A new method to measure iron absorption from the enrichment of 
57Fe and 58Fe in young erythroid cells

Ellen G. H. M. van den Heuvel,* Theo Muys, Hillie Pellegrom, Joost P. Bruyntjes, Wim van Dokkum, Steven Spanhaak, and Gertjan Schaafsma

Iron absorption can be measured by the incorporation of stable iron isotopes into erythrocytes, 14 days after isotope administration. The disadvantage of this method is the high dose of isotopes needed to obtain a sufficient enrichment. Therefore, in this study cell fractions rich in young erythroid cells were prepared by using a density separation method. From 10 women blood was taken 4, 5, and 7 days after oral and intravenous administration of 57Fe and 58Fe. In these cell fractions and in whole blood taken 14 days after isotope administration, isotope enrichment was measured and absorption calculated. Absorption calculated from the isotope enrichment in the reticulocyte-rich cell fractions (12.2 ± SEM 3.7%) was not significantly different from absorption based on whole-blood values (13.0 ± 3.3%). Because a threefold higher isotope enrichment was found in the cell fractions, the required dose of stable isotopes can be reduced to one-third of the dose used in the traditional method without loss of sensitivity.

The incorporation of two radioactive iron (Fe) isotopes, given intravenously and orally, into erythrocyte hemoglobin (Hb) is a well-known method of measuring intestinal Fe absorption and yields almost the same values as the technique based on whole-body counting [1, 2]. Because of concern regarding the use of radioisotopes in human subjects, especially in infants and pregnant women, the use of stable isotopes is gaining popularity. Barrett et al. [3] measured absorption of nonheme Fe by measuring the incorporation of an intravenously and orally administered stable Fe isotope into erythrocytes of blood sampled 2 weeks after administration. This method, however, has two disadvantages. First, relatively large amounts of isotopes are needed to sufficiently enrich the circulating red cells [4], and for the oral dose this can vary from around 10% of daily Fe intake in children to five times the daily Fe intake in adults [5]. Second, the high dose of stable isotopes needed makes the method very expensive.

To reduce the required dose of stable isotopes, an alternative method was tested. This method is based on the high rate of utilization of administered Fe isotopes within 3.5 days by young erythroid cells. About 80% to 93% of newly absorbed Fe is known to be incorporated into red blood cells within 2 weeks after administration [6, 7]. About half of that amount is present in young erythroid cells in the circulating blood after 3 to 4 days of administration [6]. As erythrocytes live about 110 days, and every day only 0.9% of this cell population is replaced, the isotopes in the newly formed erythrocytes will be diluted strongly when they enter the circulation in the form of reticulocytes. By preparing cell fractions rich in young erythrocytes it should be possible to find a higher isotope enrichment.

The aim of the present study was to investigate whether a higher enrichment value could be obtained by preparing cell fractions rich in young erythrocyoid cells. Moreover, we wished to compare Fe absorption, measured by the incorporation of stable isotopes into young erythrocyoid cells of blood sampled 4, 5, or 7 days after isotope administration, with the simultaneous measurement of Fe absorption calculated from the enrichment of stable isotopes in whole blood sampled 14 days after isotope administration. The decision to prepare reticulocyte-rich cell fractions of blood taken 4, 5, and 7 days after the last days of isotope administration was based on the enrichment of 58Fe in cell fractions isolated 2, 3, 4, 5, 6, 7, and 14 days after intravenous administration of 58Fe by
four male subjects in a pilot experiment (unpublished data). On day 4 the enrichment of $^{58}\text{Fe}/^{56}\text{Fe}$ reached a plateau that lasted until 7 days after intravenous administration of $^{58}\text{Fe}$. On day 14 the enrichment in the cell fractions decreased. By preparing the cell fractions on days 4, 5, and 7 in the present study, we could find out whether a plateau enrichment was reached for both the orally and the intravenously administered isotope.

Materials and Methods

SUBJECTS

Ten healthy women ages 20 to 30 were selected for the study [body weight (mean ± SD) 62.7 ± 7.4 kg, height 172 ± 6.4 cm]. Women were chosen because they show a wide range in Fe status and therefore also in absorption. Normal health was assessed at prestudy screening, which included a medical history and physical examination. None of the subjects was taking dietary supplements or had donated blood in the month before the study. Two were smokers but agreed not to smoke during the study. The study protocol was approved by the TNO external Medical Ethics Committee and all subjects signed informed consent forms.

STABLE ISOTOPES

The stable isotopes were obtained from Chemgas in the form of Fe metal. The abundances of the different Fe isotopes in the metal, as measured by inductively coupled plasma mass spectrometry (ICP-MS), were: enriched $^{57}\text{Fe}$: 0% $^{54}\text{Fe}$, 3.0% $^{56}\text{Fe}$, 95.15% $^{57}\text{Fe}$, and 1.85% $^{58}\text{Fe}$; enriched $^{58}\text{Fe}$: 0.01% $^{54}\text{Fe}$, 0.03% $^{56}\text{Fe}$, 8.07% $^{57}\text{Fe}$, and 91.9% $^{58}\text{Fe}$.

Before administration, these isotopes were prepared as $^{57}\text{Fe}$ or $^{58}\text{Fe}$ ferric sulfate, according to the method of Barrett et al. [3]. $^{57}\text{Fe}$ and $^{58}\text{Fe}$ were mixed with 0.5 mol/L H$_2$SO$_4$ and heated to 50 °C until dissolved. To the $^{57}\text{Fe}$ and $^{58}\text{Fe}$ solutions deaerated, deionized water was added, giving a measured Fe concentration of exactly 1.52 g/L Fe and 126 mg/L Fe, respectively.

The solutions were sterilized by filtration into vials. In the morning of the test days, before the subjects arrived, $^{57}\text{Fe}^{2+}$ was reduced to $^{57}\text{Fe}^{3+}$ by adding 20 mg of ascorbic acid to 5 mL of $^{57}\text{Fe}$ sulfate or 10 mg of ascorbic acid to 2 mL of $^{58}\text{Fe}$ sulfate.

STUDY DESIGN

To avoid possible saturation of the mucosal cells with Fe, the subjects consumed a low-Fe diet for 3 days before isotope administration. The subjects also consumed a low-Fe diet during 3 days of isotope administration, to keep total Fe intake constant. The average diet contained 61 mg of Fe and 9.5 MJ of energy, of which 13% came from protein, 31% from fat, and 56% from carbohydrates, as computed from the Dutch Food Composition Table [8].

In the morning of days −3 to −1 after an overnight fast, the subjects came to the research institute. The first day a blood sample of 40 mL was taken, of which 30 mL was put in a lithium heparin tube for measurement of basal Fe-isotope ratios. The remainder was divided over sample tubes for measurement of Hb concentration, serum ferritin concentration, and transferrin saturation.

After blood sampling, 150 mL of orange juice containing ~7.5 mg of enriched $^{57}\text{Fe}^{2+}$ was drunk at the beginning of a standardized light breakfast, which consisted of white bread with sweet dressing and contained 0.8 mg of Fe. No coffee or tea was allowed until 2 h after lunch.

On days −2 and −1, 2 h after a similar breakfast, which included the orange juice with $^{57}\text{Fe}^{2+}$, ~250 μg of enriched $^{58}\text{Fe}^{2+}$ was injected intravenously and simultaneously with 10 mL of saline via a three-way tap. The opening used for the injection of the intravenous solution was flushed clean with saline. Both the oral and the intravenous doses were weighed carefully. The final mean oral and intravenous doses were 23.3 ± 0.2 (SD) mg of $^{57}\text{Fe}$ and 448.5 ± 6.6 μg of $^{58}\text{Fe}$, respectively.

After the isotope administrations on days −3 to −1, the subjects left the research institute with the diet for the rest of the day.

Four, 5, 7, and 14 days after the administration of the stable isotopes, 20 to 30 mL of blood was taken and transferred into a lithium heparin tube. Erythrocytes of the blood sampled on days −3, 4, 5, and 7 were separated by a Percoll density gradient to isolate reticulocyte-enriched cell fractions [9]. Enrichment of reticulocytes was determined by means of thiazole orange labeling of reticulocytes in combination with flow cytometric analysis.

A time schedule is given in Fig. 1.

Both the reticulocyte-enriched fractions of days −3, 4, 5, and 7 and the whole-blood samples of days −3 and 14 were prepared for ICP-MS analysis of the stable isotope ratios. From the enrichment of the $^{57}\text{Fe}/^{56}\text{Fe}$ and $^{58}\text{Fe}/^{56}\text{Fe}$ ratios over 4, 5, or 7 days in reticulocyte-enriched cell fractions or over the 2-week interval in erythrocytes of whole blood, Fe absorption was calculated according to the formula of Barrett et al. [3]:

$$\text{Absorption of } ^{57}\text{Fe}(\text{mg}) = \left[ \left( \frac{\Delta^{57}\text{Fe}^{56}\text{Fe}}{\Delta^{58}\text{Fe}^{56}\text{Fe}} \right) \times ^{58}\text{Fe}(\text{iv} + \text{po}) \right] - ^{57}\text{Fe}(\text{iv})$$

in which $\Delta^{57}\text{Fe}/^{56}\text{Fe}$ is the enrichment of $^{57}\text{Fe}/^{56}\text{Fe}$ ratio, $\Delta^{58}\text{Fe}/^{56}\text{Fe}$ the enrichment of the $^{58}\text{Fe}/^{56}\text{Fe}$ ratio, iv intravenous, and po the percentage of absorption of $^{58}\text{Fe}$ given orally. The percentage of oral absorption is calculated by:

$$\text{Percentage of Fe absorption} = \frac{\text{amount of } ^{57}\text{Fe} \text{ absorbed (mg)}}{\text{amount of } ^{58}\text{Fe} \text{ given (mg)}}$$

ANALYSIS OF IRON STATUS VARIABLES

After lysing the erythrocytes, Hb was determined by spectrometric measurement of Hb cyanide with a Sysmex K-1000 Hematology Analyzer (Toa Medical Electronics). Serum ferritin was measured by an enzyme immunoassay
(sandwich) with an AIA-600 (Tosoh Corp.). Serum Fe was measured with Ferrozine (Hach Chemical Co.) without deproteinization (Boehringer Mannheim Diagnostica). Total Fe-binding capacity (TIBC) was determined by saturating transferrin with an excess of Fe$^{3+}$ ions, followed by the addition of magnesium carbonate, which removes all the Fe not bound to serum transferrin (Boehringer Mannheim Diagnostica). Percentage of transferrin saturation was calculated as serum Fe/TIBC × 100.

**DENSITY SEPARATION**

To separate young and old erythrocytes, red blood cells were fractionated by discontinuous density gradient with Percoll (Pharmacia Biotech Benelux). A bovine serum albumin (BSA)-HEPES-buffered solution and a BSA-Percoll-HEPES-buffered solution, as described by Salvo et al. [9], were mixed to form six solutions with a final Percoll concentration of 57%, 58.5%, 60%, 61.5%, 63%, and 64.5%. Discontinuous six-layer gradients were prepared by superimposing 6 mL of each Percoll solution in a 50-mL conical tube (Becton Dickinson Labware) starting with the 64.5% solution. Optimum conditions were obtained when a quantity of 4 mL of whole blood was layered on top of the gradient. Centrifugation was carried out at 1000 rpm for 10 min at 20 °C [9]. Cell fractions from the layers with the lowest Percoll concentration were obtained with a Pasteur pipette and washed once with HEPES-buffered isotonic saline, followed by centrifugation at 300g for 10 min at 4 °C to remove Percoll. For each volunteer four blood samples of 4 mL were processed by density separation on day −3. The cell fractions for each subject were combined to obtain a cell fraction with a sufficient amount of Fe for measurement by ICP-MS. Because the combined cell fraction obtained on day −3 for some of the subjects contained a low amount of Fe, cell fractions of six 4-mL blood samples were isolated and combined on days 4, 5, and 7.

**DETERMINATION OF ERYTHROID CELLS**

To 5 μL of each combined cell fraction or whole blood, 1 mL of thiazole orange (Becton Dickinson) was added. After 30 min of incubation in the dark, the percentage of reticulocytes was measured with an Epics Elite flow cytometer (Coulter) on the basis of their fluorescence signals at 525 nm after excitation with 488-nm light. On the basis of the fluorescence and forward scatter characteristics, leukocytes and platelets were discriminated from erythrocytes. The percentage of reticulocytes gave an impression of the efficiency of the separation.

About 2.5 mL of each remaining part of the six cell fractions was resuspended in 0.5 mL of HEPES-buffered isotonic saline. Of these mixtures, the recovery of the total number of erythrocytes was determined with a cell counter (Charles Goffin, CC180). The total amount of erythrocytes gave an impression of the amount of Fe.

**ICP-MS**

After destruction of the cell fractions and whole-blood samples in a Teflon tube, Fe was isolated by selective extraction with sodium diethyldithiocarbamate (trihydrate) into CCl$_4$ and back-extraction into HNO$_3$ and prepared for ICP-MS (Perkin-Elmer Sciex, Elan 500) measurement [10].

The Fe concentration in the HNO$_3$ solution was measured by atomic absorption spectrometry. A Fe concentration of about 1.0 mg/L was necessary to produce a signal to obtain a high precision and to avoid saturation of the detection system. ICP-MS plasma was optimized to reduce the possible molecular interference at m/z 56 of ArO$^+$ [11].

During each ICP-MS run, solutions of an Fe calibrator (1.0 mg/L), blank, HNO$_3$, and a sample were analyzed for the $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe isotope ratios. The results of the samples were corrected for the small interference of ArO$^+$ at $^{56}$Fe. Minor adjustments in bias were made for unknown factors by comparing calibrators with accepted natural abundances. All samples were measured in duplicate. All cell fractions and whole-blood samples from one subject were analyzed within 1 day to reduce variability. Therefore, the measurement of basal ratios in whole-blood or cell fractions of all subjects had to be spread over several days.

The precision of the $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe isotope ratios in cell fractions was tested in a prestudy in which the basal ratios in three cell fractions of three subjects were isolated and analyzed in duplicate by ICP-MS within 1 day. The mean basal $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe ratios of these isolated cell fractions were 0.0245 (CV 1.1%) and 0.00305 (CV 2.0%) and the within-subject CV was 0.31% and 0.98%, respectively.

**STATISTICS**

Statistical comparisons between Fe absorption calculated from the enrichment measured in whole blood on day 14 and Fe absorption based on the isolated cell fractions were made by using the technique of Bland and Altman [12]. Briefly, comparison of Fe absorption determined by the two methods ($x_a$ and $x_b$) is made by calculating the
Because the differences are normally distributed, 95% of them will lie within 2 SD from the mean difference ($d_{ab}$). Two methods are said to give results that agree when the 95% confidence limits are sufficiently narrow.

Moreover, the correlation between Fe absorption determined by the two methods and the correlation between serum ferritin and Fe absorption were calculated by correlation analyses \[13\]. Because serum ferritin did not have a symmetric distribution, the logarithm was used in statistical analysis.

### Results

All subjects completed the study. Because of illness (cystitis and fever) from day \(-6\) to day 6, subject 6 did not adhere to the distributed diet and no blood was sampled on day 4. We have no evidence that fever and cystitis affect iron incorporation into erythroid cells. The administration of $^{58}$Fe to subject 3 was only partly intravenous.

Table 1 shows the mean percentage of reticulocytes, the enrichment values of the $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe ratios in whole blood, and the cell fractions and the corresponding absorption values.

The reticulocyte percentage of the cell fraction prepared on day \(-3\) was 3.8 ± 1.8 and differed significantly from the percentages of reticulocytes present in the isolated cell fractions prepared on days 4, 5, or 7. The mean amount of Fe per erythrocyte was 8.8 fg.

The enrichment of $^{58}$Fe/$^{56}$Fe correlated significantly with the reticulocyte percentage in the cell fractions isolated on days 4, 5, and 7 (n = 29, \(r = 0.74, P < 0.001\)). Also, the amount of $^{58}$Fe incorporated into the erythroid cells correlated significantly with the reticulocyte percentage (n = 29, \(r = 0.41, P < 0.05\)).

Table 2 shows the individual absorption percentages determined by the two different methods. In Fig. 2, the y-axis shows the difference between Fe absorption calculated from the enrichment of Fe isotopes in whole blood and Fe absorption calculated from the isotope enrichment in the isolated cell fractions of days 4, 5, or 7, respectively.

The average of the two methods is shown on the x-axis. The ±2SD confidence interval for the differences is also shown. The results indicate that the Fe absorption calculated from the enrichment in the isolated cell fractions of days 4, 5, or 7 could be expected to differ by as much as \(\pm 4.6\%\), \(\pm 3.9\%\), and \(\pm 2.9\%\), respectively, from the “old” method. No significant differences were found between the “new” and “old” methods. As expected, Fe absorption calculated from the isotope enrichment in cell fractions isolated on days 4, 5, or 7 correlated significantly with Fe absorption calculated from the isotope enrichment in whole blood (\(r = 0.96, r = 0.93,\) and \(r = 0.96,\) respectively).

Among subjects a broad range in Fe status was found. The mean (±SD) Hb, serum ferritin, TIBC, and transferrin

### Table 1. Percentage of reticulocytes and enrichment of $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe in whole blood and cell fractions from which absorption was calculated.

<table>
<thead>
<tr>
<th></th>
<th>Isolated cell fractions</th>
<th></th>
<th>Whole blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4(^{c})</td>
<td>Day 5</td>
<td>Day 7</td>
</tr>
<tr>
<td>Reticulocytes in whole blood, %</td>
<td>1.0 ± 0.1 (0.5–1.7)</td>
<td>1.1 ± 0.1 (0.5–1.9)</td>
<td>1.0 ± 0.1 (0.5–1.8)</td>
</tr>
<tr>
<td>Reticulocytes in cell fractions, %</td>
<td>6.6 ± 1.1 (2.8–10.9)</td>
<td>6.3 ± 1.2 (1.7–13.1)</td>
<td>5.6 ± 1.0 (1.8–12.0)</td>
</tr>
<tr>
<td>Enrichment of $^{57}$Fe/$^{56}$Fe, %</td>
<td>16.5 ± 5.4 (1.8–47.9)</td>
<td>15.6 ± 4.7 (1.2–43.9)</td>
<td>16.7 ± 4.9 (1.9–47.8)</td>
</tr>
<tr>
<td>Enrichment of $^{58}$Fe/$^{56}$Fe, %</td>
<td>22.8 ± 2.6 (12.3–36.0)</td>
<td>20.5 ± 3.0 (7.8–36.0)</td>
<td>18.6 ± 1.6 (10.7–25.9)</td>
</tr>
<tr>
<td>Absorption, mg</td>
<td>2.7 ± 0.8 (0.2–7.5)</td>
<td>2.9 ± 0.8 (0.1–7.7)</td>
<td>3.1 ± 0.8 (0.2–8.5)</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± SEM (range).

\(^{b}\) n = 9.

\(^{c}\) Significantly different from the cell fractions (\(P < 0.05\)).

\(^{d}\) Significantly different from the cell fractions (\(P < 0.001\)).

### Table 2. Individual absorption percentages, calculated with the enrichment of $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe in whole blood and in the isolated cell fractions obtained several days after isotope administration.

<table>
<thead>
<tr>
<th>Subject</th>
<th>New method</th>
<th>Traditional method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4</td>
<td>Day 5</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>31.9</td>
<td>32.8</td>
</tr>
<tr>
<td>3</td>
<td>7.5</td>
<td>6.9</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
<td>5.3</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>14.3</td>
</tr>
<tr>
<td>6</td>
<td>5.1</td>
<td>3.8</td>
</tr>
<tr>
<td>7</td>
<td>10.4</td>
<td>12.7</td>
</tr>
<tr>
<td>8</td>
<td>25.6</td>
<td>28.0</td>
</tr>
</tbody>
</table>

Mean ± SEM 11.6 ± 3.6 12.5 ± 3.4 13.4 ± 3.5 13.0 ± 3.3
saturation were 130.2 ± 7.1 g/L, 27.0 ± 26.6 μg/L, 75.6 ± 16.6 μmol/L, and 22.4 ± 8.6%, respectively. Fe absorption calculated from the enrichment of $^{57}$Fe/$^{56}$Fe and $^{58}$Fe/$^{56}$Fe in the cell fractions of days 4, 5, and 7 and whole blood correlated significantly with serum ferritin (see Fig. 3). No significant differences were found between these correlation coefficients.

**Discussion**

In the present study, reticulocyte-rich cell fractions were obtained a few days after isotope administration to find out whether a higher enrichment of the isotopes in these fractions can be achieved than in whole blood, so that the dose of isotopes can be reduced. To determine the efficiency of the density separation method the reticulocyte percentage was measured in the prepared cell fractions. A four- to sixfold higher percentage of reticulocytes was found in the cell fractions than in whole blood. The lower reticulocyte percentage in the cell fractions obtained on day −3 as compared with the cell fractions obtained on days 4, 5, and 7 could be attributed to the difference in total amount of blood separated on day −3 vs days 4, 5, or 7. Moreover, the density of the batch of Percoll (1.130 kg/L) used for density separation on days 4, 5, and 7 was 0.003 kg/L higher than the Percoll density used on day −3.

In the study of Barrett et al. [3] the average enrichment of the $^{58}$Fe/$^{56}$Fe and $^{57}$Fe/$^{56}$Fe ratios in whole blood of five women after intravenous and oral administration of ~520 μg of $^{58}$Fe and 8.5 mg of $^{57}$Fe was 11.9% (range 9.6–13.8%) and 4.8% (range 1.9–7.4%), respectively. In the present study the enrichment of the $^{58}$Fe/$^{56}$Fe ratio in whole blood was lower because of a lower dose of $^{58}$Fe and because subject 3 got the injection only partially intravenously. Without subject 3, the average enrichment of the $^{58}$Fe/$^{56}$Fe ratio was 7.5% (6.0–9.6%), resulting in an average Fe incorporation of 82.8% (69–99%), which is comparable with the average incorporation of $^{58}$Fe of 80.8% (range 68–93%) found by Barrett et al. [3]. Although the incorporation of $^{58}$Fe in subject 3 was only 40.2%, the enrichment of the $^{58}$Fe/$^{56}$Fe ratio in subject 3 was included in the calculation of absorption, because no estimation of incorporation of absorbed Fe in the cell fractions is available as it is for the incorporation in whole blood after 14 days of isotope administration. Therefore, the absorption value is probably not the correct percent-
In the cell fractions a threefold higher isotope enrichment was found than in whole blood. The enrichment of the $^{58}\text{Fe}/^{56}\text{Fe}$ and $^{57}\text{Fe}/^{56}\text{Fe}$ ratios in the cell fractions did not differ between days 4, 5, or 7. This indicates that each of these days is equally suitable for blood sampling. As could be expected, the incorporation of $^{58}\text{Fe}$ and $^{57}\text{Fe}$ into erythroid cells was related to the percentage of reticulocytes in the cell fractions.

Because the enrichment of both $^{57}\text{Fe}/^{56}\text{Fe}$ and $^{58}\text{Fe}/^{56}\text{Fe}$ was measured in the same cell fraction, the calculated absorption percentages are independent of the number of young erythroid cells in these cell fractions. Therefore, no significant difference was found between absorption percentages calculated from the isotope enrichment in the cell fraction of days 4, 5, and 7. The standard deviation of these absorption percentages was 1.95% absorption and is in accordance with the precision of the ICP-MS analyses.

Fe absorption calculated from the enrichment of both $^{57}\text{Fe}/^{56}\text{Fe}$ and $^{58}\text{Fe}/^{56}\text{Fe}$ measured in cell fractions of days 4, 5, or 7 did not differ significantly from Fe absorption calculated from the enrichment of the isotope ratios in whole blood. Moreover, a clear correlation was found between serum ferritin and Fe absorption on the basis of isoencephalonic enrichment in cell fractions or whole blood, indicating that both methods measure Fe absorption to be expected on a physiological basis, as Fe absorption is inversely related to Fe status [14]. The differences between Fe absorption percentage on the basis of the cell fractions of days 4, 5, or 7 and absorption on the basis of whole blood were between $-0.38\%$ and $+0.58\%$. Although a maximum enrichment should be present, to estimate absorption, the day (4, 5, or 7) on which the cell fractions are obtained does not seem to matter.

In conclusion, a threefold higher enrichment of both ratios was found in the reticulocyte-enriched cell fractions than in whole blood. This, together with the significant correlation between enrichment of $^{58}\text{Fe}/^{56}\text{Fe}$ and the reticulocyte percentage in isolated cell fractions, demonstrates that it is possible to achieve a higher enrichment with the same dose of stable isotopes by preparing cell fractions with young erythroid cells. Absorption on the basis of the isotope enrichment values in the cell fractions rich in young erythroid cells was essentially the same as absorption calculated from the isotope enrichment in whole blood. As the enrichment is three times higher, the required dose of stable isotopes can be reduced pro rata.

When labor is included, this results in a total cost savings of 35% for women and 55% for men.

We are grateful to Jan Catsburg and Wilfred Sieling for technical assistance.

References