Studies in Iron Absorption V. Effect of Gastrointestinal Factors on Iron Absorption

By S. Höglund and P. Reizenstein

Previous studies showed that general systemic factors like the hemoglobin concentration, serum iron concentration, iron binding capacity, and the plasma iron clearance rate were not correlated to the intestinal iron absorption. Neither did parenteral iron treatment normalize high iron absorption.\(^1\)

The purpose of the present study is to examine such local intestinal factors as the quantity and quality of the iron and of the food present in the intestinal lumen, and also the mucosal iron. Previous studies of, e.g., the absorption of iron from various foodstuffs have been reviewed.\(^2\)\(^3\)

Materials

The effect of the iron dose and of ascorbic acid, food, and iron therapy on radioiron absorption was studied. A total of 240 absorption studies were performed in 150 persons.

All studies were performed in healthy male and female volunteers. Normal iron absorption values were established in 24 male and 33 female volunteers.\(^4\) The studies of the effect of food were performed in 33 volunteers, 29 male and 4 female.

Four qualities of iron labelled with \(^{59}\)Fe were used: Ferrous sulphate, (Abbott, specific activity 10.3 mCi/mg. iron, concentration 3.2 \(\mu\)g. iron/ml.) ferrous fumarate, and two qualities of metallic reduced iron prepared by Amersham and Studsvik, Sweden, respectively. In the "coarse" type of reduced iron 48\% of the particles were over 30 \(\mu\) and 23\% between 20 \(\mu\) and 30 \(\mu\), while the "fine" type of reduced iron had 0.1\% of the particles over 10 \(\mu\) and 97\% about 5 \(\mu\). The iron enrichment of the flour was 40 mg./Kg., and the total iron content thus became in flour 50 mg./Kg., and in bread 35 mg./Kg.

For the studies of the effect of iron dose and of ascorbic acid, ferrous fumarate was used because convenient radioactive tablets could be obtained (Ferrosan Drug Co., Malmö). The tablets were labelled with 2-3 \(\mu\)Ci \(^{59}\)Fe. One kind contained only 10 mg iron, the other also 200 mg. ascorbic acid. It has been demonstrated previously that no significant absorption difference exists between ferrous sulphate and ferrous fumarate.\(^5\)

To study the effect of sifted flour on the absorption of iron used to enrich flour, 30 Gm. slices of bread baked with sifted wheat flour were used. Each slice contained about 1 mg. iron and 1-1.5 \(\mu\)Ci \(^{59}\)Fe.

The absorption of the reduced iron customarily used to enrich flour and of ferrous sulphate was studied. The baking was performed thanks to Dr. T. Widhe, Swedish Cooperative Society.

To study the difference between the effect of a carbohydrate-rich and a fat-rich meal upon iron absorption, two standard meals were prepared and called "porridge" and "porridge and cream," respectively, where the "porridge" was prepared from nonsifted or

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Table 1.—Composition of the Meals Studied

<table>
<thead>
<tr>
<th>Meal Classification</th>
<th>Fat Gm.</th>
<th>Carbohydrate Gm.</th>
<th>Protein Gm.</th>
<th>Iron mg.</th>
<th>Iron Absorption Index</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sifted flour (60 Gm.)</td>
<td>1.9</td>
<td>31.1</td>
<td>5.1</td>
<td>1.1</td>
<td>1.041</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coarse ground flour *</td>
<td>2.6</td>
<td>30.4</td>
<td>8.7</td>
<td>1.6</td>
<td>0.226</td>
<td>0.164</td>
<td></td>
</tr>
<tr>
<td>Coarse ground flour † and cream</td>
<td>37.5</td>
<td>28.5</td>
<td>7.5</td>
<td>1.5</td>
<td>0.279</td>
<td>0.124</td>
<td></td>
</tr>
<tr>
<td>Complete meal 1</td>
<td>25.5</td>
<td>44.3</td>
<td>16.0</td>
<td>2.7</td>
<td>0.084</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 36 Gm. hulled oats and 100 Gm. skimmed milk. The hulled oats contain approximately 6.0 µg. phytic acid phosphorous per Gm.11-14
† 36 Gm. hulled oats and 100 Gm. whipping cream.
‡ Consisting of approximately 2 slices of bread (30 Gm.) and butter (10 Gm.), ham (20 Gm.) and lettuce (10 Gm.), tomato (10 Gm.), cheese (20 Gm.), 1 cup of coffee (100 Gm.) and 2 sweet rolls (50 Gm.).
§ Mean for men and women compared to normal absorption. The index is lower in women who have a higher normal absorption.

Coarse-ground flour. To study the difference between coarse-ground and sifted flour, hulled oats (porridge) and wheat bread were compared. In addition, a “complete meal” was studied, data about which were taken from a previous publication. The composition of the different meals is shown in Table 1.

The radioiron was added to the porridge in the form of ferrous sulphate. The effect of a complete meal on iron absorption was studied by giving the patients a ferrous sulphate solution immediately after eating.

The studies of the effect of the luminal iron concentration and of ascorbic acid were performed in 25 healthy female volunteers. In all these volunteers, a careful history was taken to exclude blood donors and patients receiving medical attention.

To study the effect of oral iron treatment, i.e., of a possible intracellular iron concentration increase in the intestinal mucosa, subjects with iron deficiency but otherwise healthy were desired. Twenty-six male blood donor volunteers were selected. They are described elsewhere.

Methods

The methods used for determination of serum iron concentration, total iron binding capacity, plasma iron clearance rate and iron absorption have been described, as have the statistical methods. Iron absorption was measured using radioactive iron and a whole body counter. The radioactive background of fasting subjects was registered and 0.25 mg. $^{59}$Fe administered in a drink of water. One hour after administration the 100 per cent radioactivity value was measured, and 2 weeks later the body retention of the test dose was registered.

Studies of the effect of ascorbic acid, fat and oral iron treatment on absorption were planned as crossover studies, i.e., each person served as his own control. It was not possible to perform all studies in a single group of subjects for practical reasons and because the number of permissible tracer studies in healthy controls is limited. For this reason, and because iron absorption in, e.g., men is not directly comparable to that in women, an “iron absorption index” is used. It is the ratio between the mean absorption found in the group given a particular nutrient or form of iron, and the normal mean absorption of ferrous iron in the relevant comparison group.

Results and Discussion

Luminal Iron Concentration

Previous studies of the relation between the iron absorption and the dose of iron used have been reviewed. Table 2 shows the present results, and...
Table 2.—Effect of Luminal Iron Concentration

<table>
<thead>
<tr>
<th>Oral Iron Dose</th>
<th>No. of Subjects</th>
<th>Mean Age, Years</th>
<th>Mean Serum Iron Conc. mg./100 ml.</th>
<th>Iron Absorption, Per Cent of Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>0.25 mg. iron as ferrous sulfate</td>
<td>33</td>
<td>26</td>
<td>0.116</td>
<td>43.5</td>
</tr>
<tr>
<td>10 mg. iron as ferrous fumarate</td>
<td>25</td>
<td>22</td>
<td>0.108</td>
<td>17.6</td>
</tr>
</tbody>
</table>

Fig. 1.—Absorption of iron from different iron doses. Data from literature (circles) and present investigations (squares). The oblique lines are regression lines for men (open circles) and women (closed circles) respectively. The present data, open square (men) and closed squares (women), have been superimposed.

Figure 1 shows the good agreement between present and previous data. It is also seen that the luminal iron concentration does influence absorption—the percentage absorption decreases with increasing concentration. The difference is statistically significant (P < 0.01). However, a fortyfold increase in concentration decreases absorption by little more than one half.
Table 3.—Effect of Quantity of Bread Eaten on Iron Absorption

<table>
<thead>
<tr>
<th>Form of Iron</th>
<th>Amt. of Bread (Gm.)</th>
<th>Iron Content (mg.)</th>
<th>No. of Subjects</th>
<th>Mean Absorption</th>
<th>S.E. of Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{59}$Fe-metallic</td>
<td>25</td>
<td>0.8</td>
<td>5</td>
<td>12.1</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>3</td>
<td>8</td>
<td>7.0</td>
<td>2.9</td>
</tr>
<tr>
<td>$^{59}$Fe SO$_4$</td>
<td>40</td>
<td>1.3</td>
<td>4</td>
<td>26.8</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>3</td>
<td>4</td>
<td>12.6</td>
<td>5.9</td>
</tr>
</tbody>
</table>

* Radioiron given as fine grain reduced iron used to enrich flour.

Fig. 2.—Absorption response to dose of iron salt (lines) and bread iron (circles). The oblique lines represent normal absorption of iron salt in men (left) and women (right). The figure shows that the absorption response is as good for bread iron as for iron salt, in spite of an increasing bulk of bread.

Table 3 shows the effect of food quantity upon iron absorption. Numerically, a decrease in absorption is seen for both metallic and soluble iron when the quantity is increased, but statistically it is not significant. Not only the food quantity, but also the increased iron content of the intestine, from...
Table 4—Effect of Ascorbic Acid *  

<table>
<thead>
<tr>
<th>Oral Dose</th>
<th>No. of Subjects</th>
<th>Iron Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg. ferrous fumarate</td>
<td>9</td>
<td>Mean 9.8</td>
</tr>
<tr>
<td>10 mg. ferrous fumarate +</td>
<td>200 mg. ascorbic acid</td>
<td>9</td>
</tr>
</tbody>
</table>

* Absorption given as per cent of administered dose. Iron and ascorbic acid were combined in a pharmaceutically stable tablet.

0.8 to 3.0 mg. Fe, must be considered. As may be calculated from Figure 1 and Table 2 the increased iron dose alone would be expected to cause a maximum decrease in absorption of 10 per cent of the administered dose, i.e. from 33 to 23 per cent, (Fig. 1) and it is thus probable that volume increase alone does not significantly decrease iron absorption. This is further illustrated in Figure 2 where the oblique lines indicate the correlation between FeSO₄ dose and absorption and where absorption from the different bread iron doses falls within or close to this normal area.

Ascorbic acid facilitates absorption even of soluble ferrous iron. Table 4 shows that with the pharmaceutical preparation used (Ferrocevit, Ferrosan, Malmö, Sweden) a statistically significant threefold increase in absorption is obtained in healthy controls. The effect found is larger than the 50 per cent increase described earlier, probably because of differences in method and material.

The present results do not prove that therapeutic benefit is obtained by giving combinations of iron and ascorbic acid to patients with manifest iron deficiency, who have a high absorption of iron even without ascorbic acid. However, even blood donors with a probable iron deficiency do seem to absorb more iron in combination with ascorbic acid (and succinic acid). This suggests that such a benefit may be obtained. Also, an ascorbic acid supplement in itself, especially to persons with nutritional defects, may be valuable—possibly more so than a succinic acid supplement. Since most people forget to take their tablets unless they are taken with meals, the ascorbic acid may also be useful in overcoming the absorption-inhibition caused by food.

Bread baked from sifted wheat flour did not appear to inhibit the absorption of ferrous iron (Tables 1 and 5). Since about half of the iron in Swedish diets derives from bread, and since iron deficiency is a common disease, this finding may be of relevance.

The mean iron absorption after coarse ground flour porridge was lower than that found after bread baked from sifted flour (Table 5), but these studies were not performed in the same subjects. It is nevertheless quite probable that the difference is due to the hull fraction present in the coarse ground flour.

No further decrease in absorption resulted when fat was added to the porridge in spite of the possibility that iron soaps may be formed in the intestine.

Thus, neither the lumenal iron concentration (Fig. 1), nor the food volume alone (Fig. 2), nor carbohydrates (cereals) or fat (cream) alone (Table 5)
Table 5.—Iron Absorption with Food

<table>
<thead>
<tr>
<th>Main* Food Constituents</th>
<th>Approximate Iron Quantity mg.</th>
<th>No. of Patients</th>
<th>Subject Category</th>
<th>Percentage Absorption of Iron Mean ± S.E.</th>
<th>Confidence Level Statistical Significance of Inhibition †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sifted flour</td>
<td>1</td>
<td>8</td>
<td>Men</td>
<td>17.5 ± 5.3</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Coarse ground flour</td>
<td>1.6</td>
<td>3</td>
<td>Men</td>
<td>4.3 ± 2.6</td>
<td>0.001 &lt; P &lt; 0.01</td>
</tr>
<tr>
<td>(Porridge)</td>
<td>4</td>
<td>7</td>
<td>All</td>
<td>6.5 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Coarse ground flour</td>
<td>1.5</td>
<td>3</td>
<td>Men</td>
<td>5.3 ± 3.7 †</td>
<td>0.001 &lt; P &lt; 0.01</td>
</tr>
<tr>
<td>+ fat</td>
<td>4</td>
<td>7</td>
<td>Women</td>
<td>4.9 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>(Porridge and cream)</td>
<td></td>
<td></td>
<td>All</td>
<td>5.1 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Complete meal</td>
<td>2.9</td>
<td>7</td>
<td>Men</td>
<td>1.6 ± 0.2 †</td>
<td>0.001 &lt; P &lt; 0.01</td>
</tr>
</tbody>
</table>

* Obviously some fat and protein is present in all foods studied.
† The mean difference, in the same subjects, between iron absorption from porridge and porridge and cream was 0.5 ± 3.6 per cent.
‡ The same subjects absorbed 18 ± 1.5 per cent of ferrous sulfate.6
§ All mean values compared to 24 normal men (19.0 ± 2.3) and 27 normal women (39.7 ± 4.4).

seem to explain the inhibition of absorption seen after a complete meal.6 The present studies do not show whether this inhibition is a sum effect of the effects already mentioned, or whether it is secondary to a particular food constituent not yet examined. Neither fat nor sifted flour per se decreased absorption in a statistically significant fashion, but coarse-ground flour seems to, and a complete meal does.

The sifted wheat flour contained about 0.08 mg. phytic acid phosphorous per mg. iron (an equimolar amount would be about 3.2 mg.) and the coarse ground oats contained about twice as much. It is believed that phytase, which is present in the wheat flour but not in the oats, is activated during the baking process and splits the phytic acid molecule, thereby interfering with its possibility to bind iron.

Higher extent of phytic acid and lack of phytase in coarse ground flour compared to sifted flour may partly explain the greater inhibition of iron absorption.

Iron Quality

Table 6 demonstrates that the grain size of reduced iron is important for iron absorption. The coarser the reduced iron, the less is absorbed, but even of the fine quality considerably less is absorbed than of ferrous iron. The difference is statistically significant (Table 7). These findings may be important for three reasons: Two thirds of the bread iron is derived from enrichment; bread is responsible for about 50 per cent of the iron in Swedish food; and iron deficiency is a common condition.4 It is conceivable that the decreased intake of iron secondary to decreased caloric requirements must be compensated by a qualitatively and quantitatively superior iron enrichment of the food.
Table 6.—Absorption of Iron Used to Enrich Bread *

<table>
<thead>
<tr>
<th>Form of Iron in Bread</th>
<th>Grain Size</th>
<th>No. of Measurements</th>
<th>Absorption, % M S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced iron</td>
<td>Fine †</td>
<td>13</td>
<td>9.0 ± 2.3</td>
</tr>
<tr>
<td>Reduced iron</td>
<td>Coarse ‡</td>
<td>9</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>—</td>
<td>8</td>
<td>19.8 ± 5.7</td>
</tr>
</tbody>
</table>

* 1 slice bread (30 Gm.) contains 1 mg. radioiron added to the flour.
† 0.1 per cent > 10 μ, 97 per cent about 5 μ.
‡ 48 per cent > 30 μ, 23 per cent about 25 μ.

Table 7.—Statistical Significance of Some Differences in Crossover Studies

<table>
<thead>
<tr>
<th>Effect Studied</th>
<th>Difference Between</th>
<th>No. of Subjects</th>
<th>Difference, per cent of dose ± S.E. of mean</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>Absorption with and without ascorbic acid</td>
<td>9 female</td>
<td>19.3 ± 4.2</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Form of iron in bread</td>
<td>⁵⁹Fe-absorption after FeSO₄ in bread and coarse metallic iron</td>
<td>8 male</td>
<td>17.4 ± 5.3</td>
<td>0.01 &lt; P &lt; 0.02</td>
</tr>
<tr>
<td>Complete meal</td>
<td>⁵⁹Fe-absorption in subjects fasting and after meal (6)</td>
<td>6 male</td>
<td>16.1 ± 1.3</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>“Mucosal iron”</td>
<td>⁵⁹Fe-absorption before and after oral iron treatment</td>
<td>26 male blood donors</td>
<td>19.7 ± 6.0</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

“Mucosal Iron Concentration”

The quotation marks indicate that it is only an assumption that the effect of oral iron treatment upon iron absorption can be attributed to “mucosal iron concentration.” The assumption is supported by the finding that neither a fortyfold increase of the lumenal iron concentration (Table 2) nor parenteral iron treatment had a comparable effect.

The results of the oral treatment are seen in Table 7. Although the calculated increase in body iron after a month of treatment is only about one fortieth of the normal total body iron, and although a similar increase by parenteral treatment did not affect absorption significantly, iron absorption is halved. The decrease in iron absorption was statistically highly significant (Table 7). The relation between this decrease and concomitant changes in general systemic factors is discussed elsewhere.¹ A further study is in progress to examine the time relation between oral treatment and the normalization of absorption.

Summary

1. Since previous studies could not demonstrate that any of several general plasma factors played a major role in intestinal iron absorption, local intestinal factors were examined in 240 iron absorption studies on 150 healthy subjects.
2. When the iron dose was increased 40 times, from 0.25 to 10 mg, the percentage absorption was halved.
3. Trebling the quantity of food (bread) in the intestine did not significantly decrease absorption.

4. Ascorbic acid in the intestinal lumen trebled the absorption even of ferrous iron. A stable pharmaceutical combination of iron and ascorbic acid was tested.

5. Sifted flour did not seem to inhibit the absorption of ferrous iron, but coarse ground flour did. When fat was added, no further decrease in absorption was found although iron soaps may be formed.

6. A further decrease in absorption was found after a complete meal.

7. When fine grain reduced iron was used to enrich flour—this is done in all Swedish flour—absorption was 50 per cent lower, and when a coarser grain reduced iron was used 85 per cent lower, than when ferrous sulfate was used for enrichment.

8. When oral iron treatment was given to persons with high iron absorption, absorption was decreased to normal.

**SUMMARIO IN INTERLINGUA**

1. Viste que previe studios non succeedeva a demonstrar que ulle de piure general factores plasmatic ha un rolo major in le absorption intestinal de ferro, local factores intestinal esseva examinate in 240 studios del absorption de ferro in 150 subjectos normal.

2. Quando le dose de ferro esseva augmentate ab 0,25 ad 10 mg, i.e., per un factor de 40, le absorption procentual esseva reducite per un medietate. Le triplication del quantitate de alimento (pan) in le intestino non reducea le absorption de maniera significative.

3. Acido ascorbic in le lumine intestinal triplicava le absorption mesmo de ferro ferrose. Un stabile combination pharmaceutic de ferro e acido ascorbic esseva testate.

4. Farina cribrate non pareva inhibir le absorption de ferro ferrose, sed farina molite plus grossiermente habeva iste effecto. Quando grassia esseva addite, nulle declino addicional in le absorption esseva trovate, ben que le formation de sapon a ferro occurreva.

5. Un declino additional in le absorption esseva trovate post un repasto complete.

6. Cuando un reduce ferru a grano fin esseva usate pro inricchir le farina — isto es le costume in Sveda pro omne farina — le absorption esseva plus basse per 50 pro cento, e quando reduce ferru a grano plus grossier esseva usate, illo esseva plus basse per 85 pro cento que quando sultato ferru esseva usate in le inricchimento.

7. Quando un tractamento oral a ferro esseva applicate a subjectos con un alte absorption de ferro, le absorption esseva reducite a nivellos normal.

**REFERENCES**


9. Zackrisson, L., and Reizenstein, P.: A method for mapping relations between clin-
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Studies in Iron Absorption V. Effect of Gastrointestinal Factors on Iron Absorption

S. HÖGLUND and P. REIZENSTEIN