Iron nutrition in Indian women at different ages


Summary
The iron status of 320 Indian women living in Chatsworth, Durban, who had volunteered for iron absorption studies, was assessed using a number of measurements. These included: radio-iron absorption, the transferrin saturation, the serum ferritin concentration and the haemoglobin concentration. In the sample as a whole, the prevalence of iron deficiency anaemia (haemoglobin concentration <12 g/dl, with two or more abnormal measurements of iron status) was 14.4%. A further 26% had depleted iron stores (serum ferritin <12 µg/l) and 8.4% also had evidence of iron-deficient erythropoiesis (serum ferritin <12 µg/l and transferrin saturation below 16%). A profile of iron status based on the cumulative frequency distribution of iron stores showed that the sample, with calculated median iron stores of 150 mg and lower and upper 10 percentiles of -355 mg and 655 mg respectively, was significantly more iron deficient than a sample of women studied in Washington, State, USA. Of interest was the observation that all measurements of iron status were better in the older age groups, presumably as a result of the cessation of menstruation. In addition, there was evidence that the duration of menstruation, as volunteered in a brief history, had a significant effect on several measurements of iron status. This was particularly true of the serum ferritin concentration and radio-iron absorption, both of which reflect the size of the iron stores.

Iron deficiency frequently occurs in women during their reproductive years. This is due to the fact that menstruation, pregnancy and lactation increase their physiological losses, while their food intake, and hence iron intake, is less than that of men.1 Iron deficiency occurs in women during their reproductive years. This is due to the fact that menstruation, pregnancy and lactation increase their physiological losses, while their food intake, and hence iron intake, is less than that of men.1 A previous investigation in Durban revealed that the prevalence of anaemia (haemoglobin <12 g/dl) in Indian women was 38% and that it was mostly due to iron deficiency.2 Over the last several years a number of iron absorption studies have been carried out in Chatsworth, Durban, by members of the joint University of the Witwatersrand-South African Medical Research Council Iron and Red Cell Metabolism Unit. In the present article the iron status of the Indian women who took part in these studies is evaluated, with particular reference to the effects of age.

Subjects and methods
Subjects
The 320 women who took part in this study came from a population of largely Indian descent living at sea level in Chatsworth, near Durban. All had volunteered to take part in a series of studies on iron absorption, published elsewhere,3 and the various indices of iron status were measured in the course of these studies. Samples were taken after informed consent had been obtained and apart from excluding children and pregnant or lactating mothers no further selection was applied.

Methods
Each of the subjects drank 50 ml of a solution containing 30 mg ascorbic acid and 3 mg iron as 59FeSO4·7H2O (3 µCi 59Fe per subject) after an overnight fast. No food or drink was allowed for 4 hours after drinking the solution. The 59Fe activity in a 4 ml blood sample taken 2 weeks later was assessed against suitable standards using a scintillation spectrometer (Auto-Gamma Tri-Carb Spectrometer Model No. 3001, Packard Instrument Co.). The percentage absorption of the iron salt was calculated on the assumption that all the absorbed radioactivity was present in the haemoglobin of circulating erythrocytes, and that the blood volume of each subject was 65 ml/kg. Haemoglobin concentrations were measured by the cyanmethaemoglobin method, serum iron concentrations by the ICSH method4 and the unsaturated iron-binding capacity by the method of Herbert et al.5 The serum ferritin concentrations were measured by radio-immunoassay using the method of Deppe et al.6

Statistical analyses
Serum ferritin concentrations followed a skewed distribution, which was normalized by logarithmic transformation. All serum ferritin results were therefore expressed as geometric means and standard deviation ranges. Standard parametric statistical methods were applied to the data except in correlation analyses, when the Spearman rank correlation coefficient (r) was used.7,8 Individual measurements of iron status were considered to be normal at the following levels: transferrin saturation below 16%,9 serum ferritin below 12 µg/l,10 radio-iron absorption above 40%,11 and haemoglobin below 12 g/dl.12

Results
The effect of age on the various measurements of iron status is shown in Fig. 1. After the age of 45 years the mean serum ferritin...
concentration rose twofold. This was mirrored by a fall in the percentage of abnormal values (<12 µg/l). Between 44% and 50% of women under 45 years, but only about 20% of older women, had abnormal serum ferritin concentrations. This increase in iron stores with age was also reflected by a fall in the mean percentage absorption of radio-iron. It was above 40% in age groups under 45 years, but fell to 32% in the 46-55-year age group and to 26% in the over 55-year age group. Similarly, the proportion of women in each age group with radio-iron absorption measurements compatible with iron deficiency (>40%) fell from more than 50% to 32% in the 45-55-year age group and to 17% in those over the age of 55 years. The effect of age on the transferrin saturation was less marked. While the percentage of abnormal values (saturation less than 16%) was lower in the older age groups, the mean transferrin saturation varied only between 22% and 29%. Haemoglobin concentrations in the anaemic range (<12 g/dl) were encountered in about 20% of the women below 45 years and in about 10% of older women. The changes which occurred after the age of 45 years in each of the indices of iron nutrition were found to be statistically significant for each of the measurements (Table I).

In a further analysis the prevalence of iron deficiency was calculated on the basis of the findings with each of the measurements. Of the 320 women in the study, 118 (36.9%) had an abnormal serum ferritin concentration (<12 µg/l), 74 (23.1%) had a transferrin saturation less than 16%, 137 (42.8%) had a radio-iron absorption in the iron-deficient range (>40%) and 52 (16.3%) were anaemic (haemoglobin <12 g/dl). Seventy-two of the subjects had values in the iron-deficient range for only one of the measurements (radio-iron absorption, serum ferritin or transferrin saturation) used to assess iron status. Radio-iron absorption was increased in 43, while 21 had a decreased serum ferritin concentration and 8 a decreased transferrin saturation. Of these 72 subjects only 3 (4.2%) were anaemic, as compared with a figure of 16.3% in the whole sample. On the other hand, when two of the measurements were abnormal the prevalence of anaemia rose to 27.9% and when three were abnormal the figure was 67.5%. It was therefore apparent that anaemia can be ascribed to iron deficiency only when at least two other measurements of iron status are abnormal.

When the group was assessed as a whole, 46 (14.4%) had iron deficiency anaemia on the basis of a haemoglobin concentration below 12 g/dl accompanied by two or more abnormal measurements of iron status. Of the remaining individuals, 83 (25.9% of the total) had a serum ferritin concentration below 12 µg/l, which indicated that their iron stores were depleted. Twenty-seven (8.4% of the total) of these subjects also had a low transferrin saturation, which meant that they had reached a stage of iron deficiency where erythropoiesis was starting to be compromised. The remaining 191 (59.7%) had normal iron status. A gradual fall in the prevalence of iron deficiency was noted with advancing age. Below the age of 45 years the prevalence of iron deficiency anaemia was 18.5%, as compared with 6.9% in older women. Corresponding figures for subjects with a normal iron status were 50.0% and 76.5% respectively. As more than 80% of the women over 45 years were menopausal or had had a hysterectomy, as compared with only 8% of women younger than 45 years, a further analysis was undertaken to assess the importance of menstrual blood loss. No definitive measurements of blood loss were available, but a brief menstrual history was obtained at the time when the subjects volunteered for iron absorption studies. On this basis, the women were divided into four groups consisting of those who were postmenopausal or hysterectomized, those in whom the duration of menstrual flow was less than 3 days, those in whom it was 3-5 days, and those in whom it was more than 5 days (Table II). The menstrual history had a significant effect on each of the measurements of iron status. The effect was most marked for those measurements reflecting the size of the iron stores, i.e. the serum ferritin concentration (F = 13.65, P<0.0001) and the radio-iron absorption (F = 10.79, P<0.001). With each measurement there was a tendency for the iron status to deteriorate with increasing menstrual blood losses. No correction for age was made, since no significant correlation was found between age and any of the measurements in premenopausal women. However, because of the large changes in iron status that occurred after the menopause there was a weak but statistically significant overall correlation between age and both the serum ferritin concentration (r = 0.27, P<0.001) and radio-iron absorption (r = -0.24, P<0.001).

In a final analysis, a comparison was made between the different measurements of iron status (Table III). In the group as a whole and in the more iron-deficient younger age group (20-

### Table I. Comparison of Measurements of Iron Status at Different Ages

<table>
<thead>
<tr>
<th>Age group</th>
<th>20 - 25 yrs (N = 205)</th>
<th>46 - 75 yrs (N = 115)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.1 ± 1.9</td>
<td>13.8 ± 1.4</td>
<td>3.89</td>
</tr>
<tr>
<td>Serum ferritin (µg/l)</td>
<td>14.0 ± 4.4 - 44.7</td>
<td>27.8 ± 10.2 - 75.4</td>
<td>5.54</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>24.3 ± 11.3</td>
<td>27.6 ± 9.7</td>
<td>2.70</td>
</tr>
<tr>
<td>Radio-iron absorption (%)</td>
<td>43.8 ± 23.1</td>
<td>29.6 ± 22.4</td>
<td>5.38</td>
</tr>
</tbody>
</table>

* Geometric mean and SD range.
TABLE II. RELATIONSHIP BETWEEN MEASUREMENTS OF IRON STATUS (MEAN ± SD) AND MENSTRUAL HISTORIES
IN 318 WOMEN

<table>
<thead>
<tr>
<th>Measurements</th>
<th>20 - 75 yrs (N = 320)</th>
<th>20 - 45 yrs (N = 205)</th>
<th>46 - 75 yrs (N = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin &amp; haemoglobin</td>
<td>0.48</td>
<td>0.53</td>
<td>0.27</td>
</tr>
<tr>
<td>Serum ferritin &amp; transferrin saturation</td>
<td>0.52</td>
<td>0.51</td>
<td>0.50</td>
</tr>
<tr>
<td>Serum ferritin &amp; reference absorption</td>
<td>-0.53</td>
<td>-0.49</td>
<td>-0.46</td>
</tr>
<tr>
<td>Transferrin saturation &amp; haemoglobin</td>
<td>0.52</td>
<td>0.61</td>
<td>0.30</td>
</tr>
<tr>
<td>Transferrin saturation &amp; reference absorption</td>
<td>-0.43</td>
<td>-0.41</td>
<td>-0.39</td>
</tr>
<tr>
<td>Haemoglobin &amp; reference absorption</td>
<td>-0.36</td>
<td>-0.41</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

P<0.01 in all instances except 1 (P<0.05) and 1 (P<0.1).

Discussion

The most useful laboratory tests for the evaluation of the iron status of a population are the serum ferritin concentration, the transferrin saturation, the free erythrocyte protoporphyrin, and the haemoglobin concentration. The serum ferritin concentration is an indicator of the most subtle grade of iron deficiency, namely the depletion of the body iron stores, and there is now ample evidence than a linear relationship exists in adults between the serum ferritin concentration and the body iron stores. In the present study, the absorption of radio-iron provided another measurement of the size of the body iron stores, since it has been shown that the amounts of iron absorbed from the gut are inversely correlated with stores. A serum ferritin concentration of less than 12 µg/l has been shown to indicate virtual exhaustion of body iron stores, while Hallberg et al. have suggested that the absorption of a 3 mg dose of iron given as ferrous ascorbate rises above 40% when body iron stores are exhausted. In the present study both of these measurements indicated that stores were low in women younger than 45 years. The geometric mean serum ferritin concentration in this group was only 14.0 µg/l and the mean radio-iron absorption was high (43.8%). While there was some improvement with age in both measurements, the increase in serum ferritin was far less than has been reported in women living in the State of Washington, USA. Whether this was due to a lack of available iron in the diet or to malabsorption of iron could not be resolved from the available data. The fact that the inverse correlation between iron stores, as measured by the serum ferritin concentration, and iron absorption was less good than has been observed in other studies suggests that malabsorption may have been a factor. However, if this were so then it would have been expected that the correlation would have been worse in older subjects, but this was not the case (Table I). An alternative and more plausible explanation for the findings is a degree of non-compliance in the women, all of whom may not have been fasting when the radio-iron absorption studies were done.

When iron stores are depleted, the supply of iron for haemoglobin synthesis is compromised. This is reflected by a transferrin saturation below 16% and a rise in free erythrocyte protoporphyrin. In the present study the mean transferrin saturation was well above 16% in all age groups. However, more than 20% of women in the age groups under 45 years had values below 16% and there was a modest but significant rise in transferrin saturation after the age of 45 (Table I). Iron deficiency anaemia only develops when the stores have been exhausted (serum ferritin < 12 µg/l) and the supply of iron for erythropoiesis is inadequate (transferrin saturation < 16%
and/or an elevated free erythrocyte protoporphyrin). In the present study there was a significant rise in the haemoglobin concentration after the age of 45 years (Fig. 1, Table I), which was accompanied by a fall in the percentage of subjects with a haemoglobin concentration below 12 g/dl. However, consideration of the haemoglobin concentration alone can be misleading. The arbitrary cut-off point for anaemia in non-pregnant women is 12 g/dl,\textsuperscript{12} but there is considerable variation in normal subjects, so that normal and anaemic individuals occur on both sides of the cut-off limit.\textsuperscript{1} In so far as iron deficiency anaemia is concerned, the haemoglobin concentration must therefore be viewed in conjunction with other measurements of iron status, such as the serum ferritin concentration, radioiron absorption, the transferrin saturation and the free erythrocyte protoporphyrin. This was well illustrated by the findings in the present study, which corroborate those obtained in a population sample in Washington State, USA.\textsuperscript{19} In both studies the prevalence of anaemia was not increased by restricting the sample to subjects having only one of the measurements of iron status in the iron-deficient range. If, however, the sample was confined to those having two or more abnormal measurements the prevalence of anaemia was about 28%, and with all three abnormal it was above 60%. A diagnosis of iron deficiency anaemia can therefore only be made if at least two abnormal measurements of iron status and a low haemoglobin concentration are present. On this basis, more than 20% of the subjects in the present study had evidence of iron deficiency, while a further 17.5% had depleted iron stores. The prevalence of all grades of iron deficiency fell off with age, so that less than 7% of women over 45 years had iron deficiency anaemia. In fact, the vast majority (83%) had a normal iron status. Although different criteria were used, our results suggest that there has been some improvement in the iron status of women living in Chatsworth since the studies conducted by Mayet.\textsuperscript{21} At the same time, two points must be stressed. Firstly, the two samples were not age-matched, and secondly, the present sample was not randomly chosen and may therefore not reflect the overall situation in adult Chatsworth women.

The important role played by the loss of iron via menstruation in the genesis of iron deficiency was clearly illustrated by the improvement in iron status that occurred after 45 years of age (Fig. 1, Table I). Although personal estimates of monthly blood losses are notoriously unreliable,\textsuperscript{22} with many women unaware that their losses are excessive,\textsuperscript{23} results in the present study indicate that there was a significant relationship between the duration of menstruation and measurements of iron status (Table II). Not surprisingly, this relationship was most clear-cut for those measurements which reflected iron stores.

Although the prevalence of various stages of iron deficiency can be effectively assessed using the various measurements previously discussed, a better overall picture can be obtained by estimating the distribution of iron stores in the sample being tested. Using a model described by Cook and Finch,\textsuperscript{24} we constructed a profile of iron status using a cumulative frequency distribution of iron stores (Fig. 2). The lower end of the curve represents those women who fulfilled the criteria of iron deficiency anaemia. The points are based on the assumption that 1 g/dl circulating haemoglobin represents about 150 mg of body iron and therefore a deficit of at least 300 mg of storage iron must occur before the haemoglobin falls from a normal value of 14 g to 12 g/dl. In the present study, haemoglobin concentrations below 12, 11, 10 and 9 g/dl were found in 12%, 7% 4% and 2% of the women, representing deficits of iron stores of 300, 450, 600 and 750 mg respectively. In the original model the points 0 mg and -150 mg stores were determined empirically from the transferrin saturation and the free erythrocyte protoporphyrin in subjects with low serum ferritin but normal haemoglobin concentrations.

Since we did not measure the free erythrocyte protoporphyrin, we took 0 mg stores to represent the percentage of women with serum ferritin concentrations below 12 µg/l and transferrin saturations below 16%, but normal haemoglobin concentrations (> 12 g/dl). The remaining points, which represent the upper portion of the curve, are based on the assumption that 1 µg/l of serum ferritin represents 10 mg of storage iron. Thus 66%, 78%, 88% and 93% of the women had iron stores below 300, 450, 600 and 750 mg respectively. With the model it was possible to compare the profiles of iron stores in two samples of women, the one from Washington State, USA,\textsuperscript{25} and the one described in our study. The regression lines were virtually parallel (difference between slopes (b), F = 2.87, P > 0.1) but the line representing the Chatsworth sample lay significantly to the left of the USA sample, and our sample was therefore more iron deficient (F between group elevations = 31.78, P<0.01). The Chatsworth population was calculated as having median stores of 150 mg iron, with lower and upper percentiles of -355 and 665 mg iron, while the corresponding figures for the Washington sample were 220, -240 and 700 mg respectively.

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REFERENCES