaidzo IT, Gordeuk VR. Hepatocellular carcinoma and African iron overload. *Gut* 1995;**37**:727-30.
- 8 Moyo VM, Makunike R, Gangaidzo IT, et al. African iron overload and hepatocellular carcinoma. *Eur J Haematol* 1998;**60**:28-34.
- 9 Moyo VM, Gangaidzo IT, Gomo ZAR, et al. Traditional beer consumption and the iron status of spouse pairs from a rural community in Zimbabwe. *Blood* 1997;**89**:2159-66.
- 10 MacPhail AP, Simon MO, Torrance JD, et al. Changing patterns of dietary iron overload in black South Africans. *Am J Clin Nutr* 1979;**32**:1272-8.
- 11 Rabson AR, Hallett AF, Koornhof HJ. Generalised Yersinia enterocolytica infection. *J Infect Dis* 1975;**131**:447-51.
- 12 Gordeuk V, Mukiibi J, Hasstedt, SJ, et al. Iron overload in Africa: interaction between a gene and dietary iron content. *N Engl J Med* 1992;**326**:95-110.
- 13 Torrance JD, Bothwell TH. Tissue iron stores. In: Cook JD, ed. *Iron*. New York: Churchill Livingstone, 1980:90-115.
- 14 Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurement in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology* 1986;**6**:24-9.
- 15 Scheuer PJ. Disturbances of copper and iron metabolism. In: Scheuer PJ, ed. *Liver biopsy interpretation*. London: Balliere Tindall, 1980:189-99.
- 16 Sheehan DC, Hrapchak BB. *Theory and practice of histotechnology*. Columbus: Batelle Press, 1987.
- 17 Scheuer PJ, Williams R, Muir AR. Hepatic pathology in relatives of patients with hemochromatosis. *J Pathol Bacteriol* 1962;**84**:53-64.
- 18 Edwards CQ, Carroll M, Bray P, et al. Hereditary hemochromatosis. Diagnosis in siblings and children. *N Engl J Med* 1977;**297**:7-13.
- 19 Gordeuk VR, McLaren GD, Samowitz W. Etiologies, consequences, and treatment of iron overload. *Crit Rev Clin Lab Sci* 1994;**31**:89-133.
- 20 Friedman BM, Baynes RD, Bothwell TH, et al. Dietary iron overload in southern African rural blacks. *S Afr Med J* 1990;**78**:301-5.
- 21 Feder JN, Gnirke AA, Thomas W, et al. A novel NHC class 1-like gene is mutated in patients with hereditary hemochromatosis. *Nat Genet* 1996;**13**:399-408.
- 22 Bothwell TH, Isaacson C. Siderosis in the Bantu. A comparison of incidence in males and females. *BMJ* 1962;**1**:522-4.
- 23 Summers KM, Halliday JW, Powell LW. Identification of homozygous hemochromatosis subjects by measurement of hepatic iron index. *Hepatology* 1990;**12**:20-5.
- 24 Sallie RW, Reed WD, Shilkin KB. Confirmation of the efficacy of hepatic tissue iron index in differentiating genetic hemochromatosis from alcoholic liver disease complicated by alcoholic haemosiderosis. *Gut* 1991;**32**:207-10.
- 25 Bothwell TH, Abrahams C, Bradlow BA. Idiopathic and Bantu hemochromatosis: comparative histological study. *Arch Pathol* 1965;**79**:163-8.
- 26 Prieto J, Barry M, Sherlock S. Serum ferritin in patients with iron overload and with acute and chronic liver disease. *Gastroenterology* 1975;**68**:525-33.

- 27 Ford C, Wells FE, Rogers JN. Assessment of iron status in association with excess alcohol consumption. *Ann Clin Biochem* 1995;**32**:527–31.
- 28 Wapnick AA, Bothwell TH, Seftel H. The relationship between serum iron levels and ascorbic acid stores in siderotic Bantu. *Br J Haematol* 1970;**19**:271–6.
- 29 Bothwell TH, Charlton RW, Cook JD, *et al.* *Iron metabolism in man*. Oxford: Blackwell Scientific Publications, 1979.
- 30 Brittenham GM, Farrell DE, Harris JW, *et al.* Magnetic-susceptibility measurement of human iron stores. *N Engl J Med* 1982;**307**:1671–5.
- 31 Guyader D, Gandon Y, Deugnier Y, *et al.* Evaluation of computed tomography in the assessment of liver iron overload: a study of 46 cases of idiopathic hemochromatosis. *Gastroenterology* 1989;**97**:737–43.
- 32 Anderson PB, Birgerd G, Nyman R, *et al.* Magnetic resonance imaging in idiopathic hemochromatosis. *Eur J Haematol* 1991;**47**:174–8.
- 33 Finch CA, Huebers H. Perspectives in iron metabolism. *N Engl J Med* 1982;**306**:1520–8.
- 34 Gordeuk VR, Boyd RD, Brittenham GM. Dietary iron overload persists in rural sub-Saharan Africa. *Lancet* 1986;**i**:1310–13.
- 35 Ludwig J, Hashimoto E, Porayko MK, *et al.* Hemosiderosis in cirrhosis: a study of 447 native livers. *Gastroenterology* 1997;**112**:882–8.