DAIRY FOODS RESEARCH PAPERS

Bioavailability of Iron-Milk-Protein Complexes and Fortified Cheddar Cheese

DEJIA ZHANG and ARTHUR W. MAHONEY
Department of Nutrition and Food Sciences
Utah State University
Logan 84322-8700

ABSTRACT

Iron fortification is used to increase dietary iron intake. Dairy products are widely consumed but contain almost no iron. Cheddar cheese was fortified with ferric chloride or iron-casein, ferrisucrose-phosphate-whey protein, and iron-whey protein complexes. Hemoglobin regeneration efficiency was determined to evaluate iron bioavailability. Maximal and basal iron bioavailabilities were measured in anemic weanling rats fed low iron diets (about 22 mg iron/kg) and normal adult rats fed high iron diets (about 145 mg iron/kg) of iron density (32 mg iron/1000 kcal) found in some high iron human diets. Maximal iron bioavailabilities for ferric chloride or iron-casein, ferrisucrose-phosphate-whey protein, and iron-whey protein complexes were 85, 71, 73, and 72%, respectively, and for the respective iron-fortified cheeses they were 75, 66, 74, and 67%. Differences were not significant in maximal iron bioavailabilities among iron sources and between fortified cheeses and fortification iron sources. Basal iron bioavailabilities for 10-d feeding of the respective fortification iron sources were 5, 8, 6 and 7%, respectively, and 4, 4, 3, and 3% for 14 d feeding; the differences among the iron sources were not significant. Maximal and basal iron bioavailabilities of ferrous sulfate were 85 and 5%, respectively. Practical implications of these observations are discussed.

INTRODUCTION

Iron deficiency anemia is still the most prevalent nutritional problem in the US and world (33). Infants and children, adolescents, pregnant women, women at child bearing age, and the elderly are the population groups most vulnerable to iron deficiency (3, 10). In the British Isles, 40% of the adolescents (94 out of 234) are iron depleted based on serum ferritin concentration of less than 10 ng/ml (2). It is hard to increase iron intake by dietary manipulation because some frequently consumed foods contain very little iron. Thus, to increase dietary iron levels, iron is fortified into various food products. Dairy products are widely consumed, providing high quality proteins, vitamins, and minerals except iron. Lack of iron in dairy products decreases the iron density of diets when the proportion of dairy products in the diets increases (14), so it is logical that fortifying dairy products with iron may increase dietary iron density of the people who consume large amounts of dairy products. In a previous study, Cheddar cheese fortified with several levels of different iron sources was of good quality (41). Information is needed on iron bioavailability of cheese fortified with different iron sources. Generally, the bioavailability of iron in cow milk is lower than in human and goat milks (22, 28, 31). The effect of milk protein on iron absorption is not clear. The bioavailability of iron in fortified milk and milk products depends on the source of iron (1, 21) and on processing (36). The objectives of this study were to measure the bioavailability of iron-milk-protein complexes and Cheddar cheeses

Received February 22, 1989.
Accepted June 1, 1989.
1Paper 3771 of the Utah Agricultural Experiment Station, supported by the Western Dairy Foods Research Center, National Dairy Promotion and Research Board, and Utah Agricultural Experiment Station Project 253.
fortified with different iron sources. Maximal and basal iron bioavailabilities were measured in rats.

**MATERIALS AND METHODS**

**Experimental Design**

Bioavailabilities of four iron sources, FeCl₃, and Fe-casein, ferripolyphosphate whey protein (FIP-WP), and iron whey protein (Fe-WP) complexes, were measured directly or in fortified cheese. Ferrous sulfate was the reference iron source. Maximal iron bioavailability was measured by feeding anemic, weanling male rats the diets with an iron level below their requirement. Basal iron bioavailability was measured by feeding normal adult female rats diets with an iron level several times above their requirement, simulating normal diets of normal human subjects as discussed by Thannoun et al. (35). Hemoglobin regeneration efficiency (HRE) was measured as the criterion of iron bioavailability (23).

**Iron Sources**

Ferric chloride (Catalog No. F-2877) was purchased from Sigma Chemical Company, St. Louis, MO. Fresh FeSO₄·7H₂O (Catalog No. 1-146, Fisher Scientific Co., Fairlawn, NJ) was used because it gives uniform bioavailability values (27), a necessary attribute of a good reference source. The Fe-casein and FIP-WP precipitates were made as described earlier (41). The Fe-casein and FIP-WP precipitates (pH 4.5) were washed with lactic acid solution (pH 4) and deionized H₂O. The Fe-casein and FIP-WP contained 23 and 42 mg iron/g, respectively. The Fe-WP complex was made by adding 330 ml .5 M FeCl₃ into 4000 ml cottage cheese whey and adjusting pH to 3.5 with NaOH to precipitate Fe-WP. The precipitate was washed twice with lactic acid solution (pH 4) and then by deionized H₂O. The Fe-WP complex contained 99 mg iron/g. The recovery of iron in Fe-WP, Fe-casein, and FIP-WP was 98, 92, and 68%, respectively. The iron-protein precipitates were freeze-dried and stored in plastic bottles until use.

**Cheese Making**

Five cheeses were made in stainless steel vats, 94 × 79 × 40 cm, with a steam and water temperature control system. The milk was pasteurized at 79°C (175°F) for 29 s, cooled to 31 to 32°C (88-90°F), and 150 kg were poured per vat. Cheese culture, a mixture of lactic acid bacteria strains D 11, 52, 62, and 71 (Bio- lac™D.S.S.™ Defined Strain Starter, Miles Laboratories, Inc., Madison, WI), was added to milk (1%) during stirring. Food coloring (Annatto Food Color, water soluble, Chr. Hansens Laboratory, Inc., Milwaukee, WI; 143 ml/1000 kg milk), iron sources, and single strength calf rennet (Lot No. 18096, C. Hansens Laboratory Inc., Milwaukee, WI; 313 ml/1000 kg milk) was then added. The milk mixture was stirred for 2 min and allowed to coagulate for approximately 30 min. The coagulum was cut with a set of curd knives with horizontal and vertical wires. After heating 10 min, the curd was gradually heated to 38.9°C in 30 min and cooked at this temperature for 1 h with stirring. The whey was then drained. The cheese curd was kept in the vat at 32.2°C for 3 h and piled to three high within 2 h after draining. Before hooping, salt was added to the curd (2.75 kg/1000 kg milk) and mixed thoroughly. Cheese curd was hooped and pressed under 50 psi over night and then weighed, cut, vacuum-sealed in plastic bags, and stored in an air-circulated cool room at 4°C.

**Diets**

Sixteen diets were formulated balancing for all nutrients except iron (Table 1). Diets 5, 7, 9, and 11 were formulated with different iron sources, FeCl₃, Fe-casein, FIP-WP, and Fe-WP, respectively. Diets 13 to 16 were formulated with cheeses fortified with the respective iron sources. The iron level in these diets was below the requirement of the rats, ca. 23 mg iron/kg. Diets 1 to 3 were formulated with three levels of FeSO₄. All these diets were fed to weanling anemic rats for determining maximal iron bioavailability. Diets 4, 6, 8, 10, and 12 were made with the respective iron sources for an iron level (about 145 mg/kg) about five times higher.
TABLE 1. Formulation of diets (g/kg)\(^1\).

<table>
<thead>
<tr>
<th>Diet number</th>
<th>FeSO(_4)</th>
<th>FeCl(_3)</th>
<th>Fe-Casein</th>
<th>Fe-Polyphosphate WP</th>
<th>Fe-WP</th>
<th>FeCl(_3)</th>
<th>Fe-Casein</th>
<th>Fe-Polyphosphate WP</th>
<th>Fe-WP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
<td>5.1</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>47</td>
<td>44</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>145</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>145</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>8</td>
<td>145</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>10</td>
<td>22</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>11</td>
<td>22</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>51</td>
<td>51</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>13</td>
<td>22</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>14</td>
<td>22</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>357</td>
<td>357</td>
<td>339</td>
<td>345</td>
</tr>
<tr>
<td>15</td>
<td>23</td>
<td>165</td>
<td>583</td>
<td>583</td>
<td></td>
<td>516</td>
<td>519</td>
<td>530</td>
<td>528</td>
</tr>
</tbody>
</table>

\(^1\)All diets contained mineral mixture 11.6 g/kg. The composition of the mineral mixture (g/kg) was KCl 331.6, KI 1.1, MgCO\(_3\) 121, MnSO\(_4\)-H\(_2\)O 14.5, CoCl\(_2\)-6H\(_2\)O 7, ZnSO\(_4\)-7H\(_2\)O 25, CuSO\(_4\)-5H\(_2\)O 2, NaMoO\(_4\)-2H\(_2\)O 12, and glucose 505 to make up 1 kg. Vitimian diet fortification mixture (ICN Biochemicals, Cleveland, OH, Catalog No. 904654) was added to all diets, 20 g/kg. The composition (g/kg) was vitamin A acetate (500,000 IU/g) 1.8, vitamin D\(_3\) (850,000 IU/g) 125, DL-alpha-tocopherol acetate 22, ascorbic acid 45, inositol 5, choline chloride 75, menadione 2.25, p-aminobenzoic acid 5, niacin 4.25, riboflavin 1, pyridoxin hydrochloride 1, thiamine chloride 1, calcium pantothenate 3, biotin .02, folic acid .09, and vitamin B\(_12\) .00135. DL-Methionine was added to all diets, 3 g/kg.

\(^2\)Ferripolyphosphate - whey protein.

\(^3\)Diets with no cheese added were adjusted by butter oil to match the fat content of the diets made with iron-fortified cheese.
than the rats' requirement and were fed to normal adult rats for measuring basal iron bioavailability. All iron-protein complexes and iron-fortified cheeses were lyophilized before mixing with diet ingredients. Protein and fat in cheeses were measured and balanced across diets.

Animals

Seventy-seven weanling, male (about 60 g body weight), and 35 adult, female (about 200 g body weight) Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were housed individually in stainless steel cages with wire mesh bottoms and fronts. The weanling, male rats were made anemic by feeding a low iron diet (about 3 mg iron/kg) for 7 d and by bleeding 30 drops of blood from the retroocular capillary bed (37) on d 1 and 4. Their Hb averaged 5.4 g/dl. The mature female rats were fed a high iron diet (supplemented with 145 mg iron/kg as FeSO₄) for 7 d without bleeding. Their Hb averaged 14.5 g/dl. All rats were housed in a temperature-controlled, ventilated room with a 12 h light-dark cycle.

Body weight and Hb of rats were determined on d 8. Seventy-seven anemic weanling rats were allotted low iron diets (dietary iron below the requirement of rats) and 35 normal adult female rats high iron diets (dietary iron about five times of rats' requirement). Each group contained seven rats. The rats were assigned by their Hb level, which was balanced across the diet groups. Body weight was then balanced by moving rats with similar Hb and different body weight across the groups as needed. Fresh diet, 9 to 12 g per rat, was weighed daily for 10 d. Demineralized water was available at all times.

On d 18, all rats were weighed and Hb determined again. The anemic growing rats were then killed. The normal adult rats were fed their respective diets for 4 d longer and then weighed and Hb determined again. Blood was taken for serum iron measurement. The normal adult rats were then killed, and livers were taken for iron analysis. All spilled and refused diet was weighed and subtracted from diet given to calculate total iron intake.

Iron Bioavailability Evaluation Method

Hemoglobin regeneration efficiency was used to measure iron bioavailability. It is calculated as the percentage of consumed dietary iron incorporated into hemoglobin (Hb). The formula is as follows:

\[
\text{HRE} = \frac{\text{Final Hb-iron, mg} - \text{initial Hb-iron, mg}}{\text{Dietary iron intake, mg}} \times 100.
\]

Chemical Analysis

Hemoglobin concentration was measured colorimetrically from duplicate blood samples (8). Iron in iron-milk protein complexes, cheeses, diets, and livers was measured using Ferrozine® described by Zhang and Mahoney (41). Samples were wet ashed with concentrated nitric acid. Drops of 30% hydrogen peroxide were added if sample ashes were not white. The ash was dissolved in 1 N hydrochloric acid and then analyzed for iron. Serum iron was measured by modified Ferrozine method. Serum was obtained by centri-
fusing the blood samples at $1600 \times g$ for 20 min. The absorbances contributed by color present in serum samples were measured before adding ferrozine and subtracted from total absorbances measured after adding ferrozine to the sample solutions; this corrected the readings for any effects of hemolysis in the serum samples.

**Statistical Analysis**

The data were analyzed by ANOVA. When “$F$” was significant ($P<.05$), least significant difference values were calculated at the .05 level of probability, and the group means were compared (13). Correlation coefficients and regression were calculated for the relationship between iron intake and final Hb concentrations in rats fed FeSO$_4$ diets. The intercept of final Hb level at zero iron intake was derived from the regression equation (13).

**RESULTS**

**Bioavailability of Iron Fortified in Cheese**

Hemoglobin regeneration efficiency and related hematric values of the anemic rats fed iron-fortified cheeses are listed in Table 2. More than two-thirds of the dietary iron was incorporated into Hb in anemic rats fed on iron-fortified cheese diets. There were no statistically significant differences ($P>.05$) in HRE among the cheese diets. The HRE values of the iron-protein complexes were similar whether they were mixed directly into diets or in fortified cheese and then mixed into diets (Table 2, $P>.05$). The HRE values were the same for diets supplemented with FeSO$_4$ or FeCl$_3$ (Table 2).

**Effect of Iron Status on Iron Bioavailability**

Table 3 shows body weight and hematric values of adult rats fed high iron diets. Hemoglobin regeneration efficiency of these rats was much lower than those of anemic growing rats fed low iron diets (6% versus 77%, $P<.01$). Iron status and dietary iron level strongly affected the iron bioavailability values for all iron sources. However, differences of bioavailability among the iron sources were not significant ($P>.05$) either in anemic...
TABLE 3. Body weight and hematinic values of normal adult female rats fed diets containing different iron sources for 10 d.

<table>
<thead>
<tr>
<th>Iron sources</th>
<th>FeSO₄</th>
<th>FeCl₃</th>
<th>Fe-Casein</th>
<th>FIP-WP</th>
<th>Fe-WP</th>
<th>MSE²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet number</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>217</td>
<td>215</td>
<td>218</td>
<td>214</td>
<td>215</td>
<td>...³</td>
</tr>
<tr>
<td>Gain</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>38.4</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>14.54</td>
<td>14.57</td>
<td>14.54</td>
<td>14.54</td>
<td>14.60</td>
<td>...</td>
</tr>
<tr>
<td>Gain</td>
<td>1.43</td>
<td>1.34</td>
<td>1.56</td>
<td>1.37</td>
<td>1.65</td>
<td>.647</td>
</tr>
<tr>
<td>Hb Iron gain, mg</td>
<td>.84</td>
<td>.82</td>
<td>1.15</td>
<td>.91</td>
<td>1.03</td>
<td>.307</td>
</tr>
<tr>
<td>Iron intake, mg</td>
<td>15.37</td>
<td>15.31</td>
<td>14.33</td>
<td>15.07</td>
<td>15.11</td>
<td>...</td>
</tr>
<tr>
<td>HRE, %</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>9.62</td>
</tr>
</tbody>
</table>

¹ Ferricophosphate - whey protein.
² No statistically significant (P>.05) differences were observed among the iron sources.
³ Not calculated.
⁴ Hemoglobin regeneration efficiency.

rats fed low iron or in normal rats fed high iron diets (Tables 2 and 3).

Hemoglobin Synthesis from Body Iron

The relationship between iron intake from FeSO₄ and final Hb is illustrated in Figure 1. Within the given dietary iron levels, final Hb increased when iron intake from ferrous sulfate increased (r = .97). The intercept, zero iron intake, is 6.54 g/dl final Hb, higher than initial Hb, which averaged 5.34 g/dl. This difference indicated that the anemic rats used body iron to synthesize Hb in addition to absorbed iron. In HRE calculation, Hb synthesized using body iron should be subtracted.

Comparison of 14-d Hematinic Responses Versus 10-d Responses

The 14-d hematinic values of normal adult rats fed iron-supplemented diets are shown in Table 4. Body weight gain and hematinic values were not significantly different among rats fed diets supplemented with the different iron sources; they were similar to the values obtained from 10-d feeding (Table 3). Serum Fe and Liver Fe were not significantly different among the rats fed diets with different iron sources (Table 4). Final body weight, body weight gain, final Hb Fe, and Hb Fe gain values of the rats fed diets 14 d were not statistically different from the 10 d feeding values (P>.05), Table 5. Feeding the diets for 14 d resulted in lower HRE than feeding for 10 d (P<.05); the rats had no further increase in Hb iron during the additional 4 d of feeding.

DISCUSSION

Dairy Products and Iron Bioavailability

Dairy products contain trace amounts of iron (4, 34). The iron in cow milk is not as
TABLE 4. Body weight and hematinic values of normal adult female rats fed diets containing different iron sources for 14 d.

<table>
<thead>
<tr>
<th>Iron sources</th>
<th>FeSO₄</th>
<th>FeCl₃</th>
<th>Fe-Casein</th>
<th>FIP-WP¹</th>
<th>Fe-WP</th>
<th>MSE²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet number</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>8</td>
<td>83.9</td>
</tr>
<tr>
<td>Final Hb, g/dl</td>
<td>16.24</td>
<td>15.89</td>
<td>15.56</td>
<td>15.56</td>
<td>15.17</td>
<td>.460</td>
</tr>
<tr>
<td>Final Hb Fe, mg</td>
<td>8.16</td>
<td>7.89</td>
<td>7.94</td>
<td>7.66</td>
<td>7.62</td>
<td>.250</td>
</tr>
<tr>
<td>Fe Fe gain, mg</td>
<td>1.08</td>
<td>.84</td>
<td>.82</td>
<td>.66</td>
<td>.57</td>
<td>.228</td>
</tr>
<tr>
<td>Iron intake, mg</td>
<td>21.11</td>
<td>21.04</td>
<td>21.00</td>
<td>20.55</td>
<td>20.96</td>
<td></td>
</tr>
<tr>
<td>HRE, %</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>7.93</td>
</tr>
<tr>
<td>Serum iron, µg/ml</td>
<td>3.72</td>
<td>3.45</td>
<td>3.64</td>
<td>4.14</td>
<td>3.60</td>
<td>.526</td>
</tr>
<tr>
<td>Liver iron, mg/µg</td>
<td>1.43</td>
<td>1.58</td>
<td>1.44</td>
<td>1.50</td>
<td>1.58</td>
<td>.064</td>
</tr>
<tr>
<td>mg/g</td>
<td>.22</td>
<td>.24</td>
<td>.21</td>
<td>.23</td>
<td>.23</td>
<td>.002</td>
</tr>
</tbody>
</table>

¹Ferripolyphosphate - whey protein.
²No statistically significant differences (P>0.05) were observed among iron sources for all indexes.
³Not calculated.
⁴Hemoglobin regeneration efficiency.

TABLE 5. Body weight and hematinic values of normal adult female rats with 10- and 14-d feeding.

<table>
<thead>
<tr>
<th>Feeding duration</th>
<th>10 d</th>
<th>14 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>222</td>
<td>223</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Final Hb, g/dl</td>
<td>16.03*</td>
<td>15.68*</td>
</tr>
<tr>
<td>Final Hb Fe, mg</td>
<td>8.00</td>
<td>7.85</td>
</tr>
<tr>
<td>Hb Iron gain, mg</td>
<td>.95</td>
<td>.79</td>
</tr>
<tr>
<td>Iron intake, mg</td>
<td>15.04</td>
<td>20.93</td>
</tr>
<tr>
<td>HRE, %</td>
<td>6*</td>
<td>4*</td>
</tr>
</tbody>
</table>

¹Hemoglobin regeneration efficiency.
²Statistical significance was not calculated.
*The difference of the values in the same row is statistically significant (P<.05).

Bioavailability of iron in fortified milk and infant formula varies by iron sources and processing. In studies with rats, McMillan et al. (22) reported that ⁵⁹Fe incorporated into Hb was 14% after consuming cow milk, compared with 21% for human milk. Saarinen and Siimes (31) reported 70% of iron in breast milk and 30% of iron in cow milk were absorbed by infants 2 to 4 mo old. The iron in cow milk had lower bioavailability (HRE 13%) than iron in goat milk (HRE 50%) (28).

Milk protein was attributed to the category of inhibitors of iron absorption by Morris (26), based on one study in which Cook and Monsen (7) reported that substitution of beef by milk protein in a typical American diet reduced absorption of extrinsic labeled ⁵⁹Fe from 5.5 to 1.6%. However, Carmichael et al. (6) found that administration of nonfat milk enhanced the absorption of iron from iron(III)-nitritotriacetate chelate [Fe(III)-NTA] and did not affect the absorption of iron from Fe(II)SO₄ and Fe(III)-fructose in chickens and mice. Using the Hb repletion technique in rats, Ranhotra et al. (29) found that bioavailability of iron from milk fortified with citrate phosphate iron complex was as high as iron from FeSO₄. In the present study, the rats incorporated iron into Hb from iron-casein complex, iron-whey protein complexes, and cheeses fortified with these iron sources, similar to iron incorporation from FeSO₄ and FeCl₃.

Bioavailability of iron in fortified milk and infant formula varies by iron sources and processing. In studies with rats, Lönnerdal et al. (21) reported iron bioavailability of cow milk fortified with FeCl₂, FeSO₄, ferric nitritotriacetate, or ferric lactobionate was similar. However, the rats absorbed less iron from milk fortified with ferric EDTA and the least from ferric citrate. Anderson et al. (1) found that 65% of the FeSO₄ iron, 9% of the reduced iron, and 10% of the sodium iron pyrophosphate in fortified cereal plus milk were incorporated into Hb. In this study, iron bioavailabilities were similar among cheeses fortified with different iron-protein complexes. Theuer et al. (36) found that heat processing milk-based infant formula with the iron sup-

Journal of Dairy Science Vol. 72, No. 11, 1989
plement resulted in greater iron bioavailability in anemic rats than when the supplements were added to the formulas after heat treatment. Possibly, iron-protein complexes were formed in heating processing that resulted in improved bioavailability compared with the parent iron salt supplement. Similar bioavailabilities between iron fortifying sources and fortified cheeses in this study indicated that iron-protein complexes were likely incorporated into cheese without form changes during cheese processing.

The health benefits of iron-fortified dairy products are apparent. Baby pigs absorbed 30% of the iron from whole milk fortified with ferric ammonium citrate (FeAC) (20 ppm) and remained physiologically normal (38). Fortification of milk with iron increased hematinic values of the rats (11). In a field study (32), fortification of milk with FeSO₄ improved all measures of iron nutrition of infants aged 9 to 15 mo. At 15 mo of age, the percentage of anemia was reduced from 25.7% in infants fed unfortified diets to 2.5% in infants fed fortified diets. Iron-milk protein complexes used in this study were all high bioavailability iron sources.

Jones et al. (20) and Douglas et al. (12) reported that iron from FIP-WP was utilized as well as FeSO₄ when they were fortified either in chocolate or whole milk. This is consistent with our results. High bioavailability of the four cheeses fortified with FeCl₃, Fe-casein, FIP-WP, or Fe-WP indicates that cheese could be a good iron carrier, especially considering that 73% of US blacks are lactase deficient and cannot digest milk (9). Fe-casein, FIP-WP, and Fe-WP would be good iron sources for fortifying other foods because of their high bioavailabilities and stable chemical properties.

### Effect of High Dietary Iron Intake on Iron Status of Normal Adult Rats

Humans typically consume 10 to 18 mg iron/d, but their daily iron requirement is only 1 to 2 mg. In this study, normal adult rats were fed the iron sources at about five times their dietary iron requirement (145 mg iron/kg, about 32 mg iron/1000 kcal). Farley et al. (14) reported that iron density ranged up to 30 mg iron/1000 kcal in diets of normal adult humans. The RDA of women of child-bearing age is 9 mg iron/1000 kcal. The diets in this study would have needed about 40 mg iron/kg to provide 9 mg iron/1000 kcal. White (39) reported that the average daily iron intake of females 15 to 54 yr old was 5.3 to 6.7 mg iron/1000 kcal, close to the 4.9 to 5.3 mg/1000 kcal in the diets of the anemic rats in this study.

The HRE values of normal adult rats were lower after 14 d than after 10 d, a result of exceeding the dietary iron requirement and an adequate iron status of the rats. These data indicate the rat's ability to regulate iron absorption when an excess of dietary iron is consumed from these fortification sources at an iron intake relative to requirement that is similar to human consumption patterns. However, a longer period and more observations are needed to test the extent of ability to regulate absorption from these iron sources. The capacity to regulate iron absorption is important in considering the potential for iron overload in long-term high iron consumption. Although serum Fe concentrations of rats in the present study were higher than those reported by Rosenmund (30) and Itzhaki and Belcher (19), the saturation of iron in serum was less than 70%, as calculated using the latter authors' values of serum total iron-binding capacity of rats, 530 to 610 µg/dl. Liver iron concentration was similar to those of normal humans reported by Worwood (40). Hemoglobin concentration was also similar to normal human values.

There is a consideration of potential iron overload due to iron fortification, but no report has confirmed any evidence of the risk. Iron overload is mostly related to inborn metabolism disorders (16). The only population observed with iron overload from dietary source was the people in subSaharan Africa who consumed beer made in steel drums, resulting in a high amount of soluble iron (17). Our fortification level of Cheddar cheese was low, 9 mg/1000 kcal, close to the iron level in meat. Thus, from this perspective, there should be no risk of causing iron overload.
Maximal, Practical, and Basal Iron Bioavailability

Nonheme iron may not be in the same gastrointestinal pool, and the absorption of inorganic iron salts and nonheme iron complexes may be regulated in different ways. Iron from inorganic salts such as FeSO₄ can be absorbed by passive diffusion in the intestinal mucosal cells whereas nonheme iron complexes are most likely absorbed by a regulated carrier system (42). Information on safety of iron sources depends on how well absorption is regulated, thereby preventing iron overload of iron-sufficient subjects at high iron intakes; information on bioavailability depends on how well the sources cause iron repletion of subjects with low iron status at modest iron intakes.

Iron bioavailabilities are affected by iron status, maturity of subjects, and ratio of dietary iron level to requirements. These variables are usually different among experiments when the same products are determined. To understand the meaning of iron bioavailabilities of different experiments and to make comparisons among them, these factors should be controlled. This necessity has been shown in this study; iron-deficient growing rats fed low iron diets had iron bioavailabilities 12 times higher than iron-sufficient adult rats fed high iron diets for the same iron source. This is consistent with human data that iron absorption values of iron-depleted human subjects was up to nine times higher than those of normal subjects (18). For this reason, three iron bioavailability categories: maximal, practical, or basal iron bioavailability may be introduced to control the non-experimental variables.

Maximal iron absorption measures total absorbable iron. Iron requirement of the subjects should exceed the amount of iron in the diet. Anemic subjects and low dietary iron concentration are applied. We found that 66 to 75% of the iron consumed from fortified cheeses was incorporated into hemoglobin under these conditions, which was about 80% of that of FeSO₄, the reference source. Thus, the maximal bioavailabilities of these iron-fortified cheeses were approximately equivalent to beef (15, 25, 42), turkey (24), and egg (25) relative to FeSO₄. Practical iron absorption measures iron absorption of subjects who need more iron to avoid anemia. Borderline iron-deficient subjects (hemoglobin concentration normal but body iron stores low) are fed a practical human dietary iron level, 6 to 9 mg iron/1000 kcal, to generate absorption values. Basal iron absorption measures the ability of subjects with high body iron stores to regulate the absorption of iron sources. Iron-sufficient subjects are given dietary iron more than five times their requirement for a longer period. For humans and rats, this dietary iron level would be about 30 mg iron/1000 kcal. Basal iron absorption studies can answer questions about which sources of supplemented iron might increase risk of iron overload. We found that the basal absorption of iron from the fortification sources was very low and similar to that from FeSO₄. This is consistent with what Buchowski et al. (5) found for FeSO₄, liver, and plant iron fed to normal rats under similar conditions. Thus, absorption from these iron fortification sources seems to be regulated well and should not cause iron overload.

CONCLUSIONS

Although bioavailability of iron in cow milk is lower than that of human and goat milks, evidence shows that dairy products fortified with iron could provide sufficient iron to meet human needs. Cheeses fortified with FeCl₃ or iron-protein complexes have all shown high iron bioavailability similar to FeSO₄. This indicates that cheese fortified with iron could be a good iron source to promote human dietary iron intake.

REFERENCES

5 Buchowski, M. S., A. W. Mahoney, M. P. V. Kalpa-


